
# High sensitive CRP (hs-CRP) as a marker for Coronary Heart Disease (CHD) in Ramallah area- Palestine.

By

Husam Aref Thaher

Supervisors

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Birzeit –Palestine

# High sensitive CRP (hs-CRP) as a marker for Coronary Heart Disease (CHD) in Ramallah area- Palestine.

# س.ر.بي الحساس كعامل لأمراض القلب في منطقة رام الله - فلسطين.

By

Husam Aref Thaher

This thesis was successfully defended and approved on 24/1/2008

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# Dedication

I dedicate this thesis to my Parents, my wife Enas, and my children Luna, Dania, Aref, Joud, and Saed who gave me the reason to succeed.

HAT

## Acknowledgments

I would like to acknowledge Dr. Tamer Essawi, the founder and head of the Master degree in Medical Laboratory science; for his tremendous efforts and guidance. I would like to thank Dr.Lina El-Khairy and Dr Johny Khoury my advisors for their help and support to accomplish this work and for their efforts and supervision during this study. Finally, I would like to thank all the people who helped me specially Medical Relief Prevention and Diagnostic Center of Cardiovascular Diseases – Ramallah, Specialized medical Laboratory in Ramallah, and other laboratories for their support and help in specimen collection, and processing.

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# Abbreviations

**hs-CRP**: high sensitive C- reactive protein.

**CHD**: coronary Heart Disease.

**CAD**: Coronary artery disease.

**CVA**: Cardio vascular disease.

**MI**: myocardial Infarction.

**ACS**: acute coronary syndrome.

**LDL-C**: low density lipoprotein cholesterol.

**HDL-C**: high density lipoprotein cholesterol.

**OR**: Odds ratio.

**RR**: relative risk.

**FRS**: Framingham risk score.

**PHS**: physician Health study.

**WHS**: women's Health study.

**MCR-1**: Macrophage chemoattractant protein 1.

**IL-1**: interleukin -1

**IL-6**: Interleukin -6.

**TNF-α**: Tumor necrosis Factor.

**NO**: Nitric oxide.

**eNOS**: endothelial nitric oxide synthase.

**ET-1**: endothelin – 1.

**VCAM-1**: Vascular cell adhesion molecule -1.

**ICAM-1**: Intra cellular adhesion molecule -1.

**MCP-1**: monocyte chemotactic protein.

**MMR**: matrix metalloproteinase.

**PAI**: Plasminogen activator Inhibitor.

**AT1R**: Angiotensin type 1 receptor.

**ملخص الدراسة:**

**تعتبر الوفيات الناتجة عن أمراض شرايين القلب من أعلى النسب في العالم، ومن المعروف أن هذه الأمراض لها علاقة مع ارتفاع نسبة الكولسترول في الدم وبخاصة الكولسترول المنخفض الكثافة ( الكولسترول السيئ). إن حوالي 50-60 % من الأمراض المرتبطة بالقلب يساعد فحص نسبة الكولسترول السيئ في الدم في تشخيصها. هذا الفحص المسحي للكولسترول المنخفض الكثافة فشل أحيانا في تشخيص الأشخاص الذين عندهم عوامل خطرة تهيء للإصابة بأمراض القلب وخاصة عندما يكون الكولسترول السيئ ضمن المعدل الطبيعي. من هنا يحاول الاختصاصيون التعرف على عوامل أخرى تسهم في تشخيص هذه الفئة من الأشخاص . إن ظاهرة تصلب الشرايين بحد ذاته هي أنها مرض التهابي ولهذا فانه يرافق هذا المرض عوامل التهابية تساعد في تشخيصه من أهمها سي، ر، بي آو سي ريا كتف بروتين الذي يتم قياسه بالطرق الحساسة والدقيقة جدا ويعتبر من أهم هذه العوامل المصاحبة لأمراض القلب المستقبلية. لقد تم في هذه الدراسة دراسة حوالي 160 حالة( 80 حالة مرضية مشخصة على أنها تعاني من أمراض القلب و 80 حالة لا تعاني من أية أمراض في القلب) من رام الله – فلسطين . إن كلا المجموعتين تتعرض لكافة العوامل التي قد تسبب في تصلب الشرايين مثل التدخين والضغط والسكري والكولسترول. لقد تم قياس س.ر.بي الحساس بواسطة طرق اليزا للمجموعتين لإيجاد أي علاقة ما بين هذا البروتين وأمراض القلب. وفي المقابل تم المقارنة ما بين هذا البروتين والكولسترول السيئ لمعرفة الأفضل منهما في اكتشاف أمراض القلب المستقبلية. لقد تم إيجاد علاقة إحصائية قوية بين وجود سي, ر, بي الحساس وأمراض القلب $P< 0.005$ باستعمال عامل t- test الإحصائي. وقد كانت OR (odds ratio) لفئات سي,ر,بي الثلاث ما بين المرضى وغير المرضى 4.25**

**95% CI (2.34-7.22) . سي,ر,بي الحساس أكثر من 3 ملغم لكل ليتر والكولسترول السيئ اقل من 130 ملغم لكل ديسيليتر كانت (OR 7.66 95% CI (2.5-23.4 بالمقارنة مع المجموعة المرجعية (س.ر.بي قليل مع الكولسترول السيء قليل).**

**إن هذه النتائج تشير إلى أهمية إضافة فحص س.ر.بي إلى فحص الكولسترول السيئ المسحي لتشخيص الأشخاص المعرضين لأمراض القلب المستقبلية حيث ثبت فعاليته أكثر من فحص الكولسترول السيئ لوحده فقط. ويمكن إضافته أيضا في المختبر إلى جانب نسبة الكولسترول الكلي على الكولسترول الجيد مما يزيد من نسبة الكشف عن حالات الأمراض القلبية المستقبلية.**

# High sensitive CRP (hs-CRP) as a marker for Coronary Heart Disease (CHD) in Ramallah area- Palestine.

## Abstract

Mortality from (CHD) is the highest worldwide. For long time it has been associated with high lipids concentration, mainly LDL-C. About 50-60% of cases associated with CHD were diagnosed by lipid screening profile. This profile failed to diagnose people who had a high risk for CHD in certain cases, and in some cases associated with normal LDL-C. Because of this, scientists are trying to identify other risk factors that may help in diagnosing people with high risk for developing future CHD. Atherosclerosis is considered an inflammatory and atherogenic disease. As a result, inflammatory factors are considered as the most important factors in this aspect. C - reactive protein (CRP) measured by high sensitive methods is considered the most predictive factor associated with future coronary heart disease. In this case –control study, we have selected 160 subjects (80 cases and 80 controls) from the West Bank. Controls were free of CHD, while cases were diagnosed to have CHD. Both groups had the same conditional risk factors, such as smoking, hypertension, diabetes, and dislipedemia. hs-CRP measured by ELISA methods for both groups to find out the association between levels of hs-CRP and CHD. In addition a comparison between hs-CRP and LDL-C in

predicting future detection rate of CHD were studied. It was found that there was a statistically significant relationship between hs-CRP and CHD, $P < 0.005$ using independent t-test. The odds ratio for equal quartiles of hs-CRP between control and cases were found to be *4.25 95% CI (2.34-7.22)*. hs-CRP greater than 3.0 mg/L and LDL-C less than 130 mg/dl odds ratio was *7.66 with 95% CI (2.5-23.4)* compared to the reference group( Low CRP/ Low LDL-C). These finding indicated the importance of adding hs-CRP to lipid screening profile. In this study it was found that hs-CRP can be considered a good predictor for events associated with CHD more than LDL-C, and clinically can be added with T-cholesterol/HDL-C ratio to predict the risk for CHD.

# Introduction

Coronary heart diseases (CHD) are the most prevalent causes of death in the industrialized nations worldwide. Half of all events associated with CHD were reported in apparently healthy individuals. Those individuals have normal or low cholesterol levels and have none of the traditional risk factors associated with CHD [(1)]. Therefore, attention has been focused on the role of other factors, such as inflammatory markers in patients at risk for atherosclerosis and CHD [(2).] The goal to search for inflammatory biomarkers was to improve the detection of CHD risk among seemingly healthy individuals [(3)]. Several studies [(1, 6, 9)] have shown that hs- CRP, a protein found in blood circulation, can be predictive of future (CHD) events, and a marker of inflammation in detection patients at increased risk for CHD. Several prospective studies [(1, 10, 11, 12, and 21)] have demonstrated that hs-CRP is an independent predictor of future risk for cardiovascular events among healthy individuals, as well as among patients with acute coronary syndromes. hs-CRP can add prognostic information on risk at all levels of low-density lipoprotein cholesterol (LDL-C), Framingham Risk Score, and at all levels of metabolic syndrome [(3, 33)]. The center for Disease Control and prevention (CDC) and the American Heart Association (AHA) recommended that hs-CRP should be measured among individuals without known CHD twice and results used to evaluate the risk for the purpose of primary prevention and risk detection [(2)]. Most (CHD) events begin with

atherosclerosis early in life and can progress in the body silently for decades without symptoms [4]. Recent laboratory and clinical evidence revealed that systemic inflammation plays a major role in the initiation, progression, and destabilization of atheromas [5]. Cholesterol and (LDL-C) were used as screening tools for identifying individuals at increased risk of developing future coronary events. Although this approach has been useful, it fails to identify about one-half of the 1.3 million individuals who develop myocardial infarction (MI) in the United States (US) each year whose cholesterol level is normal or moderately elevated [5] . In a recent analysis of about 27939 healthy American females, it was found that about 77% of future myocardial infarction (MI), strokes and vascular related deaths occurred among women with LDL-C levels less than 160 mg/dl, and about 46% of events occurred among women with LDL-C less than 130 mg/dl. [6] . This has given rise to extensive study to add another inflammatory risk factor mainly hs-CRP to predict future CHD. High sensitive assays for determining hs- CRP concentration in blood to predict first cardiovascular events were developed. The aim of this research is to find out if hs-CRP considered as a marker of CHD in Ramallah area -Palestine.

## C-Reactive Proteins

C - reactive protein (CRP) is a member of the hepatic pentraxin family of proteins, a group so named because they are composed of five identical subunits. Each subunit is composed of 206 amino acids with

molecular weight of 23027 Daltons arranged around a central pore as shown in Figure (1) [7].

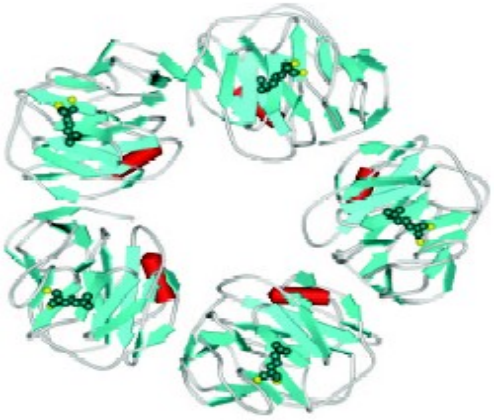


*Figure (1): CRP pentamere particle.* [7]

CRP is an acute phase plasma protein, it was discovered in 1930. William Tillet and Thomas Francis define CRP as a substance present in the serum of patients with acute inflammation and reacted with the 'C' polysaccharide of pneumoccocus [7, 9]. CRP is a member of a special response type protein called 'Acute phase reactants'. CRP appears in serum in response to a variety of inflammatory stimuli including myocardial inflammation [7]. CRP has a relatively stable concentration in the body and a long half-life in the absence of major infection or inflammation that may influence its concentration. The concentration of CRP in the blood measured in milligram per liters (mg/L). In a comprehensive study hs-CRP mean level was found to be 1.8 mg/L for men and 2.0 mg/L for women. Furthermore, hs-CRP levels was found to increase with age, approximately 30% of those subjects in the study showed hs-CRP concentrations greater than 3.0 mg/L. [7] . Blood concentration of CRP can become elevated several folds during an

infection, inflammation or neoplastic diseases. Following tissue injury, CRP levels begin to rise within 4-6 hours, doubling every 8-9 hours, reaching peak within 24 hours. Conditions that can elevate CRP levels in the body include rheumatoid diseases, vasculitides and chronic infections such as tuberculosis. Other conditions that fall in this category are allograft vasculopathy and graft occlusion, connective tissue diseases, coronary artery diseases, obesity, sepsis, smoking and vasculitis [7, 9]. Frequent exercise, aspirin and statins (3-hydroxy-3-methylglutaryl) are shown to exert inhibitory effect on the production of CRP resulting in a reduced concentration of this protein in the blood [9] . During major trauma or infection, inflammatory cells (neutrophils granulocytes etc) and red blood cells in response to the inflammation, secrete large amounts of cytokines. The most prominent secreted cytokines are the Interleukins 1, 6 and 8 (IL-1, IL-6 and IL-8) and the tumor necrosis factor-alpha (TNF-alpha). These cytokines then stimulate the liver to produce large amount of C-reactive proteins (CRP) [7, 8, and 9]. In CHD, current events suggest that atherosclerotic lesions generate CRP, but the liver produces the bulk of CRP [7] . Other proteins secreted by the liver in response to inflammatory stimulation includes, serum amyloid A, fibrinogen and mannan-binding lectin. Since these proteins are released in response to acute inflammation, they are appropriately referred to as 'acute phase reactants'. Of all acute phase reactants, CRP has stood out as a novel biomarker for predicting the development and progress of several disease conditions, mainly cardiovascular events [9, 10, and 11]. As part of its

inflammatory response functions, CRP has been shown to stimulate monocyte to release pro-inflammatory cytokines such as interleukin-1b (IL-1b), IL-6, and tumor necrosis factor – alpha (TNF). It also mediates monocytes chemotactic protein-1(MCP-1) induction in endothelial cells and causes expression of intercellular adhesion molecule -1(ICAM-1) and vascular cellular adhesion molecule-1(VCAM-1) by endothelial cells [7, 12, 13]. It appears that CRP has direct interaction with LDL-C [9]. The binding of atherogenic lipid in vitro and opsonization of LDL-C by CRP mediates uptake of LDL by macrophages [12]. CRP is also a factor in the development of atherosclerotic plaque. Although CRP was believed to be a marker of vascular inflammation, recent research indicates that it plays an active role in atherogenesis. It is detectable in the early stages of plaque development and believed to be involved throughout the atherogenic process, facilitating the whole process starting with recruitment of leukocytes to the arterial wall and eventually rupture of the plaque [7, 12] . Calabro et al. have proposed that the smooth muscle cells of human coronary arteries may also produce CRP as a local response to inflammatory cytokines [8]. They further noted that this locally produced CRP might participate in the atherogenic process [8]. In addition, Khreiss et al. have suggested that loss of the pentameric symmetry of CRP can result in a modified or monomeric CRP, which may be the major CRP promoter of the proinflammatory response in the coronary arteries [13]. As CRP concentration is traced back to inflammation, its measurement is used to detect the presence of active inflammation. Latex,

immunoturbidimetric or immunoelectrophorectic assays are used for the measurement of CRP. The normal serum concentration of CRP measured by these procedures is from 3 mg/L to above 200 mg/L [9] . This range is not sensitive enough to predict cardiovascular risk in seemingly healthy men and women. Scientists had developed an improved and modified technique to measure hs-CRP. The new hs-CRP assay has the ability to detect concentrations of CRP below 0.2 mg/L. The technique relies on the use of labeled monoclonal or polyclonal antibodies to CRP in Enzyme linked Immunosorbent assay (ELISA) or immunofluorescent assay [7].

## Coronary Heart Diseases

Coronary heart disease (CHD), also known as Coronary artery disease (CAD) is the most common cause of heart disease. This condition is due to problems with the coronary arteries i.e. arteries supplying blood to heart. These arteries become narrowed and hardened due to the build up of fat deposits and plaques on the inner walls resulting in atherosclerosis [14]. The cross section of a normal vein and artery are shown in Figure (2). Arteries carry oxygenated blood away from the heart while veins carry non-oxygenated blood back to the heart. The blue colored veins indicate the transport of blood with relatively low content of oxygen and high content of carbon dioxide. The red colored arteries indicate the transport of blood with relatively high content of oxygen and low content of carbon dioxide.

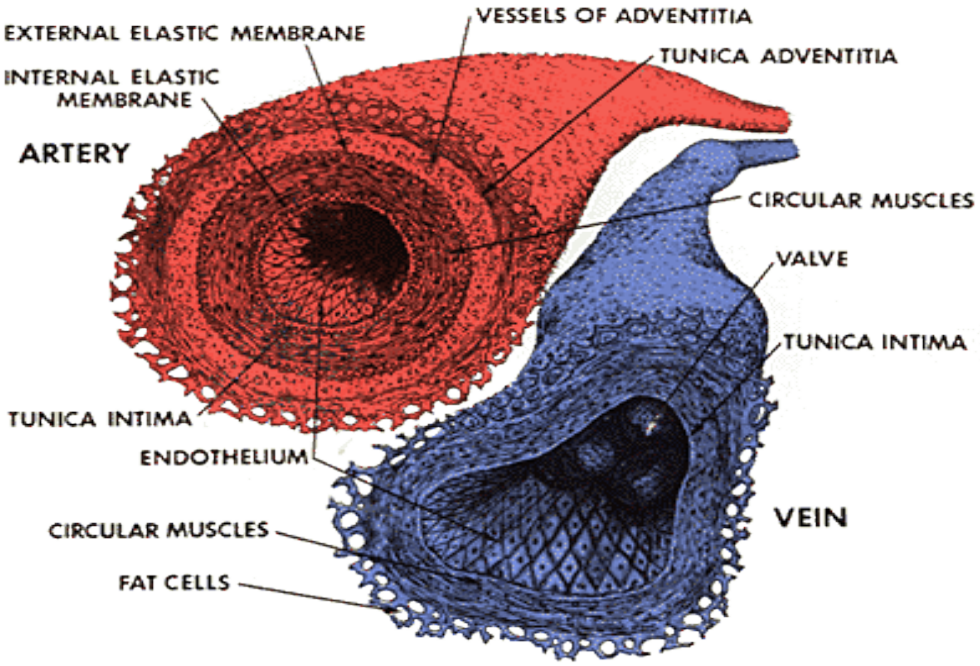



*Figure (2): Cross section of a normal vein and artery* [15] .

The common clinical manifestations and complications that correlate with CHD are the following: [14]

- Stable Angina.
- Unstable Angina.
- Acute Myocardial Infarction.
- Heart failure.
- Arrhythmia.
- Sudden death.

## *Angina*

***Stable angina***: It is ischemia due to fixed atheromatous stenosis of one or more coronary arteries, and it is known as angina pectoris. Stable angina is chest pain or discomfort that occurs when your heart muscle does not get enough oxygen-rich blood. There is a pressure or a

squeezing pain in the chest and in the shoulders, arms, neck, jaw, and back. This pain tends to get worse with activity and go away when you rest. Emotional stress also can trigger the pain. These symptoms occur when the vessels that carry blood to the heart become narrowed due to atherosclerosis, as shown in the Figure (3) [14, 15].

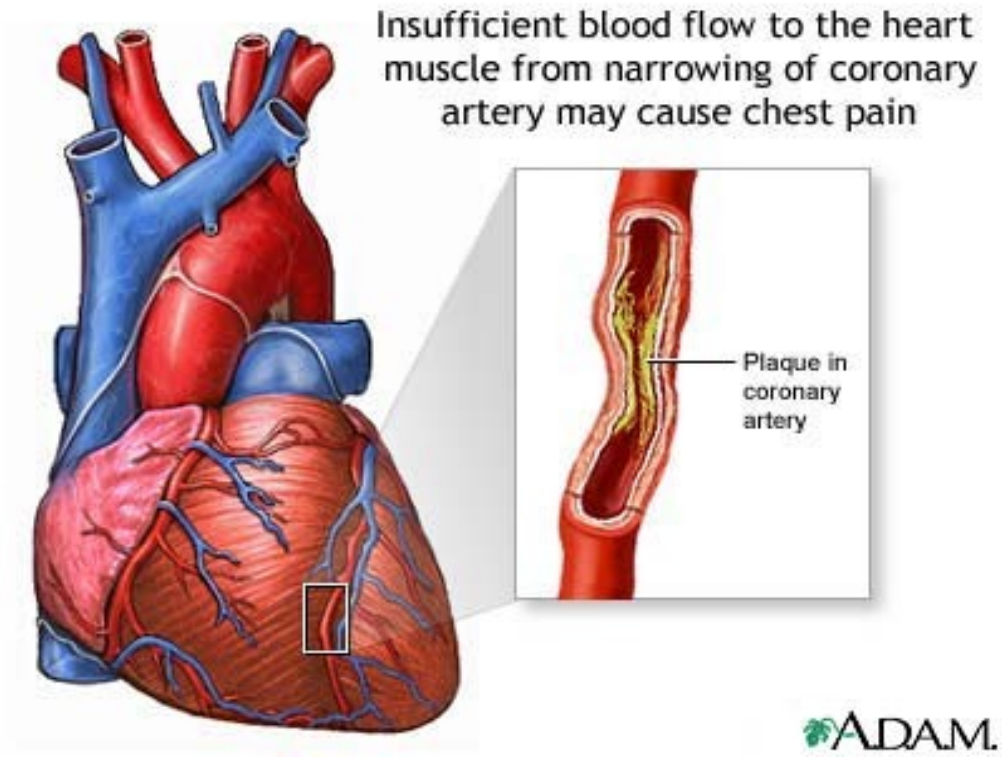



*Figure (3) Angina.*[15]

***Unstable angina***: A clinical syndrome that is intermediate between stable angina and myocardial infarction. Chest pain occurs at rest or with less exertion more often than stable angina. It is less responsive to medication. Unstable angina and myocardial infarction known as acute coronary syndromes (ACS). While stable angina considered as a chronic condition [15].

## *Acute Myocardial Infarction:*

Acute myocardial infarction (MI) or heart attack occurs when one of the arteries that supply the heart muscle becomes blocked. This happens when an area of plaque in a coronary artery breaks apart, causing a blood clot to form. The blood clot cuts off the blood to the part of the heart muscle that has fed by that artery. Cells in the heart muscle die because they do not receive enough oxygen-rich blood. This can cause irreversible lasting damage and necrosis to that part of the heart tissue as shown in the figure (4) [15].

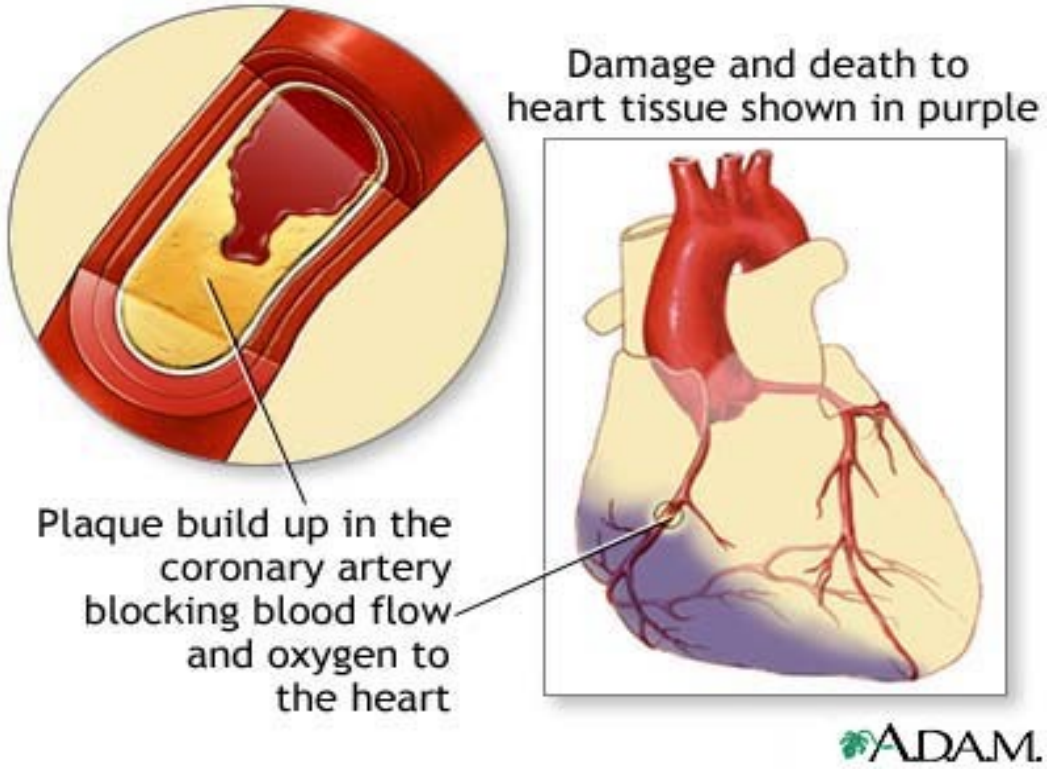



*Figure (4) Acut Myocardial Infarction* [15] .

### *Cardiovascular Risk Factors*

Risk factors are conditions or behaviors that increase the chance of getting a certain disease. These factors are different types: [14].

### *Nonmodifiable Risk Factors:*

- Age: The risk increases after age 45 in men, and after age 55 in women.

- Gender: Male sex develops higher risk than female, but by age 60-70 years old they become equal frequency while after that age women became higher in risk in comparison to men.

- Family history of early heart disease.

Increased risk if first-degree blood relatives have had coronary heart disease before the age 55 years for a male or 65 years for a female.

### *Modifiable Risk Factors:*

- Smoking.

- Hypertension.

- Dyslipidemia high total cholesterol, LDL- cholesterol and triglyceride.

Levels and low level of HDL cholesterol

- Obesity.

- Physical inactivity.

- Diabetes Mellitus.

- Unhealthy diet.

Novel Risk Factors

- Inflammation: Elevated C - reactive protein (CRP)

- Homocystine.

CHD is diagnosed by medical history, physical examination, and diagnostic tests include non- invasive and invasive test, the non-invasive tests include Electrocardiogram (ECG), exercise stress ECG test by treadmill, pharmacologic stress tests, and stress echocardiography [14].

Invasive tests such as cardiac catheterization (arteriography) used as diagnostic test to diagnose CHD [14]

## Atherosclerosis.

Atherosclerosis is a disease in which plaque builds up on the insides of the blood arteries that carry oxygen-rich blood to the heart and other parts of the body. Plaque is made up of fat, cholesterol, calcium, and other substances found in the blood. These fatty materials deposited in the vessel wall, resulting in narrowing, hardening of the artery. Severely restricted blood flow in the arteries to the heart muscle leads to certain problems such as angina and heart attack. Atherosclerosis shows no symptoms until complications occur [15].

> Atherosclerosis is a vascular inflammatory disease characterized by activation of the endothelium followed by cellular infiltration and cytokine production. This process leads to the formation of foamy macrophages, formation of atheromatous plaques, and atherothrombotic disease. [9] .

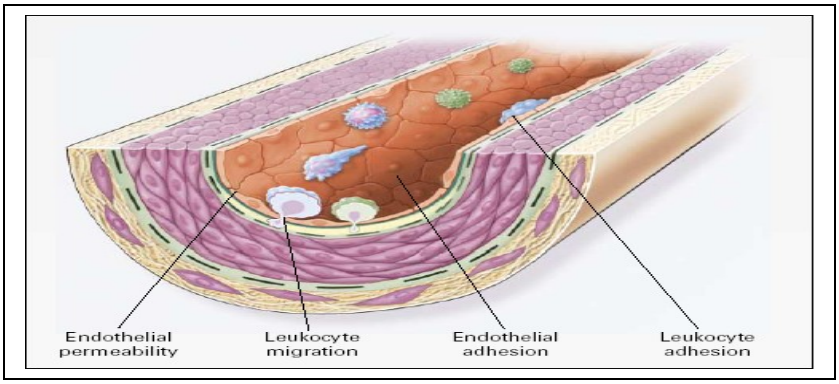
Cigarette smoking, limited exercise, obesity, and a diet high in carbohydrate or high in cholesterol and saturated fat are manageable high-risk factors for atherosclerosis. Some people are genetically susceptible to formation of plaques, while others are not genetically susceptible. Those that are susceptible form fatty streaks in the tunica intima at a young age. A fatty streak emerges when the endothelial cells subjected to large concentrations of cholesterol and lipoproteins in the blood. Prolonged exposure to cholesterol and lipoproteins transforms the fatty streaks into a plaque [15]. Recent medical advancement suggests that atherosclerosis and atherogenesis is due to more than just lipid deposits. Research studies define the process of atherosclerosis as (a progressive inflammatory disorder of the arterial wall that is characterized by focal lipid rich deposits of atheroma that remain clinically silent until they become large enough to impair arterial perfusion or disruption of the lesions results in thrombolic occlusion or embolisation at the affected vessel ) [14]. This process is divided into four stages as shown in figure 5: [4]

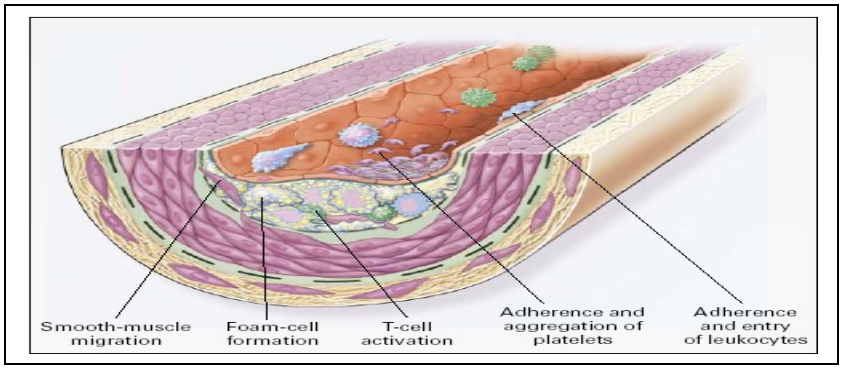
1: Endothelial dysfunction.

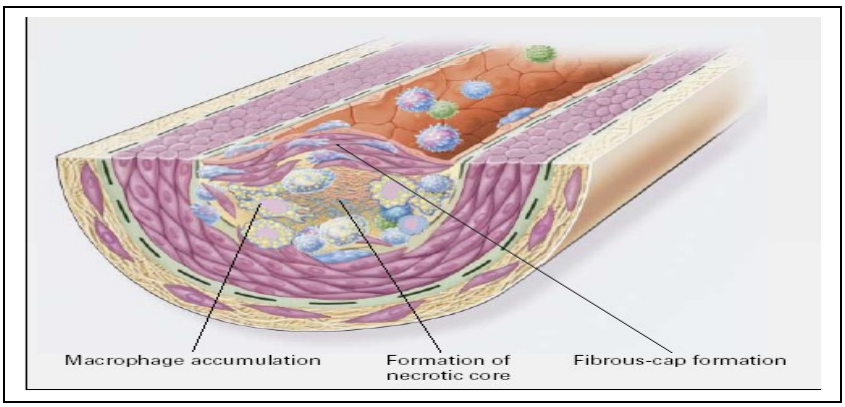
2: Fatty streak formation.

3: Formation of an advanced, complicated lesion of atherosclerosis.

4: Rupture of the fibrous cap or ulceration.



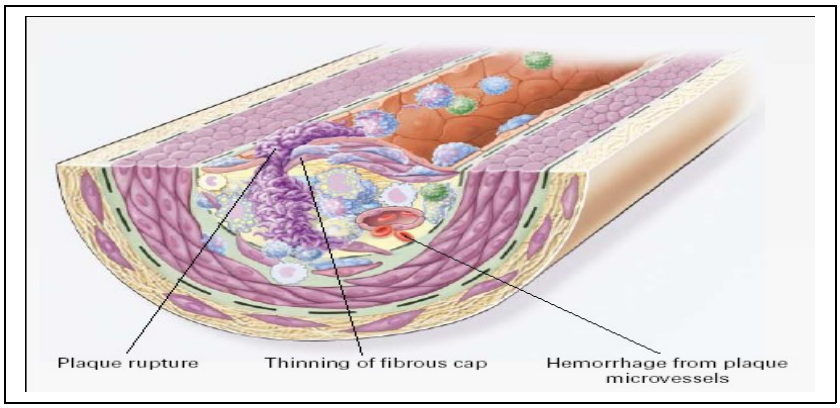




*Figure 5 : different stages of atherosclerosis.* [Ref (4)]

Several proinflammatory mediators considered in atherosclerosis process:

1- Prolonged high levels of LDL particles in blood stream infiltrate the arterial intima.

2- Lipoprotein particles undergo oxidation or other modification give rise to proinflammatory and pro- oxidant derivatives.

3- Cytokines, such as interleukins-1(IL-1), tumor necrosis factor (TNF), and angiotensin II.

4- Adipose tissue may drive inflammatory responses in the artery wall.

5- A failure of counter regulatory mechanism, example HDL particles may function as carriers for anti inflammatory and anti-oxidant mediators [7, 14] .

## CRP and Atherosclerosis

Recently hs-CRP linked to future development of (CHD) and the baseline levels of hs-CRP in the absence of any infection or tissue injury used in cardiac risk assessment [7] . Khreiss at al. provided the first evidence suggesting structural modification of pentameric CRP to monomeric subunits which is a potent atherogenic in human aortic endothelial cells [13] . Figure (6) illustrates the potential role of CRP in atherogenesis that is composed of four stages: [7]

***1- Endothelial dysfunction:*** CRP in this stage inhibits the generation of endothelial nitric oxide synthase (e-NOS), nitric oxide, and

prostacyclin. CRP also stimulates endothelial cell apoptosis and the production of vasoconstrictor endolthelin-1 (ET-1) and IL-6 resulting in blunting of endothelial vasoreactivity.

***2- Endothelial activation:*** CRP activates factor NFkB and stimulates production of IL-6, IL-8, vascular cell adhesion molecule (VCAM-1), Intracellular adhesion molecule (ICAM-1), and E-selectin. The result of this activation is monocyte adhesion and recruitment to the endothelium.

***3- Plaque formation:*** CRP stimulates the expression of monocyte chemotactic protein (MCP-1) which promotes the transmigration of monocyte to arterial wall. CRP also promotes LDL uptake by macrophages and formation of foam cells and generation of both reactive oxygen species and cytokines that fuel plaque formation. CRP may promote plaque remolding and maturation by stimulating smooth muscle cell proliferation and migration via the angiotensin type1 receptors (AT1R) which regulated by CRP.

***4- Plaque rupture:*** CRP promotes matrix metalloproteinase (MMP) activity, which denatures collagen and destabilizes the plaque. CRP inhibits (NO) and prostacyclin release and endothelial cell migration. On the other hand, stimulate the release of tissue factors from monocytes and the synthesis of plasminogen activator inhibitor (PAI-1). These conditions create a prothrombolic environment and favors plaque rupture and vessel thrombosis.

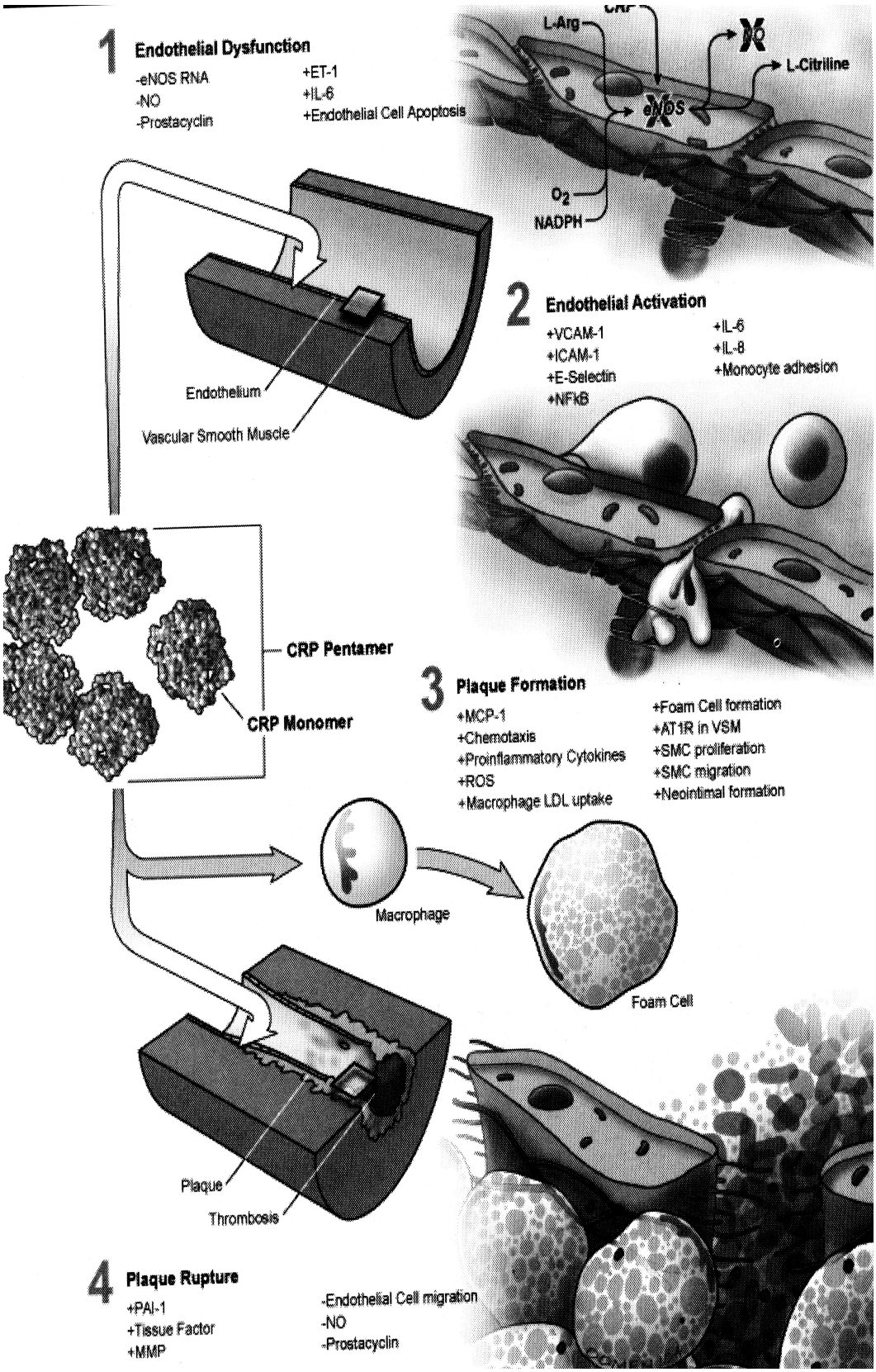



*Figure (6) the potential role of CRP in atherogenisis. Adopted from Ref.* [7 p 56]

# hs-CRP as an Independent Risk Factor

A number of large prospective epidemiologic studies have indicated that hs-CRP is a strong independent predictor of future cardiovascular events.

These events include myocardial infarction, ischemic stroke, peripheral vascular disease, and sudden cardiac death. In a Women's Health Study (WHS), it was found that hs-CRP compared to LDL-C was the most powerful predictor of future vascular events. Among 27939 healthy females studied and followed over 8 years period for future vascular events and evaluated for a full lipid panel as well as baseline hs-CRP levels, they found that there is a minimal relationship between hs-CRP and LDL-C (r=0.08) and thus the inflammatory process was providing information on plaque rupture separately from that of lipid evaluation [(6)] . The WHS study established a clinical cut points for hs-CRP as mentioned above less than 1.0, 1.0-3.0, and greater than 3.0. These cut points, can predict vascular risk across all levels of LDL-C after adjustment for other risk factors and across all levels of the Framingham Risk score [(6)] . Paul Ridker, and others in a prospective study among women shows the independence of hs-CRP and LDL-C as risk factors for cardiovascular disease [(16)]. In another study that are more recent quartiles for hs-CRP shown to provide additive prognostic information across all levels of LDL-C, apolipoprotien B100, T-Cholesterol/HDL ratio even after full adjustment for traditional risk factors [(17)] . The association between elevated hs-CRP levels and future CHD events has generally been consistent among different studies. In a cohort study of 1086 apparently healthy middle-aged men, subjects with baseline levels of hs-CRP that were in the highest quartile had a twofold increase in risk of ischemic stroke or peripheral vascular disease (*P*=.02), and a threefold increase in

risk of myocardial infarction (*P*<.001) relative to subjects in the lowest quartile. These effects were independent of other cardiovascular risk factors, including lipid levels and smoking [18] . The Honolulu Heart Program analyzed frozen serum samples to assess the relationship of hs-CRP to the development of myocardial infarction in clinically healthy men over a follow-up period of 20 years. Overall, hs-CRP levels in this study were associated with coronary events that occurred as many as 15 years later. As early as five years into follow-up, the risk of myocardial infarction grew with increasing hs-CRP levels (*P*=.009). At 10 to 15 years into follow-up, the relative odds of myocardial infarction in the highest hs-CRP quartile were 2.1 times that of the lowest quartile, after adjustment for such risk factors as total cholesterol, hypertension, and type 2 diabetes mellitus (*P*=.016) [19] . Nested case-control analyses of 121,700 women in the Nurses' Health Study and 51,529 men in the Health Professionals. Follow-up Study of 239 women and 265 men free of cardiovascular disease supported the results of the Women's Health Study, it was found that hs-CRP is a predictor of CHD and it is independent of other cardiovascular risk factors [20] . In contrast to the results of these and many other trials, a nested case –control study of 157 elderly subjects from the Rotterdam Study in 2003 raised concerns about whether hs-CRP adds predictive value to traditional risk factors. In this study measurements of CRP adds no additional value to traditional risk factors [21] . This concern raised again in 2004 by a cohort study of the Reykjavik trial that questioned the usefulness of CRP over more

established risk markers. In this study, 2459 patients diagnosed with CHD after enrollment matched with 3969 controls that did not have a CHD event. After adjustments for smoking status and other established CHD risk factors, patients whose baseline hs-CRP levels were in the top quartiles (cutoff value=2.0 mg/L) had an odds ratio for CHD of 1.45 (95% CI, 1.25-1.68) compared with those in the bottom quartiles (cutoff value=0.78 mg/L). The Reykjavik investigators concluded that hs-CRP added only little predictive value of established risk factors [22] .

## *Relationship between hs-CRP and Coronary Heart Diseases*

Recent clinical and laboratory studies have shown that atherosclerosis, the precursor to most coronary heart diseases, is not simply a disease of fat deposit and inflammation plays a crucial role in the progression and development of this disease [4]. Attention turned towards studying the role played by C-reactive protein (CRP) in atherosclerosis and several results emphasized the relationship between hs-CRP and (CHD), and its prognostic value in acute coronary syndromes and the ability to effectively predict a future coronary event in seemingly healthy men and women. CRP extensively researched over the last couple of years as a surrogate marker of other inflammatory mediators like IL-6, IL-1, and TNF-alpha to understand the inflammatory component of atherosclerosis. These barrages of studies have shown that the level of CRP in the blood might be a yardstick to measure or predict the possibility of cardiovascular events both in acute coronary syndromes and in seemingly healthy individuals [23] . Elevated hs-CRP shown to be a strong predictor

of future cardiovascular risk in patients with established CHD, with or without a previous CHD. In a study shows the relation between high CRP and thromboembolic stroke, it was found that elevated CRP considered as important risk factor in healthy men for those people. In this study, patients in the highest quartile for hs-CRP levels had the highest risk of stroke compared to those in the lowest quartiles [24] . Furthermore, Blake and Ridker had shown that elevated hs-CRP could predict risk of cardiovascular events (including death, acute myocardial infarction, and need for revascularization procedures) in patients with acute coronary syndromes (ACS) [12] . Morrow and his mates were able to demonstrate in a study that elevated CRP in patients with unstable angina or non-Q wave myocardial infarction (NQMI) is correlated with increased 14-day myocardial infarction even in patients with a negative rapid cardiac specific troponin T (cTnT) [25] . A study by Liuzzo and his colleagues showed that in 32 patients with chronic stable angina, 31 patients with severe unstable angina, and 29 patients with myocardial infarction (MI) hs-CRP level greater than 3mg/L on admission was associated with an increased incidence of recurrent angina, myocardial infarction and even cardiovascular death [26] . Also, Bholasing, de Winter and others in a separate study demonstrated that hs-CR concentrations greater than 5mg/L on admission of 150 patients with acute coronary syndrome was associated with an increased incidence of major cardiac events within 6months, irrespective of cardiac troponin I values [27] . To further emphasize the relationship between hs-CRP and coronary heart events, A

Report from a study carried out by the Multiple Risk Factors Intervention Trial (MRFIT) showed that a direct positive association exists between hs-CRP and coronary heart disease mortality in seemingly healthy smoker men followed over a 17-year period [28] .

## hs-CRP as global risk factor

Using traditional risk factors, clinicians can predict approximately 50% to 60% of future coronary heart event in healthy individuals. The addition of hs-CRP to current strategies for global risk assessment, such as the Framingham Risk Score (FRS), may therefore have the potential to increase the accuracy of cardiovascular risk prediction. The most surprising fact about the predictive utility of hs-CRP is that the results and estimates derived from most of the above-mentioned studies were independent of other recognized cardiovascular risk factors. For instance, result from the Physicians' Health Study (PHS) demonstrated a significantly higher predictive value associated with hs-CRP compared to that attributed to traditional biochemical coronary heart disease (CHD) risk markers such as Total Cholesterol (TC), (HDL-C) and (LDL-C). In a study carried out by Ridker and his colleagues on post menopausal women, hs-CRP was shown to predict cardiovascular risk among women with LDL-C value of lesser than 130mg/L, a concentration that considered to be normal for LDL-C [16] .

Besides lipids, it appears that some coronary heart disease risk factors affect inflammation response and hence hs-CRP concentrations.

One of such outstanding facts is the relationship between obesity and hs-CRP. Obesity has a direct association with increase concentration of hs-CRP, Interleukin 6, the primary stimulant of the hepatic synthesis of CRP secreted by adipose tissues. This logically explains the mechanism by which weight loss and diet reduces cardiovascular risk – due to reduction in inflammatory response [29, 30]. In a different study, Festa, and Ford reported increased hs-CRP concentrations in diabetic patients, which considered as a direct relationship between the insulin resistance syndrome and hs-CRP [31]. In addition, Liu et al. and McCaron et al. in different experiments were able to demonstrate the effect of high blood pressure on hs-CRP. The report of their experimental study showed that high blood pressure promotes endothelial cytokines causing inflammatory activation in rats [32]. Recent evidence suggests that hs-CRP plays a major role in the physiologic processes associated with the metabolic syndrome. High levels of hs-CRP shown to be an independent predictor of cardiovascular risk for all degrees of severity of the metabolic syndrome [33] . Albert et al., demonstrated that hs-CRP levels are correlated with the calculated 10-year FRS in men, as well as in women not taking hormone replacement therapy [34] . Data from the Augsburg cohort of the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study also showed that hs-CRP enhances the assessment of global coronary risk as measured by the FRS, particularly in persons at intermediate risk for CHD [35]. In the Women's Health Study, both very low (<0.5 mg/L) and very high (>10 mg/L) levels of hs-CRP were useful

for risk prediction across a full range of FRS. Women with hs-CRP levels of less than 0.5 mg per liter had the lowest risk of future cardiovascular events. Women with hs-CRP levels of greater than 20 mg per liter had a risk almost 8 times higher than normal women did. In a reanalysis of the WHS data, a linear response between hs-CRP and vascular risk was observed even among those with levels of hs-CRP greater than 20 mg/L. Thus, those patients recognized as very high-risk patients (Figure 7) [36].

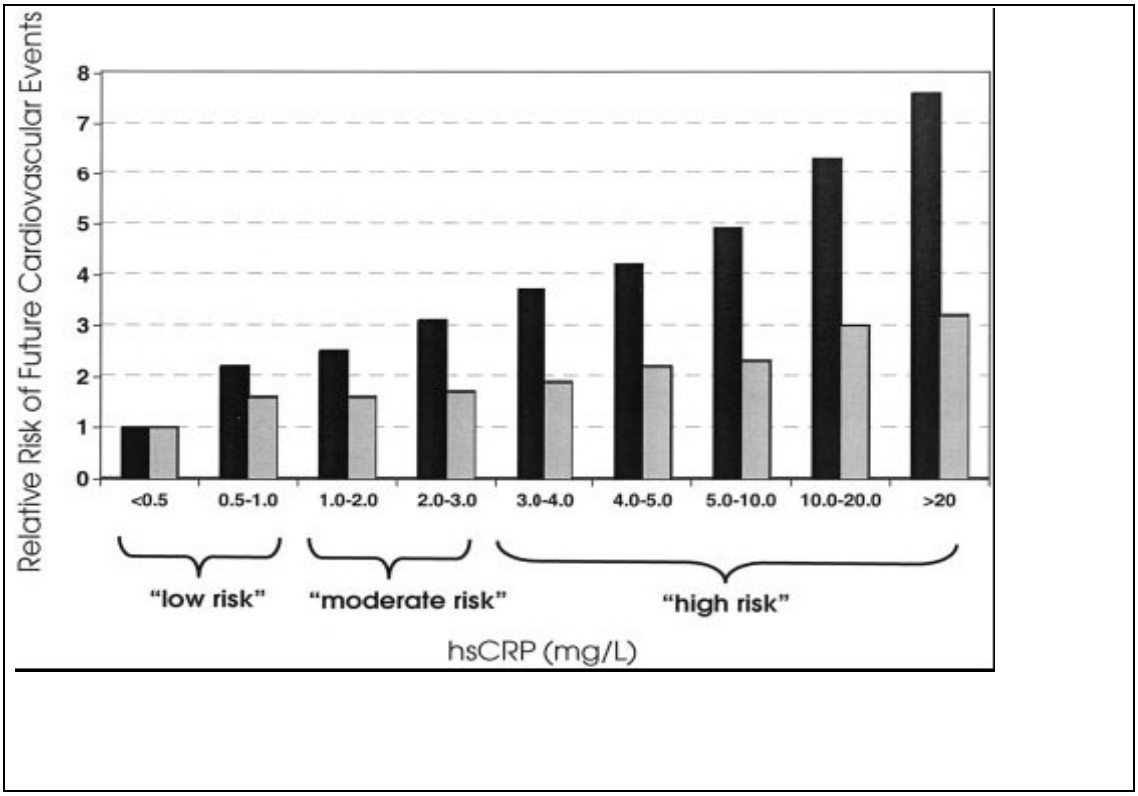



*Figure (7): Relative risk of future cardiovascular events across a full clinical range of hs-CRP values. Black bars represent crude relative risks and gray bars risk adjusted to FRS. Ref. [36]*

hs-CRP modified as global risk factor by calculating the Framingham Risk Score based on current scoring system and divide patients into three groups with 10-year risks of less than 5 percent, 5-10 percent, and 10-20 percent. Then hs-CRP data utilized in five gradations (<0.50, 0.5- <1.0,

1.0- <3.0, 3.0- <10, and ≥10.0) to generate an "hs-CRP Modified Global CHD Risk" (Figure 8) [37].

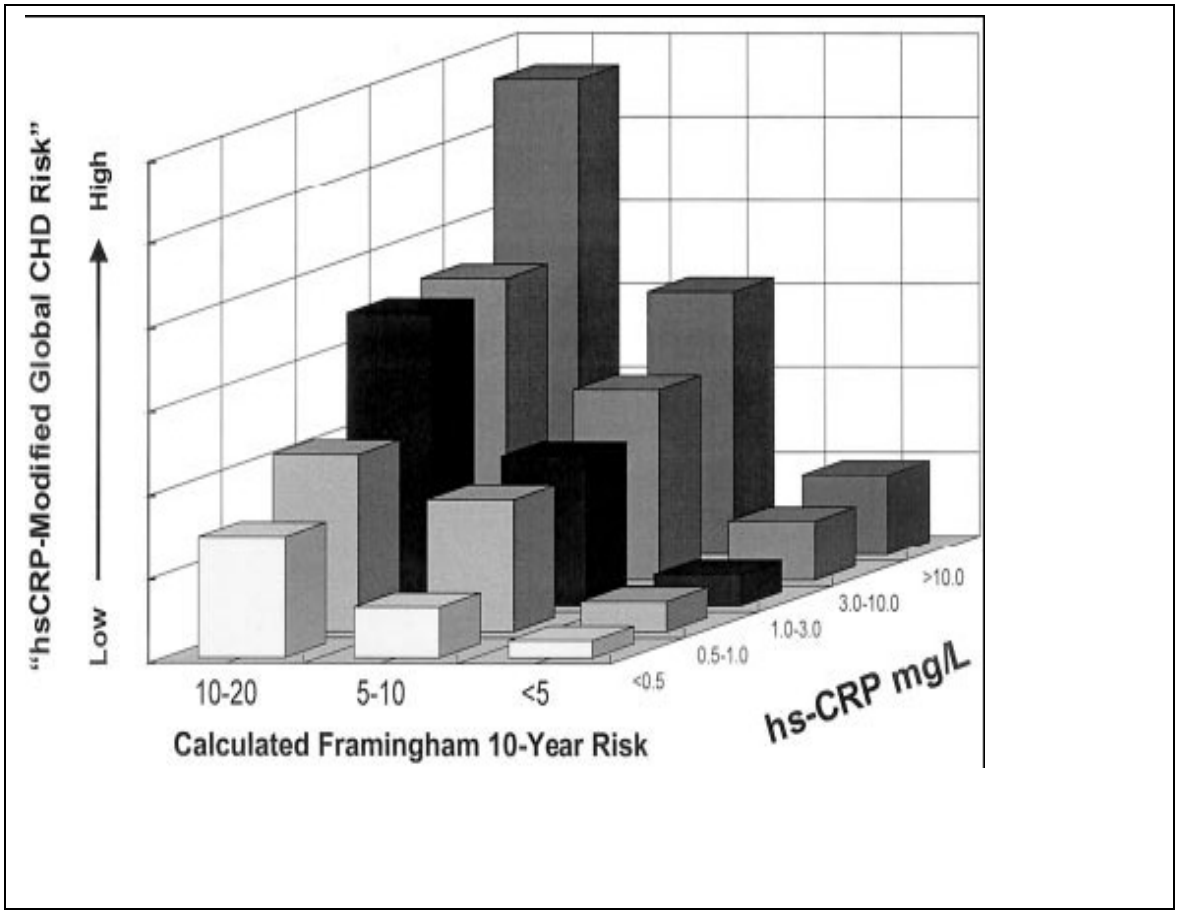



*Figure (8): Relative risks of future vascular disease using baseline levels of hs-CRP in addition to calculated 10-year Framingham risk. Ref [37]*

Clinical trials have shown that statins reduce patient levels of CRP by 15% to 28% as early as six weeks after treatment begins independent of the magnitude of reduction in LDL-C levels. Although statin therapy showed to benefit individuals with elevated hs-CRP levels, not known whether aggressive statin therapy can reduce the risk of a first cardiovascular event in persons with low LDL-C but high hs-CRP [38].

## Guidelines for Use of hs-CRP in risk assessment

In January 2003, guidelines from the Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) named hs-CRP as the inflammatory marker of choice to assess cardiovascular risk [2]. The guidelines support the use of hs-CRP in primary prevention and set cutoff points according to relative risk categories: low risk (<1.0 mg/L), moderate risk (1.0-3.0 mg/L), and high risk (>3.0 mg/L) [2] . Recently, Ridker and Cook suggested that even though levels of very low < 0.5 mg/L, and very high >10 mg/L of hs-CRP uncertain to give positive predictive value for CHD. These values of hs-CRP would provide clinicians with additional prognostic information on cardiovascular risk [36]. The CDC-AHA guidelines state that the optimal use of hs-CRP is to help guide the evaluation and therapy for primary CHD prevention for patients at intermediate risk [2,]. The guidelines also consider measurements of hs-CRP as a possible predictor of recurrent events in patients with stable coronary disease or ACS [2] . The use of hs-CRP as an adjunct to lipid screening in primary prevention intended to improve global risk prediction in patients not clearly identified as being at high risk by cholesterol levels alone. This adjustment to the screening procedure is especially important for individuals with low LDL-C levels (<130 mg/dL) but high hs-CRP levels (>3 mg/L). Preliminary data suggest that patients with low LDL-C and high hs-CRP levels may benefit from pharmacologic intervention, preferably with statin therapy [3] . The addition of hs-CRP levels to standard cholesterol evaluation protocols improves the predictive value of lipid parameters for determining future

risk of myocardial infarction. Men with high levels of both hs-CRP and total cholesterol had a 5.3 times greater relative risk of a future myocardial infarction ($P$<.001) than did men with either high total cholesterol or high hs-CRP levels alone [23]. Furthermore, women with high hs-CRP and low LDL-C levels had a higher absolute risk of a future CHD event than did women with low hs-CRP and high LDL-C levels, despite the fact that high LDL-C is traditionally targeted for aggressive intervention in primary prevention [6]. Many studies suggests that CRP might have implications for future risk assessment, especially in intermediate risk group , and adding it to Total Cholesterol/HDL ratio will independently related to incident coronary events [35] .

# Hypothesis and Specific aims

## *Hypothesis*

We hypothesize that hs-CRP is a marker of Coronary Heart Disease (CHD) among Palestinian people in the Ramallah area, Palestine.

### *Specific Aims*

The specific aims of this Case –control study are:

- To evaluate the association between hs-CRP and the coronary Heart disease (CHD) in West Bank _Palestine.
- To compare hs-CRP and LDL-cholesterol as risk factors associated with CHD.
- To establish a relative risk for a CHD based on Total Cholesterol/HDL ratio and hs-CRP.

## Materials and Methods

A signed consent was obtained from the patients prior to enrollment in this study.

***Subjects:*** Subjects enrolled in this study were divided into two groups; the first group consisted of 80 patients (64 males and 16 females) diagnosed to have CHD by a cardiologist. The second group consisted of 80 healthy subjects (49 males and 31 females) with no symptoms of CHD prior to the study. All subjects are from the West Bank selected from the Medical Relief Prevention and Diagnostic Center of Cardiovascular Diseases –Ramallah during the period from September 2005 until Feb. 2006. Risk factors for coronary heart disease such as smoking, hypertension, diabetes, and dyslipidemia were equal in both groups. The cardiologist, who diagnosed these cases, completed a written questionnaire for all patients and controls with comprehensive information. For each patient, the medical record was reviewed to determine the presence of risk factors for atherosclerotic cardiovascular disease. The Total Cholesterol, HDL-cholesterol, LDL-cholesterol, and Triglycerides were assayed using standard manual methods for all subjects. hs- CRP was determined using Enzyme Immunoassay (ELISA) technique and the results for hs-CRP were calculated in mg/L. The data was analyzed to find if there is significant difference between patients and control group.

***Sample collection***: Samples were collected from fasting subjects in 10-ml plain glass tubes (Becton Dickinson Vacutainer Systems). The samples were allowed to clot at room temperature for 30 min. The serum was then separated by centrifugation. Each specimen was divided into two

aliquots, one for lipid profile, and the other was kept at-20 C for hs-CRP testing. Repeated freezing and thawing was avoided, and hemolysed samples were rejected.

**Methods**

***CRP assay:*** CRP was assayed by a sandwich enzyme immunoassay (ELISA) kit (CRP DiaMed EuroGen, Lot No. 98104666 and expiry date in Sep/2007) Belgium. The minimal detectable concentration for the assay was determined by the manufacturer to be less than 1.0 mg/L. Standards 0.0, 1.25, 2.5,5.0,10,25,and 50 mg/L were used to plot the calibration curve to calculate the patients results. The assay was performed according to manufacturer instructions.

***Principle of the test:*** CRP in the patient serum samples and diluted standards react with polyclonal anti-CRP antibodies, immobilized on the solid phase of micro titer plates. Following an incubation period of 30 min at RT, unbound serum components were removed by a wash step. After removal of the unbound serum proteins, the antigen- antibody complex in each well was detected with specific peroxidase conjugated antibodies. After the removal of the unbound conjugate, the strips were incubated with the chromogen solution 3, 3, 5, 5-tetramethyl-benzidine (TMB) and hydrogen peroxidase. A blue color developed in proportion to the amount of immunocomplex bound to the wells of the strips. The enzyme reaction was stopped by dispensing an acidic solution (H2SO4) into the wells turning the solution from blue to yellow. The standard

curve was then plotted. The concentration of CRP in each sample was determined by interpolation from the standard curve.

***Total Cholesterol:*** Total Cholesterol was measured by using Randox, UK reagents. The cholesterol was determined after enzymatic hydrolysis and oxidation. Aliquetes of 10 ul of Standards, controls, and samples were added to 1000 ul of cholesterol reagents. Then the tubes were incubated for 5 min at 37º c. The absorbance of the samples and standards was determined against reagent blank using spectrophotometer within 60 minutes at 546 nm Hg. The results were calculated by dividing the absorbance of the sample against the standard and multiplying by the concentration of the standard.

***HDL – Cholesterol:*** HDL-Cholesterol was measured after precipitating LDL, VLDL, and chylomicron fractions, by the addition of phosphotungstic acid in the presence of magnesium ions (Randox). Aliquot of 500 ul of serum was added to 1000 ul of precipitant and allowed to stand for 10 min at room temperature. After centrifugation at 4000 rpm for 10 minutes, to get a clear supernatant containing the HDL cholesterol, HDL –cholesterol was determined using cholesterol reagent as mentioned previously of a Total Cholesterol.

***LDL –Cholesterol:*** LDL- cholesterol was measured after precipitation by heparin at their isoelectric point using Randox reagents. After centrifugation, the HDL and VLDL remain in the supernatant and were

measured by a spectrophotometer at 415 nm. The LDL-cholesterol was then calculated according to the following formula:

LDL –cholesterol = Total cholesterol – Cholesterol in the supernatant.

100 ul of serum was added to 1000 ul precipitation reagent and allowed to stand for 10 min at room temperature. The tubes were then centrifuged for 15 min at 4000 rpm. The cholesterol concentration of the supernatant was determined using cholesterol reagents.

***Triglycerides:*** Randox reagent was used to assay triglycerides. The triglycerides were determined after enzymatic hydrolysis with lipases. Serum specimens were collected after fasting 12-14 hours. 10 ul of standards, controls, and samples were added to 1000 ul of triglyceride reagents. Then they were incubated for 5 min at $37^{o}$ C. The absorbance was determined for both standards and samples against reagent blank at 500 nm. The results were determined by interpolation from the standard curve.

# Statistical Analysis

The statistical analysis was performed using the Statistical Package for Social Sciences Software (SPSS for windows version 12).

Means and proportions for risk factors for cardiovascular events at baseline were calculated for both cases and controls. The means for hs-CRP, Total Cholesterol, LDL-cholesterol, HDL-cholesterol, and Triglycerides were compared between the two groups by using 2-sample t-test. The proportions were compared by using the chi-square test. Analysis of trends was used to test for associations between increasing levels of each plasma variable and the risk of cardiovascular events. The study subjects were divided into groups according to low, medium, and high levels of LDL-cholesterol, and hs-CRP. Logistic regression was performed to evaluate relative risk or odds ratio of cardiovascular events in each of these groups. The odds ratio of different quartiles of hs-CRP, and the odds ratio of combination between quartiles of hs-CRP and LDL-C were calculated. The likelihood-ratio test was used to determine whether logistic-regression models that include LDL-C and hs-CRP provide a significantly better fit than did logistic-regression models limited to LDL-C or hs-CRP alone. All *P* values were two-tailed, and a value of less than *0.05* was considered to indicate statistical significance. All confidence intervals (CI) were calculated at the 95 percent level.

# Results

In this case control study we investigated hs-CRP as a marker of CHDin patients from Ramallah area- West Bank –Palestine. This was done by defining two groups: one control group free of CHD, and the other group is the patients group diagnosed to have CHD. Both groups (the controls and the patients) were similar in age, and in the presence of other CHD risk factors such as smoking, hypertension, diabetes, and dyslipedemia. The results of the different variables, lipid profile tests as well as hs-CRP for patients and controls are summarized in Tables 1 and 2. The means ± SD for the different variables in this study of both groups with the significant *P* value are shown in Table 3. Our results showed that there is a significant difference for hs-CRP concentration between controls and patients (*P<0.001*). The mean ± SD for hs-CRP for patients and controls was 5.90±3.1 and 2.62 ±1.59 respectively (Table 3). LDL- C showed significant difference (*P = 0.003*) between the patients and controls (Table 3). There was no significant difference for total cholesterol (*P<0.072*), HDL-C (*P 0.728*), and TG (*P=0.355*) between patients and controls. Other factors evaluated such as age, sex, hypertension, diabetes reflected a significant difference (*P<0.05*) between patients and controls. There was no significant difference for smoking (*P = 0.124*), and dyslipidimia (*P=0.115*) between two groups tested. Analysis of hs-CRP to find out the odds ratio was found to be 1.88 95% CI (1.54-2.3), compared to LDL-C odds ratio 0.98 CI 95 % (0.97-0.99). These values indicates the increased risk per unit for both hs-CRP and LDL-C.

*Table (3) - Mean and standard deviation of the different variables for both patients and control, and statistical significance (P trend).*

| Variable | Control N=(80) | Cases N=(80) | *P* trend |
|---|---|---|---|
| Age, mean ±SD. | 51.1±13.6 | 60.7±11.9 | 0.000 |
| Sex, % male<br><br>% Female | 49(61.3 %)<br><br>31 (38.3%) | 64 (80%)<br><br>16 (20%) | 0.009 |
| Smokers, % | 26.3 | 16.3 | 0.124 |
| Hypertension,% | 16.3 | 56.3 | 0.000 |
| Diabetes, % | 35 | 60 | 0.001 |
| Dyslipedemia, % | 86.3 | 93.8 | 0.115 |
| Total Cholesterol<br><br>Mean ± SD | 220±38.3 | 209.0±36.1 | 0.072 |
| LDL-C | 141.5±25.1 | 128.2±31.0 | 0.003 |
| HDL-C | 36.4±7.4 | 36.5±7.0 | 0.728 |
| TG | 143±56 | 153.8±81.2 | 0.355 |
| hs-CRP | 2.62±1.59 | 5.90±3.1 | 0.000 |

In this study, we grouped our results for hs-CRP, LDL-C to three quartiles. The Ratio of T-cholesterol/HDL-C was calculated and divided into five quartiles as in Table 4. These different quartiles were used to find out the odds ratio for both hs-CRP, and LDL-C quartiles. In addition, these groups were used to evaluate interaction between hs-CRP and LDL-C.

*Table (4).Different quartiles of hs-CRP, LDL-C, and T-cholesterol/HDL ratio.*

| | | CRP Quartiles | | | Total |
|---|---|---|---|---|---|
| | | 1.00 | 2.00 | 3.00 | |
| Cases | control | 10 | 43 | 27 | 80 |
| | patient | 4 | 13 | 63 | 80 |
| Total | | 14 | 56 | 90 | 160 |
| | | LDL Quartiles | | | Total |
| | | 1.00 | 2.00 | 3.00 | |
| Cases | control | 24 | 37 | 19 | 80 |
| | patient | 42 | 27 | 11 | 80 |
| Total | | 66 | 64 | 30 | 160 |

| | T-Cholesterol/HDL ratio | | | | | Total |
|---|---|---|---|---|---|---|
| | 1.0 | 2.00 | 3.00 | 4.00 | 5.0 | |
| Cases control | 3 | 4 | 8 | 14 | 51 | 80 |
| patient | 1 | 7 | 17 | 11 | 44 | 80 |
| Total | 4 | 11 | 25 | 25 | 95 | 160 |

*CRP (1.0) represents <1.0 mg/L, (2.0): 1.0-3.0 mg/L, and (3.0): >3.0 mg/L. For LDL-cholesterol (1.0) less than 130 mg/dl, (2.0): 130-160 mg/dl, and (3.0) greater than 160 mg/dl.And for T-chol/HDL ratio (1.0) : <3.4, (2.0): 3.4-4.0, (3.0); 4.1-4.8, (4.0): 4.8-5.50, and 5.0 >5.5.*

The odds ratio (OR) and the confidant interval (CI) for hs-CRP were 4.25 95% CI (2.34-7.72), *P* < 0.001. These finding for hs-CRP before adjustment of other risk factors evaluated in the study. hs-CRP odds ratio after adjustment with LDL –C was 5.93 and the 95% CI (3.0-11.6), *P< 0.001*. After adjustment with LDL-C and T-cholesterol/HDL ratio it was *6.5* 95% *CI (8.1-13.4), P <0.001*. Odds ratio for hs-CRP adjusted with other risk factors such as age, smoking, hypertension, diabetes, and dyslipedemia, was (*4.0)* 95% *CI (2.0-7.9)* as shown in Table (5). In comparison to hs-CRP, LDL-C odds ratio was *0.584* 95% *CI (0.35-0.84), P< 0.006*, which is significant ratio but the odds ratio was less than one, indicating a decrease in risk.

*Table (5) (OR) Odds Ratio, (CI) confidant Interval and P trend for different quartiles of hs-CRP and other variables associated with CHD risk factors before and after adjustment.*

| Test | Odds Ratio | CI | *P* trend |
|---|---|---|---|
| hs-CRP | 4.25 | 2.34 -7.72 | 0.000 |
| hs-CRP * | 5.93 | 3.0-11.6 | 0.000 |
| hs-CRP ** | 6.5 | 8.1-13.4 | 0.000 |
| hs-CRP *** | 4.0 | 2.0-7.9 | 0.000 |

| | | | |
|---|---|---|---|
| LDL-C | 0.543 | 0.35-0.84 | 0.006 |
| T-cholesterol/HDL ratio | 0.90 | 0.76-1.0 | 0.202 |

** LDL-C adjustment.*
*** LDL-C & T-cholesterol/HDL ratio adjustment.*
**** Adjustment with Age, Sex, Diabetes, HTN, Smoking, and Dyslipedemia.*

To explore that hs-CRP added to the predictive value of lipid-based screening, several additional analyses were performed. First, we computed the OR of LDL -cholesterol quartiles as well as hs-CRP (Table 6). We found that there was an increase in OR between different quartiles of hs-CRP compared to LDL-C quartiles in this study. The odds ratio for CRP group ( > 3.0 mg/L) was *5.25* 95% *CI ( 1.522-18.1), P < 0.009* as compared to reference group CRP (< 1.0mg/L) which was significant. The second group for hs-CRP (1.0-3.0 mg/L) the odds ratio was *0.813 and 95% CI (0.218- 3.0)* compared to reference group and it was not significant. In our study, all quartiles of LDL-C showed significant *P* values < 0.05, and the odds ratio showed a decrease in risk for these different quartiles (Table 6).

*Table (6): Odds Ratio for hs-CRP and LDL-C quartiles of both groups before and after adjustment of risk factors.*

| Test | Odds Ratio | CI | P value |
|---|---|---|---|
| Quartiles of hs-CRP<br>CRP<1.0) * | 1.0 | | |
| CRP(1.0-3.0) | 0.813 | 0.218-3.0 | 0.757 |
| CRP( >3.0) | 5.25 | 1.52-18.1 | 0.009 |
| CRP (1.0-3.0) after | 0.466 | 0.101-2.15 | 0.328 |

| | | | |
|---|---|---|---|
| adjustment | | | |
| CRP( >3.0) after adjustment | 3.68 | 1.06-15.1 | 0.049 |
| Quartiles of LDL-C (LDL<130) * | 1.0 | | |
| (LDL 130-160) | 0.381 | 0.185-0.783 | 0.009 |
| (LDL >160) | 0.400 | 0.171-0.933 | 0.034 |
| LDL (130-160) after adjustment | 0.291 | 0.116-0.728 | 0.008 |
| (LDL >160) after adjustment | 0.192 | 0.062-0.596 | 0.004 |

** Reference group. , (CI): confidant Interval.*
*Low hs-CRP < 1.0 mg/L, high hs-CRP >3.0 mg/L. Low LDL-C < 130 mg/dL, high LDL-C > 160 mg/dL.*

To test if both hs-CRP and LDL-C interact to increase the risk of disease, different combinations of hs-CRP and LDL-C were studied (Table 7). The OR was computed for each combination using the low levels of hs-CRP and LDL-C as the reference. The first quartile (hs-CRP low <1.0 and LDL-C low < 130 mg/dL) was considered as reference group (OR 1.0). The second quartile (hs-CRP high >3.0 mg/L, and LDL-C high > 160 mg/dL), the OR was (2.0) with CI (1.01-3.9) and *P* 0.025 before adjustment. After adjustment with risk factors the odds ratio was still significant *2.3 CI (1.05-5.9) P* 0.036. Table 7 and Figure 9. The most significant group was the third quartile, (high hs- CRP and low LDL-C). Patients with LDL-C levels < 130 mg/dl and high levels of hs-CRP >3.0 mg/L have higher risk *(OR 7.66) CI (2.5-23.4)* than patients with low hs-CRP, and high LDL-C, *(OR 5.2) CI (0.61-46.0) Table 7.* A correlation between hs-CRP and LDL cholesterol for both groups was performed and r= 0.174 (*P 0.028*). This value considered low but there is a significant correlation between hs-CRP and LDL-C. The correlation between hs-

CRP and LDL-C in controls 0.429, $p < 0.05$. Where the correlation in patients 0.385, $p < 0.05$.

*Table (7) Odds ratio of different combinations of hs-CRP & LDL-C quartiles.*

| TEST | OR | CI | P value |
|---|---|---|---|
| Low hs-CRP/Low LDL-C * | 1.0 | | |
| Low hs-CRP/high LDL-C | 5.278 | 0.61-46.1 | 0.133 |
| high hs-CRP/low LDL-C | 7.66 | 2.5-23.4 | 0.00 |
| high hs-CRP/high LDL-C | 2.0 | 1.01-3.9 | 0.025 |
| Low hs-CRP/high LDL-C** | 1.69 | 0.162-17.7 | 0.659 |
| high hs-CRP/low LDL-C ** | 7.52 | 1.94-29.0 | 0.003 |
| high hs-CRP/high LDL-C ** | 2.3 | 1.05-5.19 | 0.036 |

** Reference group*
*** after adjustment with age, sex, Diabetes, HNT, smoking, and dyslipid.*

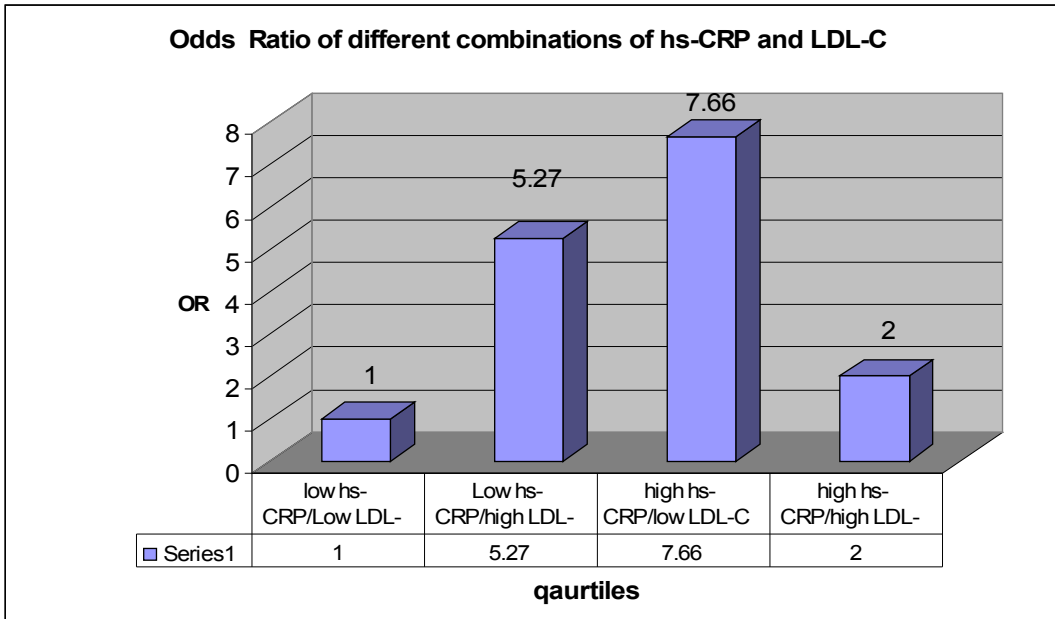



Figure *(9) Odds Ratio for combination of different quartiles of hs-CRP & LDL-C*

The likelihood-ratio tests were used to compare the fit of predictive models that were based on measurement of hs-CRP in combination with LDL-C, and the fit of models based on LDL-C measurements alone. The likelihood ratio Chi-square statistics was higher for the models based on hs-CRP than that based on LDL-C (54.35 vs.15.35, both with 2 df). The

addition of CRP to the model based on LDL-C was stronger (chi- square = 51.3, 3 df, and *P <0.001*) than the addition of LDL-C to the model based on CRP (chi square = 44.6, 3df, and *P<0.001*). On the other hand, addition of CRP to the T-Cholesterol/HDL ratio was stronger (chi square 41.5, df 3, *P<0.001*) than addition of the T-cholesterol/HDL ratio to CRP (chi square 38.74, df 5, P< 0.001).

# Discussion

hs-CRP was found to be a significant predictor for the risk of future CHD events [1, 5, 9,and 10] . In addition, measurement of this marker increased the predictive value of models based only on standard lipid screening. Among different parameters measured, and evaluated in many studies hs-CRP was the most significant predictor of the risk for CHD events; when measured with a highly sensitive assay. This marker distinguished between persons at high risk and those at low risk, even in the subgroup of persons with LDL cholesterol levels below 130 mg /dl [6].

Liuzzo and his colleagues showed that in 31 patients with no evidences of myocardial infarction (MI), hs-CRP levels were greater than 3mg/L upon admission to hospital. They were associated with an increased incidence of recurrent angina, myocardial infarction and even cardiovascular death [26]. Another study, done by Bholasing, de Winter and others demonstrated

that hs-CRP concentrations of greater than 5mg/L upon admission of 150 patients with acute coronary syndrome was associated with an increased incidence of major cardiac events within 6 months [27] .

The results of this study showed that there is a significant difference between the patients and control groups. The odds ratio was 1.88 CI (1.54-2.3), $p < 0.001$. This means that increased hs-CRP one unit will increase the risk factor 1.88 times. This indicates a good association between high results of hs-CRP and the CHD in West Bank –Palestine. The mean ± SD for hs-CRP for patients and controls was 5.90±3.1, and 2.62 ±1.59 respectively, which was very significant. These findings confirmed that hs-CRP marker is an important independent predictor of the risk of CHD events. The current data supported the hypothesis that atherosclerosis is, in part, an inflammatory disease [4], and hs- CRP can be used as a marker of this disease. To compare hs-CRP and LDL-C as a risk factor for CHD, different quartiles for both hs-CRP and LDL-C were done for low, moderate, and high risk. In this study, it was found that the odds ratio of all different quartiles of hs-CRP was 4.25, and 95 % CI (2.34-7.72). This means that patients group had 4-fold increase in risk for CHD than control group. After adjustment of hs-CRP with other risk factors mainly LDL-C, and total Cholesterol / HDL ratio, the odds ratio still very high and significant 5.9, and 6.5 respectively as shown in Table 5. These results was evident that hs-CRP remained a strong and independent factor for CHD even in the presence or absence of other risk

factors. In a prospective study among women, Paul Ridker, and others shows the independence of hs-CRP and LDL-C as risk factors for cardiovascular disease [16]. The addition of hs-CRP levels to standard cholesterol evaluation protocols improves the predictive value of lipid parameters for determining future risk of myocardial infarction. Men with high levels of both hs-CRP and total cholesterol had a 5.3 times greater relative risk of a future myocardial infarction ($P<.001$) than did men with either high total cholesterol or high hs-CRP levels alone [23]. In a prospective study of CRP and the risk of future cardiovascular events among healthy women, those in the highest quartiles had five fold increase in risk of any vascular events (Relative risk RR 4.8, ), and a seven fold increase in risk of MI or stroke (RR 7.3) [16]. In a cohort study of 1086 apparently healthy middle-aged men, subjects with baseline levels of hs-CRP in the highest quartile had a twofold increase in risk of ischemic stroke or peripheral vascular disease ($P=.02$) and a three fold increase in risk of myocardial infarction ($P<.001$), relative to subjects in the lowest quartile. These results were independent of other cardiovascular risk factors including lipid levels and smoking [18].

In another middle age group men, levels of CRP were positively associated with thromboembolic stroke (TE) through 20 years follow up, where the odds ratio of CRP was 3.8-fold excess by 10-15 years of follow up between the highest and lowest quartiles [24] . Several additional analysis were performed on the different quartiles of hs-CRP and LDL-C

separately. It was found that the odds ratio, for hs-CRP was 5.25 CI (1.522-18.1), $P < 0.009$ when (CRP more than 3.0 mg/L) compared to a reference group (CRP less than 1.0) which was significant. This means that a person falls in this quartile have 5-fold chance to develop CHD more than person in the first quartile does. The second quartiles for hs-CRP (1.0-3.0 mg/L) was considered not significant (Odds ratio 0.813 CI 0.218- 3.0). This means that high concentration of hs-CRP more than 3.0 mg/L can be considered predictive for future events of CHD more than the second quartile. On the other hand, all quartiles of LDL-C showed significant values $P < 0.05$, where odds ratio showed a decrease in risk for the different quartiles as shown in Table 6.

In this study we test the interaction between both quartiles of hs-CRP and LDL-C. Table 7. Low hs-CRP and Low LDL-C were considered as a reference group. It was found that the odds ratio was 7.66, 95% CI (2.5-23.4) for the quartile of hs-CRP > 3.0 mg/L, and LDL-C < 130 mg/ dl. These results support the idea that hs-CRP can predict risk if greater than 3.0 mg/L even though LDL-C less than 130 mg/dL. But in our study this rule does not fit because all the control and patients group on drug therapy for LDL-C lowering. Another group significant when hs-CRP > 3.0 mg/L, and LDL-C > 160 mg/dl. The odds ratio was 2.0, 95% CI (1.01-3.9) indicating that subject fall in the highest quartile have two times risk for CHD than subjects in the first quartile. These results for

this quartile less than the previous one because LDL-C results are not the true results, where most of the patients take medication Figure 10.

These findings indicate that lipid screening needs other risk factors to detect events of future CHD. These results for LDL-C considered not significant because both controls and patients receive therapy to lower LDL cholesterol. As a result, we need other risk factors independent on LDL-C. Our study indicated that hs-CRP was an independent factor for detection of CHD, even though there was a minimal correlation between hs-CRP and LDL-C (r = 0.174). In a similar study on 27939 healthy females, studied and followed over 10 years period for future vascular events and evaluated for a full lipid panel as well as baseline hs-CRP levels. They found that there is a minimal relationship between hs-CRP and LDL-C (r=0.08) and thus the inflammatory process was providing information on plaque rupture separately from that of lipid evaluation [16]. This correlation for the controls 0.429, and for the patients 0.385 when analysis done separately for the controls and patients. In addition, in likelihood ratio tests of the contribution of each variable, the addition of CRP to the model based on LDL-C was stronger (chi- square = 51.3, 3 df, and $P < 0.001$) than the addition of LDL-C to the model based on CRP (chi square = 44.6, 3df, and $P<0.001$). On the other hand, addition of CRP to the T-Cholesterol/HDL ratio was stronger (chi square 41.5, df 3, $P<0.001$) than addition of the T-cholesterol/HDL ratio to CRP (chi square 38.74, df 5, P< 0.001). Our study showed that CRP adds to the

predictive value of Total Cholesterol/HDL ratio in determining risk of cardiovascular disease. Where, adding hs-CRP to this ratio increases the predictive detection value. hs-CRP and T-Cholesterol/HDL ratio were determined and arranged in Figure (10) as shown below for both men and women**.** Studies using data from the PHS have demonstrated that hs-CRP is the most effective predictive marker and in combination with TC/ HDL ratio is the best overall predictor of relative risk estimates for future coronary events. The relative risk estimates for MI increased significantly with each increasing quartiles of baseline hs-CRP combined with TC/ HDL, where a relative risk (RR was 4.4) in the highest quartiles compared to the lowest quartiles (RR 1.0) (23).

Similar to men, the combination of hs-CRP and the TC/ HDL ratio provides the best predictive value for risk of future coronary events in women. Women with the highest quartiles levels of combined hs-CRP and TC:HDL ratio had a relative risk for future coronary events that were also 8.7 times higher compared to the lowest quartiles [(39)].

As a result, measuring both hs-CRP and the ratio of Total Cholesterol to HDL cholesterol allowed significantly better prediction of risk than did models based solely on this lipid ratio alone. Adding this risk factor to detect the risk for healthy people for CHD will help us in the laboratories to increase the prediction risk. Finally, we believe that these current results have public health implications both in terms of the prediction of the risk of cardiovascular events and in terms of the use of statin therapy

for primary prevention. As in our findings, which indicate that hs-CRP is a potent independent predictor of risk regardless of the LDL cholesterol level; data from the Cholesterol and Recurrent Events trial indicated that use of pravastatin resulted in decreased levels of hs-CRP in a manner largely independent of LDL cholesterol [42].

| | | | Quintile of hs-C Reactive Protein, mg/L | | | | |
|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 |
| | | | <0.7 | 0.7-1.1 | 1.2-1.9 | 2.0-3.8 | 3.9-15.0 |
| **Quintile of Chol./HDL ratio** | Men | Women | | | | | |
| **1** | <3.4 | <3.4 | **1** | **1.2** | **1.4** | **1.7** | **2.2** |
| **2** | 3.4-4.0 | 3.4-4.1 | **1.4** | **1.7** | **2.1** | **2.5** | **3** |
| **3** | 4.1-4.7 | 4.2-4.7 | **2** | **2.5** | **2.9** | **3.5** | **4.2** |
| **4** | 4.8-5.5 | 4.8-5.8 | **2.9** | **3.5** | **4.2** | **5.1** | **6** |
| **5** | >5.5 | >5.8 | **4.2** | **5** | **6** | **7.2** | **8.7** |

| **Reference Ranges of Relative Risk values for a Future Coronary Event** | |
|---|---|
| **Low** | **<1.1** |
| **Mild** | **1.1 - 1.7** |
| **Moderate** | **1.8 - 2.9** |
| **High** | **3.0 - 5.1** |
| **Highest** | **5.2 - 8.7** |

***Figure (10) shows the relative risk for future coronary events among apparently healthy men and women associated with different hs-CRP concentrations and TC: HDL ratios. (5)***

# Methodological considerations:

Several limitations to be considered for this case control study: First, our study comprised apparently healthy persons without CHD as control group and patients with CHD as case group, and the significant difference found to be between these groups. Therefore, we recommend doing a

large study for the prediction of risk factors in our population to find out who will be at increased risk for cardiovascular events. Second, we measured hs-CRP marker and thus could not evaluate the effects of changes in the levels of this marker over time. However, studies have found that levels of hs-CRP are stable over long periods, as long as measurements are not made within two to three weeks of an acute infection. Moreover, with respect to the current results, levels of hs-CRP over 20 mg/L where excluded from our study to avoid any acute cases of other diseases. Finally, the patients included in the study take medication for lowering LDL-C, as a result both LDL-C and hs-CRP marker will decrease due to this therapy. This decrease may vary depending on the test measured where LDL-C may decrease due to therapy more than hs-CRP.For this reason, the results of hs-CRP considered more predictive. For that reason to get the true level of hs-CRP to be considered as a risk factor, a large number of subjects free of CHD with follow up over a period are recommended. Another conditional factors included in this study are not significant such as smoking, this because most of the people asked if they are smoking or not during the study, not before. As a result, the percentage of smokers is very low in this study and this affect the results during analysis.

# Conclusions

In conclusion, in this study, the evaluation of hs-CRP proved to be a significant predictor of the risks of future CHD events. Thus, the data raise the possibility that the addition of hs-CRP to standard lipid screening will generate an improved method for identifying persons at high risk for future cardiovascular events, who would thus be candidates for primary-prevention interventions such as the use of statin therapy.

Testing of CRP with T-cholesterol/HDL ratio is a better criteria to predict the risk for future CHD than depending in the LDL-C and the ratio only. This factor of inflammation is an independent risk factor, and can be tested independent of other factors.

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# Birziet University
# Master program of Clinical Laboratory Science

**High sensitive CRP (hs-CRP) as a marker for coronary heart .disease in Ramallah area –Palestine**

## Questionnaire

.Questionnaire No

1
2

..…… :Name
DOB:……..Age: ……..years. Sex: M or F
.Fasting Hours: 1) less than 8 hrs 2) 8-12 hrs 3) more than 12 hrs
:Wieght: kg. Occupation
.Marital status: M/ S/ D / W
Place of living: 1) city 2) village 3) camp
Type of Diet: vegetarian 2) Non vegetarian 3) both

:Risk factors
Smoking 1) yes 2) No (1
Hypertension: 1) yes 2) No (2
Diabetes: 1) yes 2) No (3
Dyslipidemia: 1) yes 2) No (4
Family history of HTN: 1) yes 2) No DM: 1) yes 2) No (5
Dyslipidemia 1) yes 2) No
Family history of CHD: 1) yes 2) No

:Date: Patient Signature

*Table (1) Summary for all cases (80 cases), and results obtained for the patients group.*

| ID | Age | sex | Smoking | HTN | Diabetes | Dyslipid | T.choles | LDL | HDL | TG | hs-CRP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 101 | 56 | 1 | 2 | 2 | 2 | 1 | 221 | 110 | 40 | 416 | 0.99 |
| 102 | 58 | 1 | 1 | 2 | 2 | 1 | 205 | 132 | 28 | 442 | 7.1 |
| 103 | 55 | 1 | 2 | 1 | 1 | 1 | 238 | 160 | 31 | 136 | 7 |
| 104 | 64 | 1 | 2 | 1 | 2 | 1 | 324 | 174 | 25 | 167 | 7.3 |
| 105 | 50 | 1 | 2 | 2 | 1 | 1 | 181 | 93 | 37 | 183 | 2.99 |
| 106 | 75 | 1 | 1 | 1 | 1 | 1 | 232 | 174 | 44 | 166 | 4.7 |
| 107 | 60 | 1 | 2 | 1 | 1 | 1 | 247 | 141 | 34 | 167 | 6.9 |
| 108 | 62 | 1 | 1 | 1 | 1 | 1 | 139 | 58 | 39 | 210 | 2.88 |
| 109 | 71 | 1 | 2 | 1 | 2 | 1 | 188 | 84 | 42 | 311 | 2.8 |
| 110 | 80 | 2 | 2 | 1 | 1 | 1 | 188 | 87 | 33 | 139 | 4.9 |
| 111 | 71 | 1 | 2 | 2 | 1 | 1 | 241 | 154 | 37 | 97 | 7.7 |
| 112 | 65 | 1 | 2 | 2 | 2 | 1 | 202 | 118 | 32 | 183 | 5.2 |
| 113 | 50 | 1 | 2 | 1 | 1 | 1 | 235 | 126 | 39 | 140 | 8.3 |
| 114 | 65 | 1 | 2 | 1 | 1 | 1 | 161 | 92 | 39 | 147 | 5.6 |
| 115 | 44 | 1 | 1 | 2 | 2 | 1 | 221 | 155 | 46 | 98 | 7.2 |
| 116 | 79 | 1 | 2 | 1 | 2 | 1 | 193 | 142 | 33 | 53 | 2.2 |
| 117 | 73 | 1 | 2 | 2 | 1 | 1 | 189 | 112 | 47 | 125 | 5.3 |
| 118 | 69 | 1 | 2 | 1 | 1 | 1 | 222 | 158 | 46 | 91 | 7.6 |
| 119 | 80 | 2 | 2 | 1 | 1 | 1 | 250 | 89 | 35 | 140 | 9.2 |
| 120 | 67 | 2 | 2 | 1 | 2 | 1 | 251 | 184 | 34 | 86 | 14.2 |
| 121 | 43 | 1 | 2 | 2 | 2 | 1 | 192 | 140 | 32 | 101 | 7 |
| 122 | 38 | 2 | 2 | 2 | 2 | 2 | 196 | 90 | 27 | 141 | 6.8 |

| 123 | 66 | 1 | 2 | 1 | 1 | 1 | 193 | 123 | 49 | 105 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 124 | 81 | 1 | 2 | 2 | 1 | 1 | 172 | 115 | 35 | 280 | 3.5 |
| 125 | 51 | 2 | 2 | 2 | 2 | 1 | 196 | 130 | 44 | 110 | 7.4 |
| 126 | 55 | 1 | 2 | 2 | 2 | 1 | 223 | 141 | 31 | 128 | 5.9 |
| 127 | 37 | 1 | 2 | 1 | 1 | 1 | 148 | 88 | 35 | 140 | 2.65 |
| 128 | 72 | 1 | 2 | 1 | 1 | 1 | 220 | 160 | 31 | 136 | 8 |
| 129 | 67 | 1 | 1 | 1 | 1 | 1 | 241 | 135 | 28 | 145 | 13.5 |
| 130 | 59 | 1 | 2 | 1 | 1 | 1 | 161 | 92 | 39 | 147 | 5 |
| 131 | 58 | 2 | 2 | 1 | 1 | 1 | 196 | 130 | 44 | 110 | 6.6 |
| 132 | 65 | 1 | 2 | 1 | 1 | 1 | 241 | 154 | 36 | 97 | 10.7 |
| 133 | 42 | 1 | 2 | 2 | 1 | 1 | 161 | 92 | 39 | 147 | 6.2 |
| 134 | 45 | 1 | 1 | 2 | 2 | 1 | 231 | 115 | 35 | 140 | 11.6 |
| 135 | 57 | 1 | 2 | 2 | 1 | 1 | 239 | 167 | 28 | 125 | 9 |
| 136 | 64 | 1 | 2 | 1 | 2 | 2 | 193 | 123 | 49 | 105 | 8 |
| 137 | 64 | 1 | 2 | 2 | 1 | 1 | 196 | 130 | 44 | 110 | 16.3 |
| 138 | 56 | 1 | 2 | 2 | 2 | 1 | 225 | 148 | 41 | 416 | 6.6 |
| 139 | 58 | 1 | 1 | 2 | 2 | 1 | 207 | 92 | 38 | 442 | 9.7 |
| 140 | 55 | 1 | 2 | 1 | 1 | 1 | 220 | 162 | 30 | 137 | 5.9 |
| 141 | 64 | 1 | 2 | 1 | 2 | 1 | 325 | 176 | 37 | 165 | 9.7 |
| 142 | 50 | 1 | 2 | 2 | 1 | 1 | 182 | 100 | 50 | 185 | 5.9 |
| 143 | 75 | 1 | 1 | 1 | 1 | 1 | 152 | 76 | 43 | 167 | 2.88 |
| ID | Age | sex | Smoking | HTN | Diabetes | Dyslipid | T.choles | LDL | HDL | TG | hs-CRP |
| 144 | 60 | 1 | 2 | 1 | 1 | 1 | 248 | 182 | 32 | 158 | 11.8 |
| 145 | 62 | 1 | 1 | 1 | 1 | 1 | 240 | 162 | 32 | 208 | 6.8 |
| 14 | 71 | 1 | 2 | 1 | 2 | 1 | 200 | 105 | 35 | 300 | 2.2 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 6 | | | | | | | | | | | |
| 147 | 80 | 2 | 2 | 1 | 1 | 1 | 212 | 87 | 30 | 140 | 0.89 |
| 148 | 71 | 1 | 2 | 2 | 1 | 1 | 241 | 154 | 37 | 97 | 6.8 |
| 149 | 65 | 1 | 2 | 2 | 2 | 1 | 162 | 93 | 32 | 183 | 4.9 |
| 150 | 50 | 1 | 2 | 1 | 1 | 1 | 235 | 168 | 29 | 140 | 6.5 |
| 151 | 65 | 1 | 2 | 1 | 1 | 1 | 161 | 92 | 39 | 147 | 4.6 |
| 152 | 44 | 1 | 1 | 2 | 2 | 1 | 221 | 155 | 26 | 98 | 7.6 |
| 153 | 79 | 1 | 2 | 1 | 2 | 1 | 205 | 142 | 33 | 53 | 4.3 |
| 154 | 73 | 1 | 2 | 2 | 1 | 1 | 239 | 167 | 47 | 125 | 6.9 |
| 155 | 69 | 1 | 2 | 1 | 1 | 1 | 222 | 158 | 46 | 91 | 3.5 |
| 156 | 80 | 2 | 2 | 1 | 1 | 1 | 150 | 89 | 35 | 140 | 3.5 |
| 157 | 67 | 2 | 2 | 1 | 2 | 1 | 151 | 84 | 50 | 86 | 0.97 |
| 158 | 43 | 1 | 2 | 2 | 2 | 1 | 192 | 140 | 32 | 101 | 6 |
| 159 | 36 | 2 | 2 | 2 | 2 | 2 | 165 | 90 | 27 | 141 | 2.99 |
| 160 | 66 | 1 | 2 | 1 | 1 | 1 | 193 | 123 | 49 | 105 | 4.7 |
| 161 | 81 | 1 | 2 | 2 | 1 | 1 | 275 | 115 | 35 | 280 | 6.6 |
| 162 | 51 | 2 | 2 | 2 | 2 | 1 | 196 | 130 | 44 | 110 | 6.4 |
| 163 | 55 | 1 | 2 | 2 | 2 | 1 | 223 | 141 | 32 | 128 | 6.3 |
| 164 | 37 | 1 | 2 | 1 | 1 | 1 | 248 | 138 | 35 | 140 | 3.2 |
| 165 | 72 | 1 | 2 | 1 | 1 | 1 | 190 | 160 | 31 | 136 | 1.8 |
| 166 | 67 | 1 | 1 | 1 | 1 | 1 | 196 | 135 | 28 | 145 | 6.7 |
| 167 | 59 | 1 | 2 | 1 | 1 | 1 | 202 | 92 | 39 | 147 | 6 |
| 168 | 58 | 2 | 2 | 1 | 1 | 1 | 196 | 130 | 44 | 110 | 5.6 |
| 169 | 65 | 1 | 2 | 1 | 1 | 1 | 241 | 154 | 24 | 97 | 11.9 |
| 170 | 42 | 1 | 2 | 2 | 1 | 1 | 161 | 92 | 39 | 147 | 7.4 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 171 | 45 | 1 | 1 | 2 | 2 | 1 | 215 | 159 | 35 | 140 | 6.4 |
| 172 | 57 | 1 | 2 | 2 | 1 | 1 | 189 | 67 | 47 | 125 | 0.99 |
| 173 | 64 | 1 | 2 | 1 | 2 | 2 | 193 | 123 | 49 | 105 | 1.99 |
| 174 | 64 | 1 | 2 | 2 | 1 | 1 | 196 | 130 | 44 | 110 | 1.4 |
| 175 | 50 | 1 | 1 | 2 | 2 | 1 | 201 | 125 | 49 | 79 | 4.8 |
| 176 | 78 | 2 | 2 | 1 | 1 | 1 | 231 | 151 | 30 | 286 | 1.2 |
| 177 | 49 | 1 | 2 | 2 | 2 | 2 | 193 | 145 | 35 | 66 | 2.4 |
| 178 | 49 | 2 | 2 | 2 | 2 | 1 | 224 | 130 | 36 | 75 | 3.6 |
| 179 | 65 | 2 | 2 | 1 | 1 | 1 | 298 | 189 | 23 | 141 | 4.6 |
| 180 | 52 | 2 | 2 | 1 | 2 | 1 | 200 | 140 | 32 | 152 | 4.9 |

*For sex (1) indicates male (2) indicates female. Smoking, HTN, Diabetes, and dyslipid (1) means yes, and (2) means NO.*

*Table (2) Summary for all cases (80 cases), and results obtained for the control group.*

| ID | Age | sex | Smoking | HTN | Diabetes | Dyslipid | Chol | LDL | HDL | TG | hs-CRP |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 201 | 42 | 1 | 1 | 2 | 2 | 1 | 292 | 165 | 44 | 157 | 2.9 |
| 202 | 68 | 2 | 2 | 1 | 1 | 1 | 256 | 139 | 44 | 164 | 2.3 |
| 203 | 37 | 1 | 1 | 2 | 2 | 1 | 286 | 171 | 24 | 110 | 3.2 |
| 204 | 54 | 2 | 2 | 2 | 2 | 1 | 217 | 136 | 38 | 117 | 1.8 |
| 205 | 53 | 1 | 2 | 1 | 2 | 1 | 198 | 115 | 36 | 163 | 1.1 |
| 206 | 73 | 2 | 2 | 2 | 2 | 1 | 244 | 141 | 29 | 100 | 2.3 |
| 207 | 62 | 1 | 1 | 2 | 2 | 1 | 155 | 84 | 44 | 111 | 0.2 |
| 208 | 55 | 1 | 2 | 2 | 2 | 1 | 145 | 82 | 63 | 113 | 0.3 |
| 209 | 69 | 2 | 2 | 2 | 1 | 1 | 200 | 128 | 35 | 88 | 1.4 |
| 210 | 81 | 2 | 2 | 2 | 1 | 2 | 262 | 145 | 52 | 157 | 4.1 |

| 211 | 38 | 1 | 2 | 2 | 2 | 1 | 199 | 131 | 43 | 128 | 2.2 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 212 | 36 | 1 | 1 | 1 | 2 | 1 | 238 | 146 | 34 | 88 | 1.4 |
| 213 | 41 | 1 | 2 | 2 | 2 | 1 | 210 | 135 | 33 | 254 | 3.2 |
| 214 | 52 | 2 | 1 | 2 | 1 | 1 | 120 | 89 | 49 | 95 | 0.1 |
| 215 | 38 | 1 | 1 | 2 | 2 | 1 | 214 | 139 | 33 | 195 | 1.3 |
| 216 | 67 | 1 | 2 | 2 | 1 | 1 | 245 | 155 | 51 | 162 | 3.5 |
| 217 | 59 | 2 | 2 | 2 | 1 | 1 | 232 | 161 | 34 | 100 | 3.1 |
| 218 | 35 | 1 | 2 | 2 | 2 | 1 | 238 | 165 | 31 | 218 | 5.5 |
| 219 | 68 | 1 | 2 | 2 | 1 | 2 | 183 | 120 | 32 | 81 | 5.9 |
| 220 | 47 | 2 | 2 | 2 | 2 | 1 | 256 | 162 | 28 | 319 | 6.2 |
| 221 | 37 | 1 | 2 | 2 | 2 | 1 | 193 | 141 | 35 | 84 | 3 |
| 222 | 38 | 2 | 2 | 1 | 2 | 1 | 186 | 128 | 29 | 110 | 1.8 |
| 223 | 64 | 1 | 1 | 2 | 1 | 1 | 255 | 169 | 24 | 152 | 7.7 |
| 224 | 38 | 1 | 1 | 2 | 2 | 1 | 209 | 155 | 35 | 91 | 2.4 |
| 225 | 40 | 1 | 2 | 2 | 2 | 2 | 117 | 77 | 30 | 156 | 1.3 |
| 226 | 50 | 1 | 1 | 2 | 2 | 1 | 211 | 128 | 35 | 79 | 1.9 |
| 227 | 78 | 2 | 2 | 1 | 1 | 1 | 244 | 161 | 30 | 286 | 6.2 |
| 228 | 49 | 1 | 2 | 2 | 2 | 2 | 213 | 145 | 35 | 66 | 4.9 |
| 229 | 49 | 2 | 2 | 2 | 2 | 1 | 224 | 130 | 43 | 75 | 3.4 |
| 230 | 65 | 2 | 2 | 1 | 1 | 1 | 202 | 139 | 48 | 141 | 2.1 |
| 231 | 52 | 2 | 2 | 1 | 2 | 1 | 220 | 140 | 32 | 152 | 3.2 |
| 232 | 42 | 1 | 1 | 2 | 2 | 1 | 292 | 155 | 44 | 157 | 3.6 |
| 233 | 68 | 2 | 2 | 1 | 1 | 1 | 200 | 141 | 44 | 164 | 2.3 |
| 234 | 37 | 1 | 1 | 2 | 2 | 1 | 286 | 169 | 24 | 110 | 3.7 |
| 235 | 54 | 2 | 2 | 2 | 2 | 1 | 247 | 158 | 26 | 117 | 4.6 |
| 236 | 53 | 1 | 2 | 1 | 2 | 1 | 198 | 115 | 36 | 163 | 3.8 |
| 237 | 73 | 2 | 2 | 2 | 2 | 1 | 244 | 141 | 24 | 100 | 6.5 |
| 238 | 62 | 1 | 1 | 2 | 2 | 1 | 222 | 155 | 44 | 111 | 1.9 |
| 239 | 55 | 1 | 2 | 2 | 2 | 1 | 291 | 185 | 33 | 213 | 4.3 |
| 240 | 69 | 2 | 2 | 2 | 1 | 1 | 192 | 190 | 35 | 88 | 2.7 |
| ID | Age | sex | Smoking | HTN | Diabetes | Dyslipid | T.choles | LDL | HDL | TG | hs-CRP |
| 241 | 81 | 2 | 2 | 2 | 1 | 2 | 162 | 85 | 52 | 157 | 1.1 |
| 242 | 38 | 1 | 2 | 2 | 2 | 1 | 199 | 129 | 43 | 128 | 2.1 |
| 243 | 36 | 1 | 1 | 1 | 2 | 1 | 238 | 166 | 54 | 88 | 1 |
| 244 | 39 | 1 | 2 | 2 | 2 | 1 | 210 | 135 | 33 | 254 | 1.6 |
| 245 | 52 | 2 | 1 | 2 | 1 | 1 | 200 | 147 | 37 | 95 | 0.1 |
| 246 | 37 | 1 | 1 | 2 | 2 | 1 | 214 | 139 | 39 | 195 | 0.1 |
| 247 | 67 | 1 | 2 | 2 | 1 | 1 | 245 | 155 | 31 | 162 | 2.8 |
| 248 | 59 | 2 | 2 | 2 | 1 | 1 | 202 | 159 | 41 | 100 | 3 |
| 249 | 35 | 1 | 2 | 2 | 2 | 1 | 238 | 154 | 40 | 218 | 2.3 |
| 250 | 68 | 1 | 2 | 2 | 1 | 2 | 241 | 147 | 30 | 81 | 4.2 |
| 251 | 47 | 2 | 2 | 2 | 2 | 1 | 208 | 106 | 38 | 219 | 2.4 |
| 252 | 36 | 1 | 2 | 2 | 2 | 1 | 193 | 141 | 35 | 84 | 0.6 |
| 253 | 37 | 2 | 2 | 1 | 2 | 1 | 218 | 129 | 33 | 110 | 1.8 |
| 254 | 64 | 1 | 1 | 2 | 1 | 1 | 193 | 112 | 40 | 152 | 2.2 |
| 255 | 38 | 1 | 1 | 2 | 2 | 1 | 209 | 155 | 35 | 91 | 1.6 |
| 256 | 40 | 1 | 2 | 2 | 2 | 2 | 117 | 81 | 30 | 156 | 2.1 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 257 | 55 | 1 | 2 | 2 | 2 | 1 | 280 | 175 | 28 | 189 | 2.8 |
| 258 | 55 | 1 | 2 | 2 | 2 | 1 | 200 | 135 | 35 | 213 | 1.5 |
| 259 | 69 | 2 | 2 | 2 | 1 | 1 | 263 | 162 | 24 | 88 | 4 |
| 260 | 81 | 2 | 2 | 2 | 1 | 2 | 195 | 128 | 32 | 157 | 2.5 |
| 261 | 36 | 1 | 2 | 2 | 2 | 1 | 199 | 134 | 38 | 128 | 0.5 |
| 262 | 36 | 1 | 1 | 1 | 2 | 1 | 238 | 139 | 36 | 112 | 1.5 |
| 263 | 32 | 1 | 2 | 2 | 2 | 1 | 252 | 168 | 33 | 254 | 3.1 |
| 264 | 52 | 2 | 1 | 2 | 1 | 1 | 225 | 157 | 39 | 148 | 0.6 |
| 265 | 36 | 1 | 1 | 2 | 2 | 1 | 246 | 169 | 33 | 195 | 1.4 |
| 266 | 58 | 1 | 2 | 2 | 1 | 1 | 245 | 155 | 42 | 162 | 4.8 |
| 267 | 59 | 2 | 2 | 2 | 1 | 1 | 240 | 138 | 33 | 100 | 1.2 |
| 268 | 35 | 1 | 2 | 2 | 2 | 1 | 240 | 160 | 39 | 220 | 2.4 |
| 269 | 62 | 1 | 2 | 2 | 1 | 2 | 238 | 129 | 29 | 85 | 3.6 |
| 270 | 47 | 2 | 2 | 2 | 2 | 1 | 210 | 116 | 37 | 259 | 4.6 |
| 271 | 35 | 1 | 2 | 2 | 2 | 1 | 195 | 144 | 35 | 84 | 3.5 |
| 272 | 55 | 1 | 2 | 2 | 2 | 1 | 291 | 185 | 33 | 213 | 2.5 |
| 273 | 55 | 2 | 2 | 2 | 1 | 1 | 252 | 190 | 34 | 98 | 2.6 |
| 274 | 62 | 2 | 2 | 2 | 1 | 2 | 162 | 105 | 42 | 157 | 1.9 |
| 275 | 34 | 1 | 2 | 2 | 2 | 1 | 200 | 130 | 43 | 128 | 1.4 |
| 276 | 36 | 1 | 1 | 1 | 2 | 1 | 212 | 164 | 39 | 88 | 2 |
| 277 | 55 | 1 | 2 | 2 | 2 | 1 | 235 | 142 | 31 | 213 | 3 |
| 278 | 59 | 2 | 2 | 2 | 1 | 1 | 292 | 177 | 27 | 88 | 4 |
| 279 | 66 | 2 | 2 | 2 | 1 | 2 | 169 | 125 | 37 | 157 | 1.4 |
| 280 | 35 | 1 | 2 | 2 | 2 | 1 | 201 | 129 | 43 | 128 | 0.6 |

*For sex (1) indicates male (2) indicates female. Smoking, HTN, Diabetes, and dyslipid (1) means yes, and (2) means NO.*

# Appendix

# Editorial

# Novel Inflammatory Markers of Coronary Risk

## Theory Versus Practice

Peter Libby, MD; Paul M. Ridker, MD, MPH

For practitioners committed to coronary risk reduction, recent clinical trial data pose a considerable challenge. Specifically, in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), a primary prevention trial,[1] treatment with lovastatin among an apparently healthy group of individuals without traditional coronary risk factors resulted in significant reductions in future cardiovascular events. Application of the results of that trial and of the West of Scotland primary prevention trial of pravastatin[2] suggests that tens of millions of Americans without manifest atherosclerosis could benefit from lipid-lowering therapy.

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Such a blanket approach, however, may be unwise from medical as well as economic perspectives. Unnecessary exposure to pharmacological agents, even those as safe as the statins, will ultimately subject some asymptomatic and low-risk individuals to unwanted side effects. Furthermore, economic constraints dictate that primary prevention strategies with even modest cost must be limited to those individuals who are likely to gain the greatest benefit. Even when an inexpensive preventive therapy such as low-dose aspirin is proven effective,[3] behavioral barriers on the parts of both physicians and patients must be overcome if long-term compliance is to be achieved. All of these considerations highlight the need for better methods to stratify risk of atherosclerotic events in apparently healthy populations.

### New Approaches to Coronary Risk Assessment

Clinical strategies designed to improve risk prediction have taken several forms. Imaging techniques including carotid ultrasound, MRI, and electron beam computed tomography (EBCT) all hold promise for identifying "vulnerable plaques" and detecting silent atheroma. However, prospective studies demonstrating the clinical utility of these approaches are limited. For example, a recent study[4] of coronary calcification detected by EBCT has shown that this method does not accurately predict future coronary events, even in high-risk patients. The cost of these noninvasive imaging modalities may also prohibit their application for widespread screening application.

Provocative testing of endothelium-dependent vasodilation has a firm pathophysiological foundation and may also furnish information regarding an asymptomatic individual's risk for future coronary events. However, as in the case of imaging modalities, the specificity of this approach is uncertain, and practical barriers preclude its utility for screening in outpatient clinic settings. Similarly, although the rapid progress in identifying genetic polymorphisms that correlate with coronary risk holds great promise, we have much work to do before we will know how to apply these data in practice.

By contrast, several serum markers have recently come to the fore as potential solutions to the challenge of detecting high-risk individuals for primary prevention.[5] Indeed, because of their low cost and simplicity for outpatient use, the identification of a simple blood test, or a battery of such tests, has become a major initiative in preventive cardiology. In this issue of *Circulation*, Xu and colleagues[6] furnish new evidence that antibodies to heat-shock protein 65 (hsp65) are associated with increased risk of atherosclerotic events in a free-living population. What can we learn from such studies about the pathophysiology of atherosclerosis and its complications? What criteria should we use in deciding how and when to apply these new techniques to our clinical practice?

### Markers of Inflammation and Stress Furnish Insight Into Pathophysiology

Serum markers of inflammation provide an avenue of insight into the pathophysiology of atherosclerosis and its complications. High-sensitivity testing for C-reactive protein (hs-CRP), a nonspecific marker of low-grade systemic inflammation, has received much attention, and several studies now support a strong link between baseline elevations of hs-CRP and future risk of coronary events.[7,8] "Distal" indicators of inflammation likewise predict coronary risk (Figure 1). Examples include the soluble forms of leukocyte adhesion molecules, such as intercellular adhesion molecule-1 (sICAM-1).[9,10] These distal markers may reflect the consequences of elevated levels of proinflammatory cytokines. For example, interleukin-6 (IL-6) probably provokes the augmented expression of the C-reactive protein (CRP) gene in the liver. Cytokines such as tumor necrosis factor-$\alpha$ (TNF-$\alpha$) or IL-1 isoforms can in turn stimulate the expression of IL-6 and of the leukocyte adhesion molecules, such as ICAM-1 (Figure 1).

The source of these cytokines remains unclear. Increased levels of cytokines might arise from atheroma themselves,

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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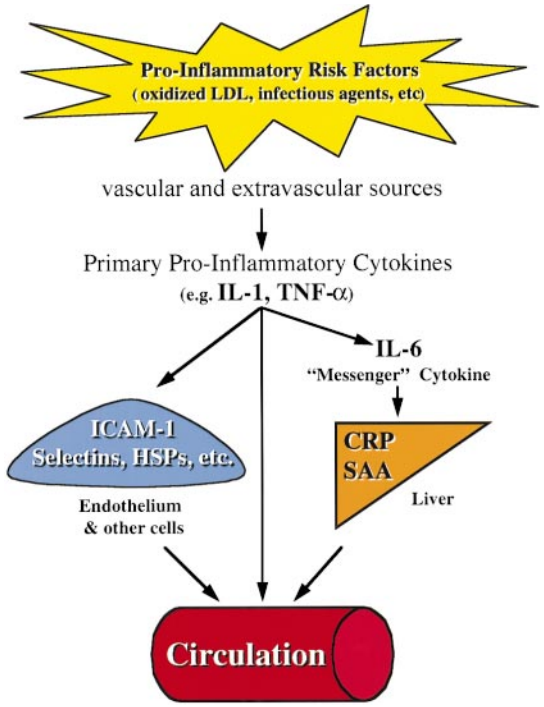


**Figure 1.** Pathways by which vascular and extravascular sources of inflammation result in circulating levels of serum markers that provide a reflection of the underlying inflammatory response. Inflammation, systemic or local, either in the blood vessel itself or elsewhere, triggers the production of multipotent proinflammatory cytokines we denote here as "primary" (eg, IL-1$\beta$ or TNF-$\alpha$). These primary cytokines can directly elicit production by endothelial and other cells of adhesion molecules, procoagulants, and other mediators that may be released in soluble form into circulating blood. Primary cytokines also stimulate production of "messenger" cytokine, IL-6, which induces expression of hepatic genes encoding acute-phase reactants found in blood, including CRP and serum amyloid-A (SAA). Thus, these markers in serum can provide a window on the inflammatory status of the individual, otherwise inaccessible in the intact subject.

reflecting their quantity (atherosclerotic burden) or quality (the degree of inflammatory activity within these lesions). The cytokines might also derive from nonvascular sources and reflect inflammatory states such as chronic infections that may accelerate atherogenesis and its manifestations.[11] Both vascular and extravascular sources of inflammatory cytokines may prove important to various degrees in different individuals. Regardless of the source of the inflammatory cytokines, emerging work on serum inflammatory markers supports the notion of a "pathway" of inflammatory activation related to acute coronary events (Figure 1). The inflammation (vascular or extravascular) begets cytokines (local and systemic), which in turn elicit the expression of acute-phase reactants such as hs-CRP and fibrinogen and of other effector molecules in the inflammatory response, such as adhesion molecules for leukocytes (Figure 1). Indeed, prospective epidemiological studies have now shown that measurements of serum inflammatory markers at each level of this pathway are associated with increased coronary risk (Figure 2).

Where do hsps fit into this schema? Cells that are stressed by thermal and other injuries augment their synthesis of a series of molecules known as hsps or chaperonins.[12] By

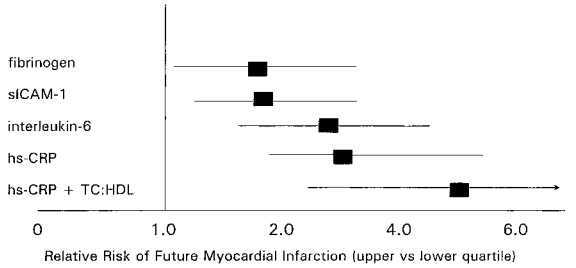


**Figure 2.** Relative risks of future myocardial infarction for apparently healthy middle aged men according to baseline plasma concentrations of fibrinogen, sICAM-1, IL-6, hs-CRP, and the combination of hs-CRP with the total cholesterol–to–HDL cholesterol ratio (TC:HDL). Data derive from participants in the Physicians Health Study.[5,7,8,10] For consistency, risks are computed for men in top vs bottom quartiles for each parameter.

binding to proteins critical to cellular function, these molecular "chaperones" can stabilize them and increase their resistance to denaturation (for example, by heat). Whatever their function, the expression of hsps does reflect cellular trauma, a process that in turn appears to activate vascular endothelium and smooth muscle cells, as well as regulate macrophage TNF-*a* and matrix metalloproteinase expression.[13,14] Furthermore, cytokines and oxidized LDLs can induce adhesion molecules such as ICAM-1 and hsp60.[15] A body of work from the laboratories of Xu and Wick has implicated hsp65 as an antigen involved in instigating the chronic immune response characteristic of human atherosclerosis. Specifically, previous cross-sectional data from this group have shown a direct relationship between antibodies against hsp60 and carotid wall atherosclerosis.[16] In the present follow-up study, these antibodies are sustained among those with the most severe degrees of underlying atherosclerosis and were demonstrated to predict 5-year mortality.[6] These new data furnish additional support for the inflammatory hypothesis of atherogenesis and of the acute coronary syndromes.

## Should We Use Novel Inflammatory Markers of Coronary Risk in the Clinic Today?

Although studies of serum markers of inflammation provide substantial insight into the pathophysiology of atherothrombosis, the clinical utility of measuring these markers remains uncertain. In general, we advocate a cautious approach for several reasons.[5] First, for a novel inflammatory marker to have a clinical role, there must be a widely available diagnostic test with reproducible assay characteristics appropriate for patient-related purposes. Of the major inflammatory markers, only CRP has an established World Health Organization standard, and high-sensitivity assays for this parameter appear to provide reliable results. Second, there must be a consistent series of prospective studies that indicate that baseline elevations of a given inflammatory marker predict future coronary events. In this regard, prospective data remain limited for the cellular adhesion molecules and for various hsps. In contrast, a remarkably consistent series of prospective data is available for both hs-CRP and fibrinogen. Third, to be of clinical use, markers of inflammation must be

shown to add substantially to our ability to predict risk beyond that achievable by use of traditional risk factors such as those incorporated into the Framingham risk algorithms or the European guidelines.[17] Although some studies suggest that inflammatory markers may well improve risk-prediction models,[7,8,18,19] we believe more data are needed before firm clinical recommendations can be made. Finally, whether inflammation per se represents a modifiable risk factor is currently uncertain, although preliminary data suggest that several common preventive therapies may work in part through anti-inflammatory targets.

As evidenced by the current study from Xu and colleagues concerning hsps, we are in a rapidly expanding phase of knowledge with respect to novel markers of vascular inflammation. Although extraordinarily valuable as research tools, we must await completion of prospective evaluations of various panels of these peripheral markers before implementing their use in daily practice. In all likelihood, a combination of genetic markers (reflecting heredity) and serum markers (reflecting the net interaction between heredity and the environment) will ultimately afford a solution to the current challenges posed in primary prevention.

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KEY WORDS: Editorials ■ atherosclerosis ■ risk factors ■ inflammation ■ cytokines ■ interleukins

# Conformational Rearrangement in C-Reactive Protein Is Required for Proinflammatory Actions on Human Endothelial Cells

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***Background***—C-reactive protein (CRP) has been suggested to actively amplify the inflammatory response underlying coronary heart diseases by directly activating endothelial cells. In this study, we investigated whether loss of the cyclic pentameric structure of CRP, resulting in formation of modified or monomeric CRP (mCRP), is a prerequisite for endothelial cell activation.

***Methods and Results***—We examined the impact of native CRP and mCRP on the production of monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8), key regulators of leukocyte recruitment, and on the expression of intercellular adhesion molecule-1 (ICAM-1), E-selectin, and vascular adhesion molecule-1 (VCAM-1) in human cultured coronary artery endothelial cells (HCAECs). Incubation with mCRP for 4 hours increased MCP-1 and IL-8 secretion and mRNA levels and expression of ICAM-1, E-selectin, and VCAM-1 protein and mRNA. Significant induction occurred at 1 to 5 $\mu$g/mL, reached a maximum at 30 $\mu$g/mL, and did not require the presence of serum. Native CRP was without detectable effects at 4 hours, whereas it enhanced cytokine release after a 24-hour incubation. An anti-Fc$\gamma$RIII (CD16) but not an anti-Fc$\gamma$RII (CD32) antibody produced a 14% to 32% reduction of the mCRP effects ($P<0.05$). mCRP but not CRP evoked phosphorylation of p38 mitogen-activated protein kinase, and inhibition of this kinase with SB 203580 reversed the effects of mCRP. Furthermore, culture of HCAECs in the presence of SB203580 markedly decreased mCRP-stimulated E-selectin and ICAM-1–dependent adhesion of neutrophils to HCAECs ($P<0.001$).

***Conclusions***—Loss of pentameric symmetry in CRP, resulting in formation of mCRP, promotes a proinflammatory HCAEC phenotype through a p38 MAPK–dependent mechanism. **(*Circulation*. 2004;109:2016-2022.)**

**Key Words:** proteins ■ cell adhesion molecules ■ signal transduction ■ endothelium ■ inflammation

Acute coronary artery diseases are associated with evidence of inflammation both systemically and in the arterial wall.[1,2] Elevated plasma levels of C-reactive protein (CRP) are predictive of subsequent acute coronary events among apparently healthy subjects and patients with stable or unstable angina.[3–5] However, the exact role and mechanisms of action of CRP as a modulator of inflammation have not been well defined, because both proinflammatory and antiinflammatory actions have been reported.[6–12] Recent results suggest that CRP may directly contribute to endothelial dysfunction by inducing cytokine release and surface expression of adhesion molecules.[13–15] Intriguingly, these actions were evident only after 12 to 24 hours of incubation, whereas maximum increases in adhesion molecule expression can be detected within 4 to 6 hours in response to proinflammatory cytokines or bacterial lipopolysaccharide (LPS). These observations raise the possibility that CRP may undergo structural changes to activate endothelial cells. Indeed, it has been proposed that distinct isoforms of CRP are formed during inflammation. Conformationally altered forms of CRP express several epitopes that are not present on native CRP[16] and display properties distinct from those of native CRP.[17–19]

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Native, pentameric CRP dissociates into free subunits within a few hours after binding to plasma membrane.[20] These subunits expressing several neoepitopes are referred to as modified or monomeric CRP (mCRP). mCRP antigens were detected in the wall of human normal blood vessels[21] and in inflamed tissues.[22]

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Dr Potempa was employed by NextEra Therapeutics, Inc, of Barrington, Ill, and is currently employed by Immtech International, Inc, of Vernon Hills, Ill. NextEra and Immtech are biotechnology companies that (in aggregate) are the primary owners of intellectual property developed on the modified form of C-reactive protein. Dr Potempa received support for his work on modified C-reactive protein from Immtech (until 1999) and from NextEra (from 1998 to 2002).

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In the present study, we investigated whether conformational rearrangement of native CRP, resulting in formation of mCRP, may be required for induction of release of interleukin-8 (IL-8) and monocyte chemoattractant protein-1 (MCP-1), key regulators of leukocyte recruitment, and expression of adhesion molecules in human coronary artery endothelial cells (HCAECs). To gain insight into the underlying mechanisms, we also examined whether the mCRP actions on HCAECs are mediated through binding to one of the IgG receptor subtypes similarly to that reported for leukocytes[18,23,24] and via activation of p38 mitogen-activated protein kinase (MAPK).

## Methods

### CRP Isoforms

High-purity (>99%) human native CRP (Calbiochem) was stored in a $NaN_3$-free buffer containing $CaCl_2$ to prevent spontaneous formation of mCRP from the native pentamer. A recombinant form of mCRP ($r_m$-CRP) that cannot rearrange into a pentameric structure was engineered as described previously.[18] Native CRP was distinguished from mCRP by binding and antigenicity differences using monoclonal antibodies described for each form of the molecule[16,17] and by their secondary structure.[18] The endotoxin levels of all peptide solutions were below the detection limit (0.125 EU/mL, corresponding to ≈0.01 ng/mL LPS) of the *Limulus* assay (Sigma).

### HCAEC Stimulation

HCAECs (passage 3, from Clonetics) were cultured in EGM-MV medium (Clonetics) supplemented with 10% FBS.[11] Monolayers of HCAECs (passages 4 through 6) in 24-well or 96-well microplates (confluence >97%, ≈28 000 cells/cm$^2$) were incubated with native CRP or mCRP. In some experiments, HCAECs were pretreated with the MAPK kinase inhibitor PD98059 (50 μmol/L), the p38 MAPK inhibitor SB203580 (0.1 to 1 μmol/L), the phosphatidylinositol 3-kinase inhibitor wortmannin (100 nmol/L), the anti-FcγRIII (CD16) antibody 3G8, anti-FcγRIIa (CD32) antibody FLI8.26, or the irrelevant antibody MOPC-21 (each at 2.5 μg/mL, Pharmingen) for 30 minutes before addition of mCRP. At the indicated times, culture supernatants were collected, and the cells were processed as described below.

### MCP-1 and IL-8 Production

The concentrations of MCP-1 and IL-8 in culture supernatants were determined in duplicate by selective ELISAs (BD Pharmingen). Intra-assay and interassay coefficients of variation were typically <4% and <6%, respectively. There was no cross-reactivity with CRP isoforms in the assays.

### Cell Adhesion Molecule Expression

After incubation for 4 hours, HCAECs were detached with EDTA (0.01% in PBS) from the 24-well microplates and then stained for intercellular adhesion molecule-1 (ICAM-1), E-selectin, or vascular adhesion molecule-1 (VCAM-1) using fluorescent dye–conjugated anti–ICAM-1, anti–VCAM-1 (Pharmingen) or anti–E-selectin (Serotec) antibodies as described previously.[19] Nonspecific binding was evaluated by use of appropriately labeled mouse IgG1. Immunofluorescence (10 000 cells for each sample) was analyzed with a FACScan flow cytometer with CellQuestPro software.

### RNase Protection Assay

For multiprobe RNase protection assays, HCAECs were lysed with 50 μL of lysis/denaturation solution (Ambion). [$^{32}$P]-labeled antisense RNA probes were generated using templates for IL-8, MCP-1, ICAM-1, E-selectin, VCAM-1, L32, and GAPDH (RiboQuant, BD Pharmingen), and the assays were performed with the Direct Protect kit (Ambion) as described previously.[25]

### Neutrophil–Endothelial Cell Adhesion Assay

The adhesion assay was performed as described previously.[11,19] In brief, monolayers of HCAECs in 96-well microplates were cultured with CRP, mCRP, or LPS (1 μg/mL, a positive control) for 4 hours at 37°C and washed extensively, and $2\times10^5$ human $^{51}$Cr-labeled neutrophils in 100 μL were then added. In some experiments, mCRP was added back together with neutrophils to mCRP-treated HCAECs. Some experiments were repeated using function-blocking monoclonal antibodies against E-selectin [ENA-2, 10 μg/mL, purified F(ab′)$_2$, Monosan], L-selectin (DREG-56, 20 μg/mL), CD18 (L130, 10 μg/mL), or the irrelevant antibody MOPC-21 (20 μg/mL, all from BD Biosciences). HCAECs were incubated with neutrophils for 30 minutes at 37°C on an orbital shaker at 90 rpm. Loosely adherent or unattached cells were removed by washing, and the endothelial monolayer and the adherent neutrophils were lysed. The number of adherent neutrophils in each experiment was calculated from the radioactivity of a control sample.

### Western Blot for p38 MAPK

Protein extracts were prepared by lysing $5\times10^4$ HCAECs in 100 μL of lysis buffer. Western blot analysis of phosphorylated and total p38 MAPK was performed using the PhosphoPlus p38 MAPK antibody kit (New England Biolabs).[18,19]

### Statistical Analysis

Results are expressed as mean±SEM. Statistical comparisons were made by ANOVA using ranks (Kruskal-Wallis test) followed by Dunn's multiple contrast hypothesis test to identify differences between various treatments or by the Mann-Whitney *U* test for unpaired observations. Values of $P<0.05$ were considered significant for all tests.

## Results

### mCRP Induces p38 MAPK-Dependent Expression of IL-8 and MCP-1 in HCAECs

Culture of HCAECs with mCRP for 4 hours resulted in concentration-dependent increases in IL-8 and MCP-1 release, whereas native CRP was without effect (Figure 1A). Significant induction was detected even with 1 μg/mL, which peaked at 100 μg/mL mCRP. The maximal effects of mCRP were ≈50% of those of LPS 1 μg/mL (IL-8, 3.9±0.2 ng/mL; MCP-1, 11.4±0.5 ng/mL). Native CRP induced significant release of IL-8 and MCP-1 at 24-hours; however, it was a considerable less potent inducer of cytokine production than mCRP (Figure 1B). The absence of serum did not affect the responses to mCRP (Figure 1C).

Preincubation of HCAECs with SB203580 concentration-dependently decreased mCRP-induced IL-8 and MCP-1 release at 4-hours, whereas neither wortmannin nor PD98059 affected the responses to mCRP (Figure 2A). Furthermore, mCRP but not native CRP induced phosphorylation of p38 MAPK relative to unstimulated controls. Phosphorylation was rapid in onset (peak at ≈30 minutes) and occurred in a concentration-dependent manner (Figure 2B).

We performed RNase protection assays on RNA extracted from HCAECs after 4 hours of incubation with mCRP. Consistent with the observations at protein levels, mCRP stimulated IL-8 and MCP-1 mRNA levels, which was suppressed by SB203580 but not by PD98059 or wortmannin (Figure 3). Native CRP did not produce detectable changes (Figure 3).

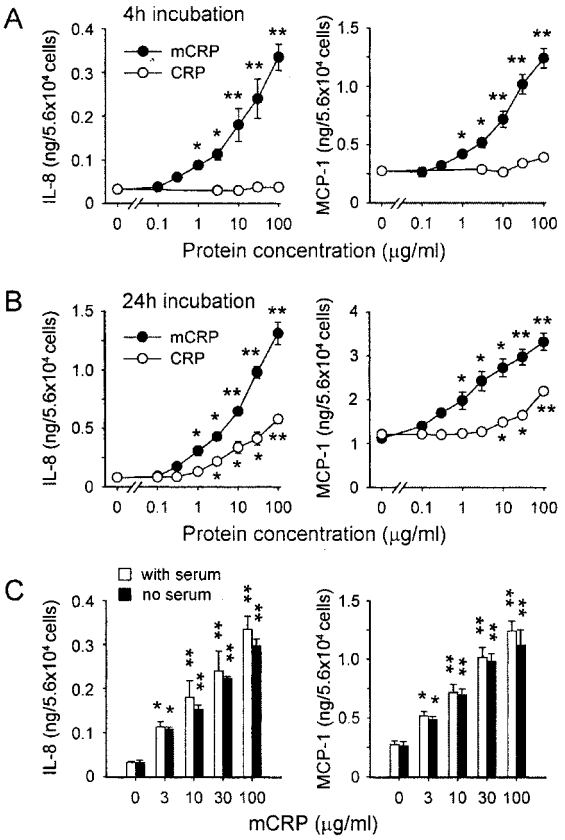


**Figure 1.** Effects of CRP isoforms on IL-8 and MCP-1 release in HCAECs. HCAECs were cultured with native CRP or mCRP for 4 hours (A) or 24 hours (B) in presence of 10% FBS. C, Comparison of effects of 4 hours of incubation with mCRP in presence of 10% FBS (with serum) or in serum-free conditions (no serum). Results are mean±SEM for 4 to 8 experiments. *$P<0.05$, **$P<0.01$ vs vehicle.

## mCRP Induces Expression of Adhesion Molecules

Under our experimental conditions, 2%, 39%, and 1% of untreated HCAECs expressed E-selectin, ICAM-1, and VCAM-1, respectively (Figure 4A). Treatment with mCRP for 4 hours evoked concentration-dependent increases in the overall expression and in the percentage of HCAECs expressing these adhesion molecules, whereas native CRP was without effect (Figure 4A). As a positive control, LPS produced on average 10-, 10-, and 5-fold increases in E-selectin, ICAM-1, and VCAM-1 expression, respectively (Figure 4A). SB203580 markedly attenuated mCRP-stimulated expression of these adhesion molecules, whereas PD98059 or wortmannin was without effect (Figure 4B). Likewise, mCRP induced increases in E-selectin, ICAM-1, and VCAM-1 mRNA levels that were markedly attenuated by SB203580 (Figure 3). After 24 hours of incubation, native CRP (100 μg/mL) increased ICAM-1 and VCAM-1 expression on HCAECs from 22.9±1.6 to 52.9±2.0 relative fluorescence units (RFU) and from 3.2±0.2 to 7.6±0.5 RFU, respectively (n=4, $P<0.05$), although it was less potent than mCRP (ICAM-1, 107.9±8.9 RFU; VCAM-1, 10.7±0.5 RFU; n=4, $P<0.05$ versus native CRP). Cell viability assessed by propidium iodide staining was >92% in all experiments.

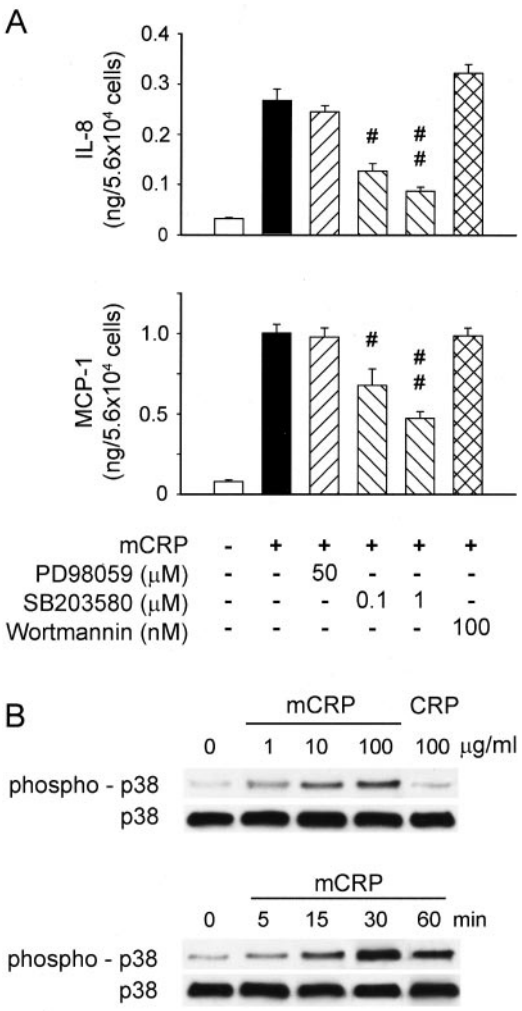


**Figure 2.** Effect of p38 MAPK inhibition on mCRP-stimulated IL-8 and MCP-1 release. A, HCAECs were preincubated with PD98059 (an MEK inhibitor), SB203580 (p38 MAPK inhibitor), or wortmannin (phosphatidylinositol-3 kinase inhibitor) for 20 minutes and then were cultured with mCRP (30 μg/mL) for 4 hours. n=5 to 6 per group. #$P<0.05$, ##$P<0.01$ vs mCRP alone. B, Time- and concentration-dependent induction of phosphorylation of p38 MAPK by mCRP. HCAECs were challenged for 30 minutes with various concentrations of mCRP or CRP or with 30 μg/mL mCRP for indicated times. Experiments were repeated 4 times with similar results.

## mCRP Promotes Neutrophil Adhesion to HCAECs

The biological significance of adhesion molecule expression was confirmed by the significant increase of adhesion of neutrophils to HCAECs cultured with mCRP (Figure 5A). Enhanced neutrophil attachment was evident with mCRP at 1 μg/mL and reached an apparent maximum at 100 μg/mL. By contrast, native CRP even at 100 μg/mL failed to promote adherence (Figure 5A). The number of adherent neutrophils to mCRP-activated HCAECs was further enhanced when the adhesion assay was performed in the presence of mCRP (Figure 5B), indicating that mCRP activates both neutrophils and HCAECs.

Because multiple receptors are involved in neutrophil adhesion to HCAECs under nonstatic conditions[11] and mCRP affects adhesion molecule expression on both neutrophils[19]

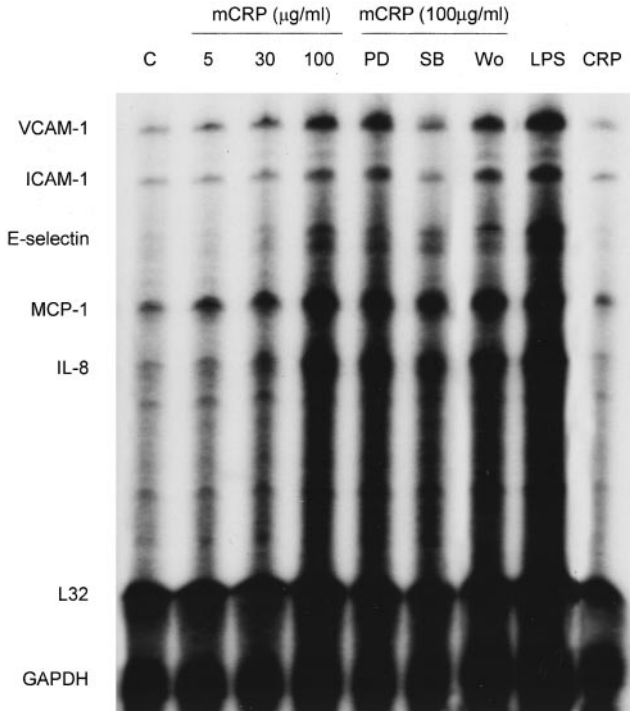


**Figure 3.** Effects of mCRP and native CRP on IL-8, MCP-1, E-selectin, ICAM-1, VCAM-1, L32, and GAPDH mRNA expression. HCAECs were cultured with CRP (100 μg/mL) or mCRP in presence of PD98059 (PD), SB203580 (SB), or wortmannin (Wo) for 4 hours. C indicates control (medium only). Effects of LPS (1 μg/mL) are shown for comparison. Shown is a representative multiprobe RNase protection assay of 4 independent experiments.

and endothelial cells (the present study), we assessed the contribution of L-selectin, $\beta_2$-integrins, and E-selectin to the binding interaction by using function-blocking monoclonal antibodies. mCRP-stimulated neutrophil attachment to mCRP-activated HCAECs was blocked by antibodies against CD18 (57±4%, n=6), E-selectin (38±3%), and L-selectin (14±2%) (Figure 5C). The combination of these antibodies inhibited neutrophil adhesion by 92±3% (Figure 5C). The number of adherent neutrophils was reduced from $2.8\pm0.2\times10^4$ cells/well to $1.4\pm0.1\times10^4$ cells/well when HCAECs were cultured with mCRP (30 μg/mL) in the absence and presence of SB203580 (n=6, $P<0.01$). Neither PD98059 nor wortmannin significantly affected the neutrophil-HCAEC attachment (data not shown).

### Search for mCRP Receptors on HCAECs

Because CRP binds predominantly to the low-affinity IgG FcγRIIa (CD32)[23,24] and mCRP utilizes the low-affinity immune-complex FcγRIII (CD16)[18] on leukocytes, we used function-blocking antibodies as competitors to assess the possible involvement of these receptors in mediating the actions of mCRP on HCAECs. Preincubation of HCAECs with the anti-CD16 antibody resulted in 14% to 32% attenuation of the responses to mCRP (Figure 6). Neither the anti-CD32 antibody (Figure 6) nor the irrelevant MOPC-21 antibody (data not shown) affected the responses to mCRP.

## Discussion

The present results provide evidence for a novel molecular mechanism by which CRP may activate endothelial cells. This bioactivity of CRP is expressed when the pentameric structure dissociates and undergoes a conformational rearrangement, resulting in formation of mCRP.

Formation of mCRP from native CRP involves the dissociation of the CRP pentameric disk. This is accompanied by a loss of predominantly β-sheet secondary structure with an increase in α-helix[18] and exposure of intersubunit contact residues, in particular residues 198 to 206, the predominant neoepitope expressed on mCRP,[16] and expression of distinct biological activities.[17–19] For instance, native CRP inhibits whereas mCRP promotes adhesion of neutrophils to LPS-activated HCAECs.[11,19] Furthermore, recent results suggest that aggregated (ie, structurally modified) CRP rather than native CRP may promote uptake of low-density lipoproteins by macrophages.[26,27] To avoid the confounding effects of spon-

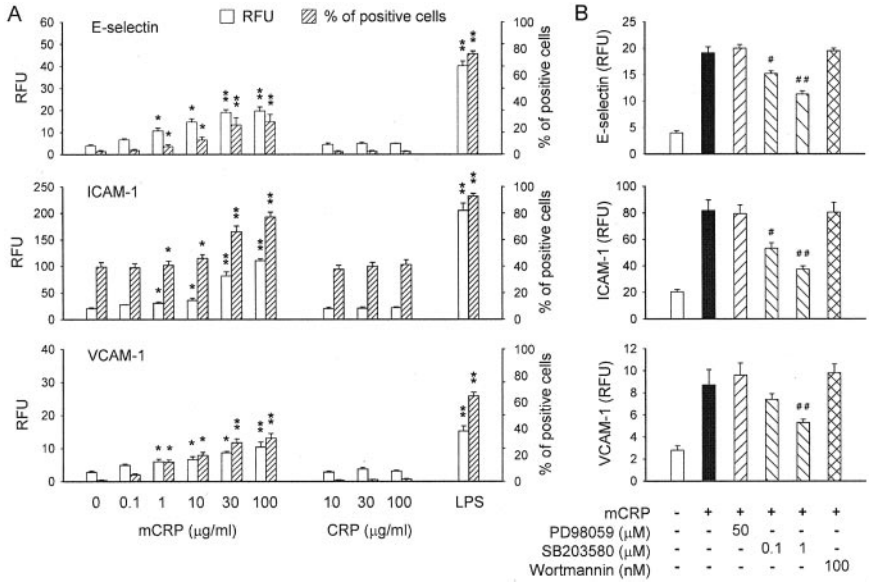


**Figure 4.** Induction of adhesion molecule expression by mCRP. A, Monolayers of HCAECs were cultured for 4 hours with mCRP, native CRP, or LPS (1 μg/mL). Adhesion molecule expression was assessed by flow cytometry and is expressed as relative fluorescence intensity (RFU) after subtracting nonspecific immunostaining. Positive cells represent percentage of HCAECs that stained positive for indicated adhesion molecule. B, HCAECs were preincubated with PD98059, SB203580, or wortmannin for 20 minutes and then challenged with mCRP (30 μg/mL) for 4 hours. Values are mean±SEM for 4 to 6 independent experiments. *$P<0.05$, **$P<0.01$ vs control (cells cultured in medium alone). #$P<0.05$; ##$P<0.01$ vs mCRP alone.

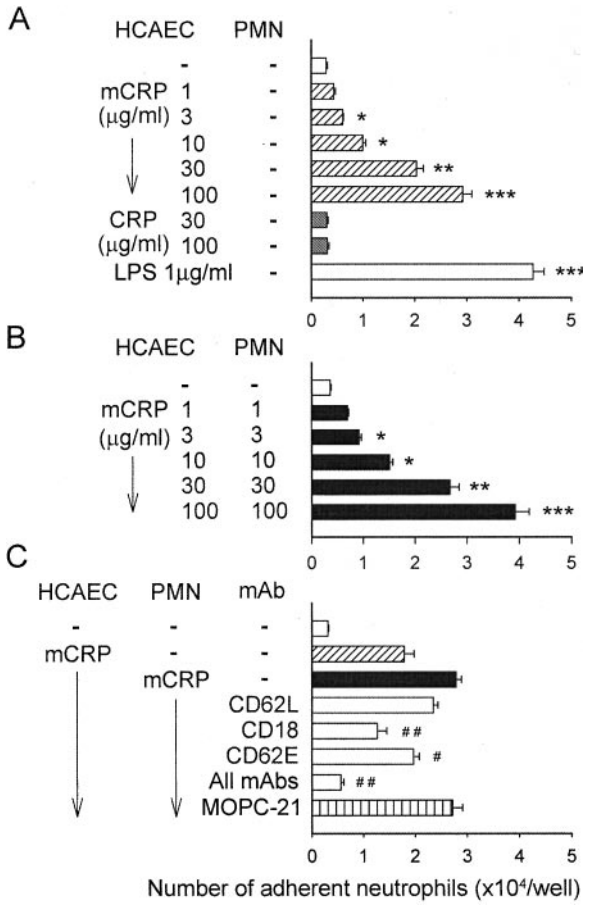


**Figure 5.** mCRP but not native CRP promotes neutrophil adhesion to HCAECs. Confluent HCAEC monolayers were cultured in medium only (control) or challenged with mCRP, CRP, or LPS for 4 hours, then radiolabeled neutrophils (PMN) without (A) or together with (B) mCRP were added and incubated with HCAECs for 30 minutes at 37°C. C, Inhibition of mCRP-stimulated neutrophil adhesion by function-blocking anti–E-selectin, anti-CD18, and anti–L-selectin monoclonal antibodies (mAb). HCAECs were cultured with mCRP (30 μg/mL, 4 hours), then neutrophils together with mCRP (30 μg/mL) were added for 30 minutes in presence of mAbs, as indicated. Irrelevant antibody MOPC-21 served as a negative control. Results are mean±SEM of 6 experiments. *$P<0.05$, **$P<0.01$, ***$P<0.001$ vs control (medium only); #$P<0.05$; ##$P<0.01$ vs mCRP without mAbs (solid column).

taneous formation of mCRP from native CRP during prolonged storage in the absence of calcium (our unpublished observations), we used CRP preparations devoid of mCRP contamination and engineered mCRP that cannot reassemble to form a pentamer.

Our study shows that mCRP, unlike native CRP, can induce cytokine release and expression of adhesion molecules on HCAECs after a 4-hour incubation period. We also examined the mechanisms of mCRP signaling in HCAECs, observing a predominant role for the p38 MAPK pathway.

At low μg/mL concentrations, mCRP induced transcription of IL-8, MCP-1, E-selectin, ICAM-1, and VCAM-1 genes within 4 hours of its addition to HCAECs. These effects were comparable in magnitude to those observed with LPS, a well-known activator of endothelial cells. Consistent with previous studies,[13–15] native CRP did not evoke detectable

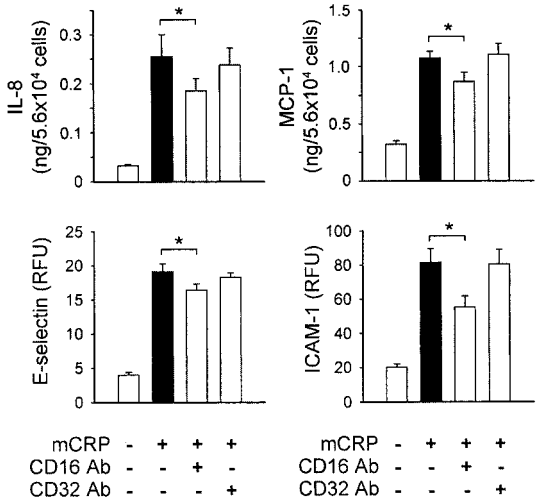


**Figure 6.** Effect of anti-CD16 and anti-CD32 antibody on HCAEC responses to mCRP. HCAECs were cultured with or without mCRP (30 μg/mL) plus an anti-CD16 antibody (Ab), anti-CD32 antibody, or irrelevant antibody MOPC-21 (all at 2.5 μg/mL). Culture medium was assayed for IL-8 and MCP-1; surface expression of E-selectin and ICAM-1 was assessed by flow cytometry. n=4 for each group. *$P<0.05$.

changes at 4 hours. The CRP induction became detectable only after 6 to 12 hours of incubation, reaching maximal effects at 24 hours,[13–15] coinciding with in vitro kinetics of dissociation into subunits.[20] Although CRP clearly enhanced IL-8 and MCP-1 production at 24 hours of culture, it was a significantly less potent inducer of cytokine production than mCRP. These observations suggest that conformational rearrangement of CRP is a prerequisite for activation of HCAECs and that the amounts of mCRP generated from CRP within 4 hours are not sufficient to evoke detectable responses. Another important difference between the actions of CRP and mCRP is that the mCRP effects do not depend on, whereas the CRP effects are dependent on, an as yet unidentified serum cofactor(s).[13] The mCRP action is based on a tissue rather than a serum environment, thus minimizing the need for serum cofactors.

The present study did not address the functional significance of mCRP-induced expression of IL-8 and MCP-1. MCP-1 and IL-8 play important roles in recruitment of monocytes into the vessel wall,[28,29] and IL-8 is a key regulator of neutrophil trafficking and activation.[30] Thus, by enhancing chemokine production, mCRP may contribute to the evolution of atherogenesis[28] and to the widespread neutrophil activation observed in patients with unstable angina.[2] The biological significance of adhesion molecule expression was confirmed by the significant increase of adhesion of neutrophils to mCRP-activated HCAECs. These observations extend previous findings that mCRP promoted neutrophil adhesion to LPS-activated HCAECs through upregulation of CD11b/CD18 on neutrophils.[19] Our results show that mCRP-induced expression of ICAM-1 and E-selectin also contributes to neutrophil-HCAEC attachment. Significantly higher numbers of neutrophils adhered to mCRP-activated HCAECs when the adhesion assay was performed in the presence than in the absence of mCRP, indicating that mCRP can promote

adhesion by activating both HCAECs and neutrophils. Leukocyte–endothelial cell interaction involves a complex interplay among adhesion molecules.[31] Indeed, the anti-CD18, anti–E-selectin, and anti–L-selectin antibody alone inhibited 57%, 34%, and 14% of neutrophil attachment, respectively, whereas combination of the 3 antibodies blocked ≈90% of adhesion. We also detected enhanced VCAM-1 expression after 4 hours of culture of HCAECs with mCRP, indicating that mCRP closely mimics the effect of 24 hours of incubation with CRP,[13,15] although the possible role of VCAM-1 in neutrophil adhesion was not investigated.

Our results indicate that mCRP activation of HCAECs involves p38 MAPK. mCRP stimulated rapid phosphorylation of this kinase, and the specific p38 MAPK inhibitor SB203580 markedly inhibited HCAEC responses to mCRP, although the inhibition was incomplete. These results are consistent with those observed with SB203580 on thrombin-induced endothelial chemokine production and ICAM-1 expression,[32,33] and suggest involvement of other intracellular signaling mechanism(s). Unlike in neutrophils,[18,19] mCRP does not appear to activate the MAPK kinase and phosphatidylinositol 3-kinase pathways in HCAECs, because there was no reduction in the presence of PD98059 and wortmannin.

Little is known at present about the CRP or mCRP receptor(s) on endothelial cells. Human aortic endothelial cells may express the receptors FcγRII and FcγRI,[34] which bind CRP on leukocytes.[23,24] In HCAECs, an anti-FcγRII antibody failed to affect the responses to mCRP, whereas an anti-FcγRIII antibody that effectively blocked the apoptosis delaying action of mCRP in neutrophils[19] produced a slight attenuation of mCRP-induced HCAEC activation. Although these observations would suggest the involvement of FcγRIII, additional studies are needed to confirm the presence of this receptor on HCAECs and to identify the major binding site(s) for mCRP on HCAECs.

Limitations of this study are that the mechanisms regulating mCRP formation in vivo are still unidentified and that mCRP levels are difficult to estimate in vivo, because, unlike CRP, mCRP is expressed on cell membranes rather than in the plasma.[21,22] Because the ratio of membrane-bound mCRP to mCRP in the culture medium is unknown, it is extremely complicated to measure the amount of native CRP that dissociated into free subunits in vitro. If indeed mCRP is a tissue-associated mediator, at the sites of injury it may come in contact with the endothelium and leukocytes, amplifying the proinflammatory response triggered by the initial endothelial injury.

In summary, the present results indicate that loss of pentameric symmetry in CRP, resulting in formation of mCRP, is prerequisite for the appearance of proinflammatory actions on HCAECs. Indeed, mCRP directly facilitates endothelial cell adhesion molecule expression, leukocyte adhesion, and MCP-1 and IL-8 production. Importantly, these effects are, in part, mediated by activation of the p38 MAPK pathway. These findings indicate that mCRP rather than native CRP may contribute to the development of vascular inflammation and suggest that inhibition of p38 MAPK may be a target for antiinflammatory strategies in vascular diseases.

## Acknowledgments

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# C-REACTIVE PROTEIN AND OTHER MARKERS OF INFLAMMATION IN THE PREDICTION OF CARDIOVASCULAR DISEASE IN WOMEN

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## ABSTRACT

***Background*** Since inflammation is believed to have a role in the pathogenesis of cardiovascular events, measurement of markers of inflammation has been proposed as a method to improve the prediction of the risk of these events.

***Methods*** We conducted a prospective, nested case–control study among 28,263 apparently healthy postmenopausal women over a mean follow-up period of three years to assess the risk of cardiovascular events associated with base-line levels of markers of inflammation. The markers included high-sensitivity C-reactive protein (hs-CRP), serum amyloid A, interleukin-6, and soluble intercellular adhesion molecule type 1 (sICAM-1). We also studied homocysteine and several lipid and lipoprotein measurements. Cardiovascular events were defined as death from coronary heart disease, nonfatal myocardial infarction or stroke, or the need for coronary-revascularization procedures.

***Results*** Of the 12 markers measured, hs-CRP was the strongest univariate predictor of the risk of cardiovascular events; the relative risk of events for women in the highest as compared with the lowest quartile for this marker was 4.4 (95 percent confidence interval, 2.2 to 8.9). Other markers significantly associated with the risk of cardiovascular events were serum amyloid A (relative risk for the highest as compared with the lowest quartile, 3.0), sICAM-1 (2.6), interleukin-6 (2.2), homocysteine (2.0), total cholesterol (2.4), low-density lipoprotein (LDL) cholesterol (2.4), apolipoprotein B-100 (3.4), high-density lipoprotein (HDL) cholesterol (0.3), and the ratio of total cholesterol to HDL cholesterol (3.4). Prediction models that incorporated markers of inflammation in addition to lipids were significantly better at predicting risk than models based on lipid levels alone ($P<0.001$). The levels of hs-CRP and serum amyloid A were significant predictors of risk even in the subgroup of women with LDL cholesterol levels below 130 mg per deciliter (3.4 mmol per liter), the target for primary prevention established by the National Cholesterol Education Program. In multivariate analyses, the only plasma markers that independently predicted risk were hs-CRP (relative risk for the highest as compared with the lowest quartile, 1.5; 95 percent confidence interval, 1.1 to 2.1) and the ratio of total cholesterol to HDL cholesterol (relative risk, 1.4; 95 percent confidence interval, 1.1 to 1.9).

***Conclusions*** The addition of the measurement of C-reactive protein to screening based on lipid levels may provide an improved method of identifying women at risk for cardiovascular events. (N Engl J Med 2000;342:836-43.)



HALF of all myocardial infarctions occur in persons in whom plasma lipid levels are normal.[1] In an effort to better identify patients at high risk for cardiovascular events, several markers of risk have been proposed for use in screening, including homocysteine and fibrinogen levels, fibrinolytic capacity, and levels of apolipoprotein A-I, apolipoprotein B-100, and Lp(a) lipoprotein. However, the clinical value of many of these markers has been limited because of inadequate standardization of assay conditions, inconsistency of prospective data, or lack of evidence of significant improvement in the prediction of risk over that afforded by standard lipid screening alone.[2]

With the recognition that atherosclerosis is an inflammatory process,[3] several plasma markers of inflammation have also been evaluated as potential tools for prediction of the risk of coronary events. Among them are markers of systemic inflammation produced in the liver, such as high-sensitivity C-reactive protein (hs-CRP) and serum amyloid A; cytokines such as interleukin-6; and adhesion molecules such as soluble intercellular adhesion molecule type 1 (sICAM-1).[4-11] However, as with other proposed predictors of the risk of cardiovascular events, the prognostic value of these markers of inflammation remains uncertain. For example, a widely held clinical view is that levels of markers of inflammation vary too greatly over time to allow accurate prediction of risk. Furthermore, few prospective studies have measured all these markers of inflammation in a single group of patients, so the relative usefulness of each marker cannot be easily evaluated. In addition, data supporting the hypothesis that markers of inflammation significantly increase the predictive value of lipid screening are scant and are limited almost exclusively to data from studies of hs-CRP in middle-aged men.[7,12] Finally, clinical application of these findings has been limited, since standardized, commercial assays for most markers of inflammation are only now being developed.

In a previous study, in which we used an experimental assay for hs-CRP, we found higher levels of this marker among healthy postmenopausal women participating in the Women's Health Study who subse-

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quently had cardiovascular events than among those who did not have such events.[13] On the basis of that finding and in the effort to address the clinical issues outlined above, we used a commercial assay to measure hs-CRP in the same cohort and simultaneously measured plasma levels of serum amyloid A, interleukin-6, sICAM-1, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, the ratio of total cholesterol to HDL cholesterol, apolipoprotein A-I, and apolipoprotein B-100. In addition, to allow comparison with other proposed markers, we measured plasma levels of Lp(a) lipoprotein and homocysteine. We thus were able to evaluate directly the relative value of each of these 12 measurements as an independent predictor of future cardiovascular events in a large cohort of apparently healthy women. We also sought to determine whether the measurement of markers of inflammation in addition to standard screening of lipid levels might provide a clinically useful method for improving overall prediction of the risk of cardiovascular events.

## METHODS

### Study Participants

We designed a prospective, nested case–control study involving participants in the Women's Health Study, an ongoing trial of aspirin and vitamin E for primary prevention among postmenopausal women with no history of cardiovascular disease or cancer.[14] Blood samples were collected in tubes containing EDTA at base line from 28,263 women (71 percent of the Women's Health Study participants) and stored in liquid nitrogen until the time of analysis.

For this analysis, case subjects were study participants from whom a base-line blood sample was obtained who subsequently had a cardiovascular event (defined as death from coronary heart disease, nonfatal myocardial infarction or stroke, or a coronary-revascularization procedure) during a mean follow-up period of three years. Myocardial infarction was classified as confirmed if symptoms met the criteria of the World Health Organization[15] and if the event was associated with abnormal levels of cardiac enzymes or diagnostic electrocardiographic changes. Stroke was classified as confirmed if the patient had a new neurologic deficit that lasted more than 24 hours. Computed tomographic scans or magnetic resonance images were available for the majority of women in whom stroke occurred. Performance of revascularization procedures was confirmed by review of hospital records. Death from coronary heart disease was confirmed by review of the autopsy report, the death certificate, medical records, or information from family members regarding the circumstances of death.

For each woman who had a confirmed cardiovascular event during follow-up, two control subjects of the same age (within one year) and smoking status (former smoker, current smoker, or nonsmoker) were selected from among the remaining study participants from whom a base-line blood sample had been obtained and who remained free of reported cardiovascular disease during follow-up. With use of these criteria, 122 case subjects and 244 control subjects were selected.

### Procedures

Base-line plasma samples from each woman with an event and each control subject were thawed and assayed for hs-CRP, serum amyloid A, and Lp(a) lipoprotein with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, Del.). Apolipoprotein A-I and apolipoprotein B-100 were simultaneously measured with this device by immunoassay. Total cholesterol, HDL cholesterol, and directly obtained LDL cholesterol levels were measured on a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis) with reagents from Roche Diagnostics and Genzyme (Cambridge, Mass.). Plasma levels of sICAM-1 and interleukin-6 were measured by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis), and the total plasma homocysteine level was measured with an IMx homocysteine assay (Abbott Laboratories, Abbott Park, Ill.) as previously reported.[16] Samples were handled in identical and in blinded fashion throughout the study. Samples were analyzed in triplicate and in random order so as to reduce systematic bias and interassay variation.

### Statistical Analysis

Means and proportions for risk factors for cardiovascular events at base line were calculated for women who had cardiovascular events during follow-up and those who did not. The significance of differences in means between the two groups was assessed with Student's t-test, and the significance of differences in proportions was tested with use of the chi-square statistic. Analysis of trends was used to test for associations between increasing levels of each plasma variable and the risk of future cardiovascular events, after the sample was divided into quartiles according to the distribution of control values for that marker. Adjusted risk estimates were obtained with use of logistic-regression models that, in addition to accounting for the variables used for matching (age and smoking status), adjusted for random assignment to aspirin or vitamin E in the Women's Health Study; several risk factors for cardiovascular events, including a history of hypertension, body-mass index, a history of diabetes, and a parental history of myocardial infarction before the age of 60 years; and other measured plasma markers.

We evaluated the combined role of lipid levels and markers of inflammation as predictors of the risk of future cardiovascular events in a series of analyses in which we explored the sensitivity and robustness of our findings from a clinical perspective. First, we used the likelihood-ratio test to determine whether logistic-regression models that included measurements of lipid variables and markers of inflammation provided a significantly better fit than did logistic-regression models limited to lipid measurements alone. Second, to estimate the clinical relevance of these effects, we computed the area under receiver-operating-characteristic curves for prediction models based on lipid measurements alone and for models based on measurements of both lipid levels and markers of inflammation. Third, we divided the study participants into nine groups according to low, medium, and high levels of total cholesterol and low, medium, and high levels of each marker of inflammation. In these analyses, logistic regression was used to evaluate simultaneously the risk of future cardiovascular events in each of the nine groups; the group of women in the lowest third for total cholesterol and in the lowest third for the respective marker of inflammation was considered the reference group. Finally, to address the clinical need for improved assessment of risk among persons with cholesterol levels currently considered safe, we performed a subgroup analysis of study participants with LDL cholesterol levels of less than 130 mg per deciliter (3.4 mmol per liter), the target level for the primary prevention of coronary heart disease according to the current guidelines of the National Cholesterol Education Program.[17]

All P values were two-tailed, and values of less than 0.05 were considered to indicate statistical significance. All confidence intervals were calculated at the 95 percent level.

## RESULTS

The base-line characteristics of the women who subsequently had cardiovascular events (case subjects) and those who remained free of reported cardiovascular disease (controls) are shown in Table 1. As expected, women who had cardiovascular events were heavier at base line than those who remained free of cardiovascular disease and were more likely to have hypertension, diabetes, or a parental history of prema-

**TABLE 1.** BASE-LINE CLINICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS.*

| CHARACTERISTIC | WOMEN WITH CARDIOVASCULAR EVENTS (N=122) | WOMEN FREE OF CARDIOVASCULAR EVENTS (N=244) | P VALUE† |
|---|---|---|---|
| Mean age (yr) | 59.3 | 59.3 | — |
| Mean body-mass index‡ | 27.1 | 26.0 | 0.04 |
| History of hypertension (%) | 55.5 | 31.3 | 0.001 |
| History of diabetes (%) | 9.8 | 2.1 | 0.001 |
| Parental history of myocardial infarction before 60 yr (%) | 21.3 | 12.7 | 0.04 |
| Smoking status (%) | | | — |
| Former smoker | 29.5 | 29.5 | |
| Current smoker | 27.9 | 27.9 | |
| Nonsmoker | 42.6 | 42.6 | |
| Frequency of exercise (%) | | | 0.9 |
| >3 times/wk | 6.6 | 8.2 | |
| 1–3 times/wk | 27.9 | 27.1 | |
| <1 time/wk | 21.3 | 20.1 | |
| Rarely or never | 44.3 | 44.5 | |
| Frequency of alcohol consumption (%) | | | 0.6 |
| Daily | 12.3 | 8.2 | |
| Weekly | 27.9 | 31.2 | |
| Monthly | 14.8 | 13.9 | |
| Rarely or never | 45.1 | 46.7 | |
| Current use of hormone-replacement therapy (%) | 44.3 | 41.0 | 0.1 |

*Because of rounding, not all percentages total 100.

†P values were not calculated for variables used in matching of case and control subjects, since the distribution of these variables was identical in the two groups.

‡The body-mass index is the weight in kilograms divided by the square of the height in meters.

**TABLE 2.** BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

| VARIABLE | WOMEN WITH CARDIOVASCULAR EVENTS | WOMEN FREE OF CARDIOVASCULAR EVENTS | P VALUE |
|---|---|---|---|
| High-sensitivity C-reactive protein (mg/dl) | | | <0.001 |
| Median | 0.42 | 0.28 | |
| Interquartile range | 0.21–0.83 | 0.11–0.55 | |
| Serum amyloid A (mg/dl) | | | 0.003 |
| Median | 0.63 | 0.52 | |
| Interquartile range | 0.45–1.01 | 0.35–0.78 | |
| Soluble intercellular adhesion molecule type 1 (ng/ml) | 349.7±121.3 | 321.3±107.4 | 0.03 |
| Interleukin-6 (pg/ml) | | | 0.003 |
| Median | 1.65 | 1.30 | |
| Interquartile range | 1.14–2.62 | 1.00–2.03 | |
| Total cholesterol (mg/dl) | 230.5±41.2 | 219.2±37.5 | 0.01 |
| LDL cholesterol (mg/dl) | 132.2±34.6 | 121.5±30.2 | 0.003 |
| HDL cholesterol (mg/dl) | 45.4±14.6 | 51.1±15.4 | <0.001 |
| Apolipoprotein A-I (mg/dl) | 163.8±40.3 | 168.5±36.1 | 0.3 |
| Apolipoprotein B-100 (mg/dl) | 128.5±31.0 | 115.0±26.7 | <0.001 |
| Lp(a) lipoprotein (mg/liter) | | | 0.3 |
| Median | 79 | 74 | |
| Interquartile range | 34–247 | 29–203 | |
| Ratio of total cholesterol to HDL cholesterol | 5.5±1.9 | 4.6±1.4 | <0.001 |
| Homocysteine (μmol/liter) | 14.1±8.0 | 12.4±5.8 | 0.02 |

*Plus–minus values are means ±SD. For normally distributed variables, P values were computed with t-tests; for non-normally distributed variables, P values were computed with the Wilcoxon rank-sum test for the difference in medians. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

ture myocardial infarction (before the age of 60 years). The frequency of exercise, the frequency of alcohol consumption, and rate of use of hormone-replacement therapy were similar in the two groups. Because of matching, the women who had cardiovascular events and the control subjects were virtually identical with respect to mean age and smoking status.

Base-line plasma levels of the inflammation markers hs-CRP ($P<0.001$), serum amyloid A ($P=0.003$), sICAM-1 ($P=0.03$), and interleukin-6 ($P=0.003$) were higher among the women who subsequently had cardiovascular events than among those who did not (Table 2). Similarly, base-line plasma levels of total cholesterol ($P=0.01$), LDL cholesterol ($P=0.003$), apolipoprotein B-100 ($P<0.001$), and homocysteine ($P=0.02$) and the ratio of total cholesterol to HDL cholesterol ($P<0.001$) were significantly higher among women with subsequent events than those without such events, whereas levels of HDL cholesterol were significantly lower among women with subsequent events ($P<0.001$). Base-line levels of Lp(a) lipoprotein were somewhat higher and levels of apolipoprotein A-I somewhat lower among the women with events than among control subjects, but these differences were not significant.

Table 3 shows the relative risks of cardiovascular events according to the quartile of each marker of inflammation or lipid measured in plasma. Measurements of hs-CRP, serum amyloid A, sICAM-1, and interleukin-6 were predictive of the risk of future cardiovascular events. Of the 12 measures, the level of hs-CRP was the most powerful predictor of risk in the univariate analysis (relative risk for women in the highest quartile as compared with the lowest quartile, 4.4; 95 percent confidence interval, 2.2 to 8.9; $P<0.001$). Of the lipid variables, the ratio of total cholesterol to HDL cholesterol (relative risk, 3.4; $P=0.001$) and the apolipoprotein B-100 level (relative risk, 3.4; $P=0.001$) were the most powerful predictors of risk. Nonsignificant trends were observed for apolipoprotein A-I and Lp(a) lipoprotein. As reported previously,[16] increasing levels of homocysteine were also associated with increased risk.

Levels of several markers of inflammation were highly correlated. For example, the correlation coefficient for the relation between hs-CRP and serum

**TABLE 3.** RELATIVE RISK OF CARDIOVASCULAR EVENTS ACCORDING TO BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

| VARIABLE | QUARTILE OF PLASMA LEVEL | | | | P VALUE FOR TREND |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| High-sensitivity C-reactive protein | | | | | |
| Median — mg/dl | 0.06 | 0.19 | 0.38 | 0.85 | |
| Relative risk (95% CI) | 1.0 | 2.1 (1.0–4.5) | 2.1 (1.0–4.4) | 4.4 (2.2–8.9) | <0.001 |
| Serum amyloid A | | | | | |
| Median — mg/dl | 0.25 | 0.43 | 0.62 | 1.17 | |
| Relative risk (95% CI) | 1.0 | 1.8 (0.9–3.6) | 1.9 (0.9–3.8) | 3.0 (1.5–6.0) | 0.002 |
| Soluble intercellular adhesion molecule type 1 | | | | | |
| Median — ng/ml | 228.7 | 273.9 | 319.1 | 439.3 | |
| Relative risk (95% CI) | 1.0 | 1.5 (0.7–3.1) | 2.0 (1.0–4.1) | 2.6 (1.3–5.1) | 0.004 |
| Interleukin-6 | | | | | |
| Median — pg/ml | 0.82 | 1.15 | 1.58 | 2.70 | |
| Relative risk (95% CI) | 1.0 | 1.3 (0.6–2.7) | 1.4 (0.7–2.8) | 2.2 (1.1–4.3) | 0.02 |
| Total cholesterol | | | | | |
| Median — mg/dl | 176 | 206 | 224 | 267 | |
| Relative risk (95% CI) | 1.0 | 1.2 (0.6–2.3) | 1.7 (0.9–3.3) | 2.4 (1.3–4.7) | 0.003 |
| LDL cholesterol | | | | | |
| Median — mg/dl | 88.4 | 108.9 | 127.4 | 156.6 | |
| Relative risk (95% CI) | 1.0 | 0.9 (0.4–1.9) | 1.7 (0.9–3.3) | 2.4 (1.3–4.6) | 0.001 |
| HDL cholesterol | | | | | |
| Median — mg/dl | 34.5 | 44.5 | 54.9 | 68.5 | |
| Relative risk (95% CI) | 1.0 | 0.5 (0.3–0.8) | 0.5 (0.2–0.8) | 0.3 (0.2–0.6) | 0.001 |
| Apolipoprotein A-I | | | | | |
| Median — mg/dl | 127 | 152 | 176 | 212 | |
| Relative risk (95% CI) | 1.0 | 0.8 (0.4–1.4) | 0.4 (0.2–0.8) | 0.8 (0.4–1.4) | 0.1 |
| Apolipoprotein B-100 | | | | | |
| Median — mg/dl | 86 | 104 | 121 | 149 | |
| Relative risk (95% CI) | 1.0 | 1.1 (0.5–2.3) | 1.6 (0.8–3.3) | 3.4 (1.8–6.8) | <0.001 |
| Lp(a) lipoprotein | | | | | |
| Median — mg/liter | 16 | 55 | 107 | 329 | |
| Relative risk (95% CI) | 1.0 | 1.0 (0.5–1.9) | 1.1 (0.6–2.1) | 1.3 (0.7–2.4) | 0.4 |
| Ratio of total cholesterol to HDL cholesterol | | | | | |
| Median | 3.06 | 4.00 | 4.80 | 6.34 | |
| Relative risk (95% CI) | 1.0 | 0.8 (0.3–1.5) | 1.7 (0.9–3.4) | 3.4 (1.8–5.9) | <0.001 |
| Homocysteine | | | | | |
| Median — μmol/liter | 8.2 | 10.3 | 12.1 | 15.7 | |
| Relative risk (95% CI) | 1.0 | 1.1 (0.6–2.2) | 1.1 (0.5–2.1) | 2.0 (1.1–3.8) | 0.02 |

*P values were calculated by logistic-regression analyses. In all models, subjects were matched according to age and smoking status, and all models were adjusted for random assignment to aspirin or vitamin E. CI denotes confidence interval, LDL low-density lipoprotein, and HDL high-density lipoprotein. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

amyloid A was 0.81 ($P<0.001$). In contrast, correlations between markers of inflammation and lipid measures were low; less than 10 percent of the variance in any marker of inflammation was explained by any of the lipid measures.

To determine the independent predictive value of each of the 12 measures, we performed a series of logistic-regression analyses that simultaneously controlled for increasing quartiles of hs-CRP, serum amyloid A, sICAM-1, interleukin-6, homocysteine, and Lp(a) lipoprotein and the ratio of total cholesterol to HDL cholesterol (because of colinearity with this ratio, levels of apolipoprotein A-I, apolipoprotein B-100, and LDL cholesterol were not included in these analyses). As shown in Table 4, only the level of hs-CRP and the ratio of total cholesterol to HDL cholesterol were found to be independent predictors of risk in models in which women were matched for smoking status and age or in models that included further adjustments for body-mass index, hypertension, diabetes, and parental history of premature coronary artery disease. In similar models that were limited to markers of inflammation, hs-CRP remained an independent predictor of the risk of future cardiovascular events. In contrast, the beta coefficients associated with serum amyloid A, sICAM-1, and interleukin-6 decreased substantially and were no longer statistically significant in analyses that included control for the quartile of hs-CRP.

To explore whether any of the markers of inflam-

**TABLE 4.** ADJUSTED RELATIVE RISK OF CARDIOVASCULAR EVENTS ASSOCIATED WITH AN INCREASE OF ONE QUARTILE IN THE CONCENTRATION OF EACH PLASMA MARKER.*

| VARIABLE | ADJUSTED FOR OTHER PLASMA MARKERS | | ADJUSTED FOR OTHER PLASMA MARKERS AND RISK FACTORS† | |
|---|---|---|---|---|
| | RELATIVE RISK (95% CI) | P VALUE | RELATIVE RISK (95% CI) | P VALUE |
| High-sensitivity C-reactive protein | 1.4 (1.1–1.9) | 0.02 | 1.5 (1.1–2.1) | 0.02 |
| Serum amyloid A | 1.1 (0.8–1.4) | 0.5 | 1.1 (0.8–1.6) | 0.4 |
| Soluble intercellular adhesion molecule type 1 | 1.1 (0.9–1.4) | 0.4 | 1.1 (0.8–1.4) | 0.6 |
| Interleukin-6 | 0.9 (0.7–1.2) | 0.6 | 0.8 (0.6–1.1) | 0.2 |
| Homocysteine | 1.1 (0.9–1.4) | 0.2 | 1.1 (0.8–1.4) | 0.6 |
| Lp(a) lipoprotein | 1.1 (0.9–1.3) | 0.6 | 1.0 (0.8–1.2) | 0.8 |
| Ratio of total cholesterol to HDL cholesterol | 1.4 (1.1–1.7) | 0.01 | 1.4 (1.1–1.9) | 0.02 |

*In all models, subjects were matched according to age and smoking status, and all models were adjusted for random assignment to aspirin or vitamin E. CI denotes confidence interval, and HDL high-density lipoprotein.

†These models were adjusted for the following additional risk factors: body-mass index (the weight in kilograms divided by the square of the height in meters), a history of hypertension, a history of diabetes, and a parental history of myocardial infarction.

mation added to the predictive value of lipid-based screening, several additional analyses were performed. First, we computed the relative risk of cardiovascular events in analyses in which study participants were stratified into nine groups according to total cholesterol level as well as each marker of inflammation. As shown in Figure 1, for each marker of inflammation included in this analysis, the risk of cardiovascular events was lowest among women with low total cholesterol levels and low levels of the marker in question. In contrast, the risk tended to be highest among women with high total cholesterol levels and high levels of a marker of inflammation. However, even among the women with low total cholesterol levels, the risk of cardiovascular events was significantly higher among those with high levels of hs-CRP and serum amyloid A than among those with low levels of these markers (Fig. 1). These associations were also evident, but to a lesser extent, for interleukin-6 and sICAM-1. In all of the analyses, these additive effects were robust with respect to the choice of cutoff point and the choice of the lipid variable analyzed. For example, the addition of hs-CRP to lipid screening produced a significant and additive predictive effect when regression analyses were based on cutoff points for quartiles (rather than cutoff points for the division of the study group into thirds) and on analysis of the ratio of total cholesterol to HDL cholesterol (rather than on total cholesterol alone).

Second, likelihood-ratio tests were used to compare the fit of predictive models that were based on measurement of a marker of inflammation in combination with lipids to the fit of models based on lipid measurements alone. In these analyses, each of the markers of inflammation significantly improved the usefulness of lipid screening in predicting risk. For example, models including both hs-CRP and total cholesterol were significantly better in the prediction of the risk of cardiovascular events than were models including only total cholesterol ($P<0.001$). Likewise, models involving both hs-CRP and the ratio of total cholesterol to HDL cholesterol allowed significantly better prediction of risk than did models based solely on this lipid ratio alone ($P<0.001$). Similar additive effects were seen for serum amyloid A, sICAM-1, and interleukin-6 when these markers were added to models based on total cholesterol or the ratio of total cholesterol to HDL cholesterol alone ($P<0.01$ for all comparisons).

Third, as a measure of clinical usefulness, we computed the area under the receiver-operating-characteristic curve associated with risk-prediction models based on lipid screening alone and compared it with those based on a combination of lipids and markers of inflammation. In these analyses, the use of hs-CRP levels in addition to total cholesterol increased the area under the receiver-operating-characteristic curve from 0.59 to 0.66 ($P<0.001$) and in addition to the ratio of total cholesterol to HDL cholesterol increased the area under the curve from 0.64 to 0.68 ($P<0.001$). Similar effects were observed for analyses that included serum amyloid A, sICAM-1, and interleukin-6: the addition of these markers to screening based on total cholesterol increased the area under the curve from 0.59 to 0.63, 0.63, and 0.64, respectively ($P<0.003$ for all three comparisons). Use of the serum amyloid A level in addition to the ratio of total cholesterol to HDL cholesterol increased the area under the curve from 0.64 to 0.67 ($P=0.007$); the use of sICAM-1 in addition to this ratio led to a smaller change (area under the curve, 0.65; $P=0.01$), as did the use of interleukin-6 (area under the curve, 0.65; $P=0.01$).

Finally, to address the clinical observation that many persons with "safe" lipid levels nonetheless have cardiovascular events, we performed a subgroup analysis limited to women whose levels of LDL cholesterol were less than 130 mg per deciliter, the target level currently recommended for primary prevention of coronary heart disease by the National Cholesterol Education Program.[17] In this analysis, women with increased base-line levels of hs-CRP, serum amyloid A, interleukin-6, or sICAM-1 were found to be at increased risk for future cardiovascular events. This effect was strongest for hs-CRP and serum amyloid A. In this subgroup, the relative risks of cardiovascular events for women in the lowest to the highest quartiles of hs-CRP were 1.0, 2.4, 2.9, and 4.1 (95 percent confidence interval for women in the highest as compared with the lowest quartile, 1.7 to 11.3; $P=0.002$; P for trend across quartiles, 0.005). After adjustment

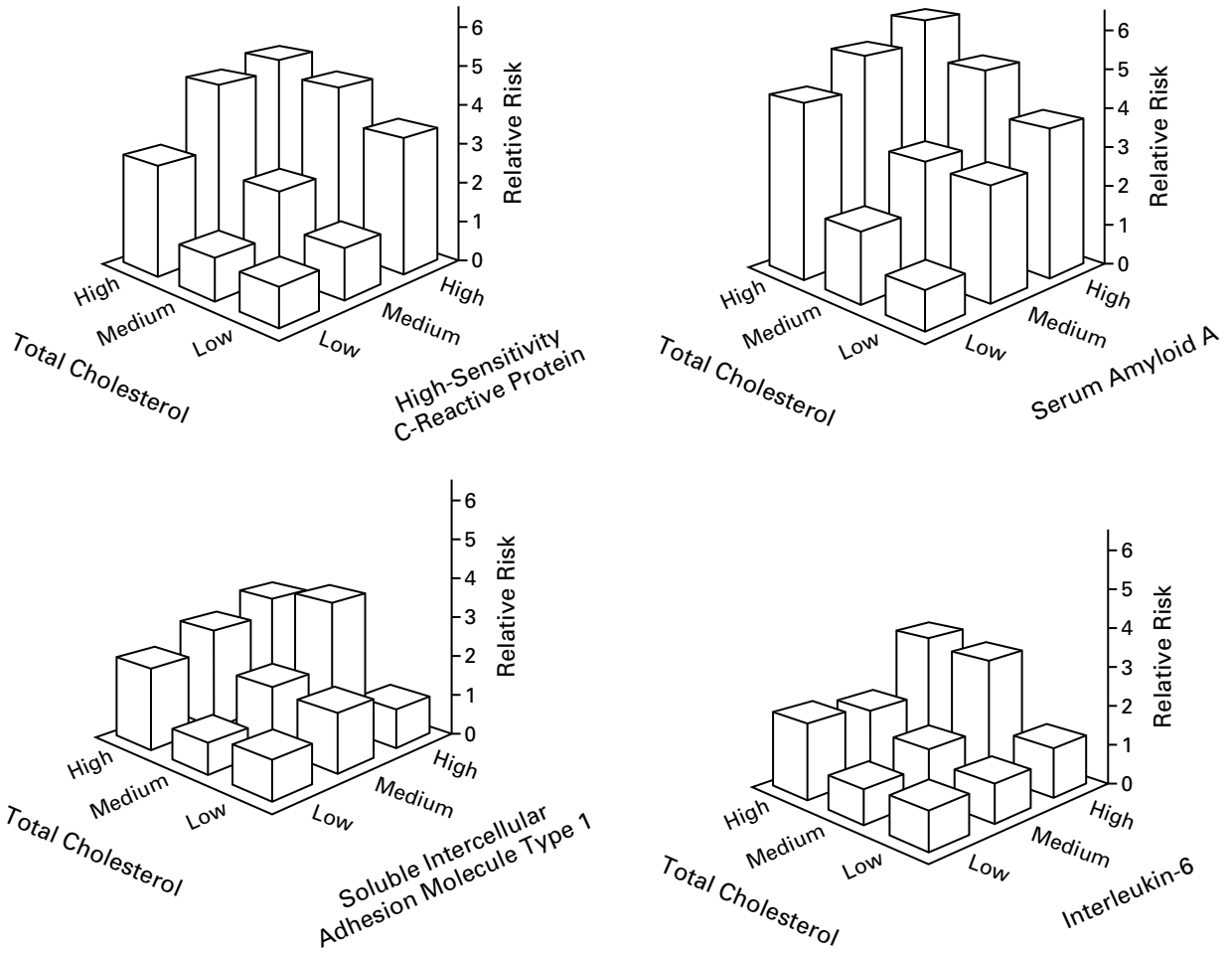


**Figure 1.** Relative Risk of Cardiovascular Events among Apparently Healthy Postmenopausal Women According to Base-Line Levels of Total Cholesterol and Markers of Inflammation.

Each marker of inflammation improved risk-prediction models based on lipid testing alone, an effect that was strongest for hs-CRP and serum amyloid A.

for body-mass index, the presence or absence of hypertension, diabetes, or a parental history of premature myocardial infarction, and the level of HDL cholesterol, the increased risk for women in the highest quartile of hs-CRP at base line remained statistically significant (relative risk, 3.1; 95 percent confidence interval, 1.1 to 8.3; P=0.03). Thus, even among women with "safe" levels of LDL cholesterol, the adjusted relative risk of cardiovascular events increased approximately 39 percent with each increasing quartile for hs-CRP (95 percent confidence interval, 13 to 89 percent; P=0.03). The mean LDL cholesterol level in this subgroup analysis was 104 mg per deciliter (2.7 mmol per liter).

## DISCUSSION

In this prospective study of apparently healthy postmenopausal women, four markers of inflammation — hs-CRP, serum amyloid A, interleukin-6, and sICAM-1 — were found to be significant predictors of the risk of future cardiovascular events. In addition, measurement of these markers increased the predictive value of models based only on standard lipid screening. Of the 12 plasma measures evaluated in this study, hs-CRP was the most significant predictor of the risk of cardiovascular events; when measured with a widely available, standardized commercial assay,[18] this marker distinguished between women at high risk and those at low risk, even in the subgroup of women with LDL cholesterol levels below 130 mg per deciliter (mean, 104 mg per deciliter), the target considered safe in the current guidelines of the National Cholesterol Education Program.[17]

The results of the current study have several important implications. First, the findings confirm that in women, markers of inflammation are important predictors of the risk of cardiovascular events. Previous data on this issue have been derived largely from

studies of middle-aged men.[4-11] Thus, from a pathophysiologic perspective, the current data support the hypothesis that atherosclerosis is, in part, an inflammatory disease.[3]

Second, because we used a commercially available assay to measure plasma hs-CRP,[18] our results provide clinically relevant confirmation of previous findings in this cohort, which were obtained with use of an experimental assay.[13] The commercial assay is inexpensive and can be used with standard hospital and outpatient laboratory equipment; thus, screening for this predictor of cardiovascular risk would be practical in many clinical settings.

Third, we believe the current results have public health implications both in terms of the prediction of the risk of cardiovascular events and in terms of the use of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase for primary prevention. Although the results of large-scale randomized trials have indicated that HMG-CoA reductase inhibition is effective even among persons at low-to-moderate risk as defined by standard lipid screening,[19,20] the large number of patients who would need to be treated and the high cost of this approach have limited the clinical application of those findings. Thus, our observation that measurement of markers of inflammation such as hs-CRP can significantly improve models for the prediction of cardiovascular risk may lead to better clinical identification of patients who might benefit from primary prevention and for whom the cost-to-benefit ratio for long-term use of statins would be improved. This issue is particularly intriguing because recent data from the Cholesterol and Recurrent Events trial indicate that long-term therapy with pravastatin significantly lowers plasma levels of hs-CRP[21] and that the efficacy of pravastatin in lowering the rate of cardiovascular events is greatest in those with increased levels of hs-CRP.[22] As in the current findings, which indicate that hs-CRP is a potent predictor of risk regardless of the LDL cholesterol level, data from the Cholesterol and Recurrent Events trial indicate that use of pravastatin resulted in decreased levels of hs-CRP in a manner largely independent of LDL cholesterol.[21]

Several limitations of these analyses merit consideration. First, our cohort comprised apparently healthy postmenopausal women, and thus the results may not apply to younger women, who may also be at increased risk for cardiovascular events. Second, we measured each marker of inflammation at study entry and thus could not evaluate the effects of changes in the levels of these markers over time. However, follow-up studies have found that levels of hs-CRP are stable over long periods, as long as measurements are not made within two to three weeks of an acute infection.[21,23] Moreover, with respect to the current results, variation over time in levels of these markers and regression dilution bias would tend, if anything, to lead to an underestimation of net effects. Finally, although base-line levels of several markers of inflammation were greater than normal among women at risk for future cardiovascular events, the mechanisms underlying these elevations remain uncertain. In this study, we did not find significant associations between cardiovascular risk and titers of IgG antibodies against *Chlamydia pneumoniae, Helicobacter pylori,* herpes simplex virus, or cytomegalovirus or between titers of these antibodies and plasma levels of hs-CRP.[24] On the other hand, markers of inflammation, including hs-CRP, interleukin-6, and interleukin-1–receptor antagonist,[25-31] have proved to have predictive value among persons with unstable angina or acute coronary syndromes. Thus, it is also possible that the inflammation that we detected in apparently healthy women who were at risk for future cardiovascular events may be an indirect marker of an enhanced cytokine response to a variety of inflammatory stimuli that ultimately prove critical at the time of acute plaque rupture.[32]

In conclusion, in this prospective evaluation of 12 plasma variables, hs-CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events. As in previous population-based epidemiologic studies, half of all cardiovascular events in our cohort occurred among women without overt hyperlipidemia. Thus, these data raise the possibility that the addition of hs-CRP to standard lipid screening will generate an improved method for identifying persons at high risk for future cardiovascular events, who would thus be candidates for primary-prevention interventions such as the use of HMG-CoA reductase inhibitors.

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Drs. Ridker and Hennekens are named as coinventors on a pending patent application filed by Brigham and Women's Hospital on the use of markers of inflammation in coronary artery disease.

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**CORRECTION**

## C-Reactive Protein in the Prediction of Cardiovascular Disease

*To the Editor:* Ridker et al. provided a stimulating article on inflammatory markers and cardiovascular disease in women (March 23 issue).[1] Not surprisingly, the popular press picked up on the article and gave their findings prominent coverage. It is not clear that Ridker et al. wanted this to happen. Their findings are cast in terms of relative risk only, not in terms of traditional predictive value; it is the latter that is more relevant to the practicing physician.[2] That is, we learn that subjects in the highest quartile for high-sensitivity C-reactive protein (hs-CRP), relative to those in the lowest quartile, had a 4.4-fold risk of cardiovascular events. However, the overall risk was just 0.4 percent (122 events in 28,263 subjects over a period of three years). We suspect that the positive predictive value (the proportion of all subjects with ``elevated´´ levels of hs-CRP who had cardiac events) in this population was low.

We were unable to calculate the conventional predictive values from the data supplied in the article. It would be instructive if the authors provided the predictive values so that readers could determine whether this new test is genuinely ready for ``prime-time´´ screening.

Even though this test performed better than measurements of conventional lipid markers such as low-density lipoprotein (LDL) cholesterol in this population (at least in terms of relative risk), there are other relevant data about LDL cholesterol that are lacking for hs-CRP. For example, we know that lowering LDL cholesterol levels has beneficial effects,[3] we have effective methods to lower LDL cholesterol levels, and we have data on the cost effectiveness of such strategies.[4]

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The authors reply:

*To the Editor:* In our study of inflammatory and lipid markers we used a matched, nested case–control analysis that allowed direct comparison of the magnitude of risk associated with various cardiovascular risk factors after age and smoking status were taken into account. Of the 12 factors evaluated — which included LDL cholesterol, high-density lipoprotein (HDL) cholesterol, Lp(a) lipoprotein, and homocysteine — hs-CRP was the strongest predictor of future cardiovascular events. Moreover, hs-CRP levels were predictive of the risk of cardiovascular events among study participants with low levels of LDL cholesterol; these data underscore the importance of the inflammatory process in atherothrombosis.

Our matched, nested case–control study was designed to maximize biologic validity. It is not, however, conducive to calculating absolute risks. We thus concur with Horowitz and Beckwith that generalizing our results to other populations must be done with caution and that studies addressing absolute risks are needed. We further concur that the reduction of lipid levels remains a fundamentally important method to reduce cardiovascular risk. At the same time, since half of all heart attacks and strokes occur among apparently healthy men and women without overt hyperlipidemia, we believe it important for clinicians to consider emerging biologic data that go beyond the use of cholesterol screening as the sole method of assessing cardiovascular risk. With regard to hs-CRP, several large-scale studies in the United States[1,2,3] and Europe[4,5] have now demonstrated the potential importance of this inflammatory marker in the detection of cardiovascular risk.

Finally, we wish to correct an error in the last sentence of the Results section of our abstract. As described in the text and in Table 4 of our article, our multivariate analysis was performed on a per-quartile basis. Thus, this sentence should read, ``In multivariate analyses, the only plasma markers that independently predicted risk were hs-CRP (increase in relative risk per quartile, 1.5; 95 percent confidence interval, 1.1 to 2.1) and the ratio of total cholesterol to HDL cholesterol (increase in relative risk per quartile, 1.4; 95 percent confidence interval, 1.1 to 1.9).´´

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# INFLAMMATION, ASPIRIN, AND THE RISK OF CARDIOVASCULAR DISEASE IN APPARENTLY HEALTHY MEN

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## ABSTRACT

***Background*** Inflammation may be important in the pathogenesis of atherothrombosis. We studied whether inflammation increases the risk of a first thrombotic event and whether treatment with aspirin decreases the risk.

***Methods*** We measured plasma C-reactive protein, a marker for systemic inflammation, in 543 apparently healthy men participating in the Physicians' Health Study in whom myocardial infarction, stroke, or venous thrombosis subsequently developed, and in 543 study participants who did not report vascular disease during a follow-up period exceeding eight years. Subjects were randomly assigned to receive aspirin or placebo at the beginning of the trial.

***Results*** Base-line plasma C-reactive protein concentrations were higher among men who went on to have myocardial infarction (1.51 vs. 1.13 mg per liter, $P<0.001$) or ischemic stroke (1.38 vs. 1.13 mg per liter, $P=0.02$), but not venous thrombosis (1.26 vs. 1.13 mg per liter, $P=0.34$), than among men without vascular events. The men in the quartile with the highest C-reactive protein values had three times the risk of myocardial infarction (relative risk, 2.9; $P<0.001$) and two times the risk of ischemic stroke (relative risk, 1.9; $P=0.02$) of the men in the lowest quartile. Risks were stable over long periods, were not modified by smoking, and were independent of other lipid-related and non–lipid-related risk factors. The use of aspirin was associated with significant reductions in the risk of myocardial infarction (55.7 percent reduction, $P=0.02$) among men in the highest quartile but with only small, nonsignificant reductions among those in the lowest quartile (13.9 percent, $P=0.77$).

***Conclusions*** The base-line plasma concentration of C-reactive protein predicts the risk of future myocardial infarction and stroke. Moreover, the reduction associated with the use of aspirin in the risk of a first myocardial infarction appears to be directly related to the level of C-reactive protein, raising the possibility that antiinflammatory agents may have clinical benefits in preventing cardiovascular disease. (N Engl J Med 1997;336:973-9.)



THROMBUS formation is the proximate cause of myocardial infarction, but atherosclerosis, the chief underlying cause, is a chronic disease that progresses over decades of life.[1] Laboratory and pathological data support the idea that inflammation has a role in both the initiation and the progression of atherosclerosis, and antiinflammatory agents may have a role in the prevention of cardiovascular disease.[2-5] However, there are few data to indicate whether inflammation increases the risk of first myocardial infarction, stroke, and venous thrombosis or whether antiinflammatory therapy decreases that risk.

C-reactive protein is an acute-phase reactant that is a marker for underlying systemic inflammation. Elevated plasma concentrations of C-reactive protein have been reported in patients with acute ischemia[6] or myocardial infarction[7,8] and have been found to predict recurrent ischemia among those hospitalized with unstable angina.[9] C-reactive protein is also associated with a risk of myocardial infarction among patients with angina pectoris[10] and with a risk of fatal coronary disease among smokers with multiple risk factors for atherosclerosis.[11] However, since concentrations of C-reactive protein and other acute-phase reactants increase after acute ischemia[6] and are directly related to cigarette smoking,[11,12] it has been uncertain whether associations observed in previous studies of acutely ill patients[9] or high-risk popula-

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tions[10,11] are causal or are due to short-term inflammatory changes or to interrelations with other risk factors, in particular smoking and hyperlipidemia.

To address these issues, we measured base-line plasma C-reactive protein concentrations in 1086 apparently healthy men participating in the Physicians' Health Study[13,14]; myocardial infarction, stroke, or venous thrombosis subsequently developed in 543. We hypothesized a priori that levels of C-reactive protein would predict the risk of myocardial infarction and stroke but not of venous thrombosis — an occlusive vascular disease generally not associated with chronic atherosclerosis. After providing base-line blood samples, study participants were randomly assigned to receive aspirin or placebo. Thus, we had the unique opportunity to evaluate directly whether aspirin, an agent with both antiplatelet and antiinflammatory properties, might modify any relation between C-reactive protein and the risk of first myocardial infarction.

## METHODS

### Study Population and Collection of Plasma Samples

The Physicians' Health Study was a randomized, double-blind, placebo-controlled two-by-two factorial trial of aspirin and beta carotene in the primary prevention of cardiovascular disease and cancer. A total of 22,071 U.S. male physicians 40 to 84 years of age in 1982, with no history of myocardial infarction, stroke, transient ischemic attack, or cancer, were assigned to one of four treatments: 325 mg of aspirin on alternate days (Bufferin, provided by Bristol-Myers), 50 mg of beta carotene on alternate days (Lurotin, provided by BASF Corporation), both, or neither. The aspirin component of the study was terminated early, on January 25, 1988, primarily because of a statistically extreme 44 percent reduction in the risk of a first infarction in the aspirin group.[13] The beta carotene component continued until the study's scheduled termination on December 31, 1995.[14]

Before randomization, between August 1982 and December 1984, potential participants were asked to provide base-line blood samples during a 16-week run-in period during which all subjects were given aspirin and none received placebo. Blood-collection kits, including EDTA Vacutainer tubes, were sent to participants with instructions for taking blood. Participants were asked to have their blood drawn into the EDTA tubes, centrifuge the tubes, and return the plasma (accompanied by a cold pack provided to participants) by overnight courier. The specimens were then divided into aliquots and stored at −80°C. Of the 22,071 participants in the Physicians' Health Study, 14,916 (68 percent) provided base-line plasma samples. Over the 14 years of the trial, no specimen inadvertently thawed during storage.

### Confirmation of End Points and Selection of Controls

We requested hospital records (and for fatal events, death certificates and autopsy reports) for all reported cases of myocardial infarction, stroke, and venous thrombosis. The records were reviewed by a committee of physicians using standardized criteria to confirm or refute reported events. Reviewers of end points were unaware of treatment assignments.

Reported myocardial infarction was confirmed if its symptoms met World Health Organization (WHO) criteria and it was associated with either elevated plasma concentrations of enzymes or characteristic electrocardiographic changes. Silent myocardial infarctions were not included, since they could not be dated accurately. Deaths due to coronary disease were confirmed on the basis of autopsy reports, symptoms, circumstances of death, and a history of coronary disease. Reported stroke was confirmed on the basis of medical records showing a neurologic deficit of sudden or rapid onset that persisted for more than 24 hours or until death. Strokes were classified as ischemic or hemorrhagic. Computed tomographic scans were available for more than 95 percent of the confirmed strokes. Reported deep venous thrombosis was confirmed by the documentation of a positive venography study or a positive ultrasound study; deep venous thromboses documented only by impedance plethysmography or Doppler examination without ultrasound were not considered confirmed. Reported pulmonary embolism was confirmed by a positive angiogram or a completed ventilation-perfusion scan demonstrating at least two segmental perfusion defects with normal ventilation.

Each participant who provided an adequate base-line plasma sample and had a confirmed myocardial infarction, stroke, or venous thrombosis after randomization was matched with one control. Controls were participating physicians who provided base-line plasma samples and reported no cardiovascular disease at the time the patient reported his event. Controls were selected randomly from among study participants who met the matching criteria of age (±1 year), smoking status (smoking currently, smoked in the past, or never smoked), and length of time since randomization (in 6-month intervals). Using these methods, we evaluated 543 patients and 543 controls in this prospective, nested, case–control study.

### Laboratory Analysis

For each patient and control, plasma collected and stored at base line was thawed and assayed for C-reactive protein by enzyme-linked immunosorbent assay (ELISA) based on purified protein and polyclonal anti–C-reactive protein antibodies (Calbiochem).[15] Antibodies were used to coat microtiter-plate wells, and biotinylated C-reactive protein, together with the patient's plasma, was diluted 1:700 in assay buffer (phosphate-buffered saline with 0.1 percent Tween 20 and 1 percent bovine serum albumin). The excess was then washed off and the amount of biotinylated protein estimated by the addition of avidin–peroxidase (Vectastain, Vector Laboratories). Purified C-reactive protein was used as the standard, with protein concentrations as determined by the manufacturer. The C-reactive protein assay was standardized according to the WHO First International Reference Standard and had a sensitivity of 0.08 μg per microliter, with a standard reference range of between 0.5 and 2.5 mg per liter. Methods used to measure plasma total and high-density lipoprotein (HDL) cholesterol, triglyceride, lipoprotein(a), total homocysteine, fibrinogen, D-dimer, and endogenous tissue plasminogen activator (t-PA) antigen have been described elsewhere.[16-20]

Blood specimens were analyzed in blinded pairs, with the position of the patient's specimen varied at random within the pairs to reduce the possibility of systematic bias and decrease interassay variability. The mean coefficient of variation for C-reactive protein across assay runs was 4.2 percent.

### Statistical Analysis

Means or proportions for base-line risk factors were calculated for patients and controls. The significance of any difference in means was tested by using Student's t-test, and the significance of any differences in proportions was tested by using the chi-square statistic. Because C-reactive protein values are skewed, median concentrations were computed and the significance of any differences in median values between patients and controls was assessed by using Wilcoxon's rank-sum test. Geometric mean concentrations of C-reactive protein were also computed after log transformation that resulted in nearly normal distribution. We used tests for trend to assess any relation of increasing C-reactive protein values with the risk of future vascular disease after dividing the sample into quartiles defined by the distribution of the control values. We obtained adjusted estimates by using conditional logistic-regression models that accounted for the matching variables and controlled for the random treatment assignment,

body-mass index, diabetes, history of hypertension, and parental history of coronary artery disease. Similar models were employed to adjust for measured base-line plasma concentrations of total and HDL cholesterol, triglyceride, lipoprotein(a), t-PA antigen, fibrinogen, D-dimer, and homocysteine. To evaluate whether aspirin affected these relations, analyses were repeated for all cases of myocardial infarction occurring on or before January 25, 1988 — the date when randomized aspirin assignment was terminated. All P values are two-tailed, and confidence intervals were calculated at the 95 percent level.

## RESULTS

Table 1 shows the base-line characteristics of the study participants. As expected, those in whom myocardial infarction subsequently developed were more likely than those who remained free of vascular disease to have a history of hypertension or hyperlipidemia or a parental history of coronary artery disease. Similarly, those in whom stroke subsequently developed were more likely to be hypertensive. Because of the matching, patients and controls were similar in age and history of smoking.

Geometric mean and median plasma concentrations of C-reactive protein at base line were significantly higher among those in whom any vascular event subsequently developed than among those who remained free of vascular disease ($P<0.001$). The difference between patients and controls was greatest for those in whom myocardial infarction subsequently developed (1.51 vs. 1.13 mg per liter, $P<0.001$), although differences were also significant for stroke ($P=0.03$), particularly ischemic stroke ($P=0.02$). In contrast, concentrations of C-reactive protein were not significantly higher among those in whom venous thrombosis subsequently developed ($P=0.34$) (Table 2).

The relative risk of first myocardial infarction increased significantly with each increasing quartile of base-line concentrations of C-reactive protein (P for trend across quartiles, $<0.001$), in such a way that the men in the highest quartile had a risk of future myocardial infarction almost three times that among those in the lowest quartile (relative risk, 2.9; 95 percent confidence interval, 1.8 to 4.6; $P<0.001$) (Table 3). Similarly, men with the highest base-line C-reactive protein values had twice the risk of future ischemic stroke (relative risk, 1.9; 95 percent confidence interval, 1.1 to 3.3; $P=0.02$). No significant associations were observed for venous thrombosis. The findings were similar in analyses limited to nonfatal events.

To evaluate whether increased base-line C-reactive protein values were associated with early rather than late thrombosis, we stratified the analysis of myocardial infarction according to the number of years of follow-up. The relative risk of future myocardial infarction that was associated with the highest quartile of C-reactive protein (as compared with the lowest quartile) ranged from 2.4 for events occurring in the first two years of follow-up to 3.2 for events occurring six or more years into follow-up (Table 4). Similarly, the relative risk of future myocardial infarction that was associated with a one-quartile change in the C-reactive protein concentration was stable over long periods (Fig. 1).

Smokers had significantly higher median concentrations of C-reactive protein than nonsmokers (2.20 vs. 1.19 mg per liter, $P<0.001$). By matching patients and controls for smoking status, we minimized the potential for confounding by smoking. To assess for effect modification, however, we repeated the analyses, limiting the cohort to nonsmokers. As Table 3 also shows, the relative risk of future myocardial infarction among nonsmokers increased sig-

**TABLE 1.** BASE-LINE CHARACTERISTICS OF THE STUDY PARTICIPANTS.

| CHARACTERISTIC | CARDIOVASCULAR DISEASE DURING FOLLOW-UP* | | | | |
|---|---|---|---|---|---|
| | NONE (N=543) | ANY (N=543) | MYOCARDIAL INFARCTION (N=246) | STROKE (N=196) | VENOUS THROMBOSIS (N=101) |
| Age (yr) | 59±9.1 | 59±9.2 | 58±8.6 | 62±9.1 | 57±9.4 |
| Smoking status (%) | | | | | |
| Never smoked | 44 | 44 | 45 | 42 | 50 |
| Smoked in the past | 41 | 41 | 40 | 40 | 44 |
| Currently a smoker | 15 | 15 | 15 | 18 | 6 |
| Diabetes (%) | 4 | 7 | 5 | 12 | 2 |
| Body-mass index† | 25±2.8 | 26±3.2 | 26±3.3 | 25±3.2 | 26±2.9 |
| History of high plasma cholesterol (%) | 9 | 13 | 17 | 10 | 7 |
| History of hypertension (%) | 16 | 29 | 27 | 35 | 20 |
| Parental history of coronary artery disease (%) | 10 | 13 | 17 | 11 | 8 |

*Plus–minus values are means ±SD.

†The body-mass index is the weight in kilograms divided by the square of the height in meters.

**TABLE 2.** BASE-LINE PLASMA CONCENTRATIONS OF C-REACTIVE PROTEIN IN STUDY PARTICIPANTS WHO REMAINED FREE OF VASCULAR DISEASE DURING FOLLOW-UP (CONTROLS) AND IN THOSE IN WHOM MYOCARDIAL INFARCTION, STROKE, OR VENOUS THROMBOSIS DEVELOPED (PATIENTS).

| CARDIOVASCULAR DISEASE DURING FOLLOW-UP | PLASMA C-REACTIVE PROTEIN | | | |
|---|---|---|---|---|
| | GEOMETRIC MEAN | P VALUE | MEDIAN | P VALUE |
| | mg/liter | | mg/liter | |
| None (n=543) | 1.10 | — | 1.13 | — |
| Any vascular event (n=543) | 1.37 | <0.001 | 1.40 | <0.001 |
| Myocardial infarction (n=246) | 1.48 | <0.001 | 1.51 | <0.001 |
| Any stroke (n=196) | 1.30 | 0.03 | 1.36 | 0.03 |
| Ischemic stroke (n=154) | 1.36 | 0.01 | 1.38 | 0.02 |
| Venous thrombosis (n=101) | 1.24 | 0.22 | 1.26 | 0.34 |

**TABLE 3.** RELATIVE RISK OF FUTURE MYOCARDIAL INFARCTION, STROKE, AND VENOUS THROMBOSIS ACCORDING TO BASE-LINE PLASMA CONCENTRATIONS OF C-REACTIVE PROTEIN.

| VASCULAR EVENT* | QUARTILE OF C-REACTIVE PROTEIN CONCENTRATION (mg/liter) | | | | P FOR TREND |
|---|---|---|---|---|---|
| | ≤0.55 | 0.56–1.14 | 1.15–2.10 | ≥2.11 | |
| Myocardial infarction (total cohort) | | | | | |
| Relative risk | 1.0 | 1.7 | 2.6 | 2.9 | <0.001 |
| 95% CI | — | 1.1–2.9 | 1.6–4.3 | 1.8–4.6 | |
| P value | — | 0.03 | <0.001 | <0.001 | |
| Myocardial infarction (nonsmokers) | | | | | |
| Relative risk | 1.0 | 1.7 | 2.5 | 2.8 | <0.001 |
| 95% CI | — | 1.0–2.8 | 1.5–4.1 | 1.7–4.7 | |
| P value | — | 0.06 | <0.001 | <0.001 | |
| Ischemic stroke | | | | | |
| Relative risk | 1.0 | 1.7 | 1.9 | 1.9 | 0.03 |
| 95% CI | — | 0.9–2.9 | 1.1–3.2 | 1.1–3.3 | |
| P value | — | 0.07 | 0.02 | 0.02 | |
| Venous thrombosis | | | | | |
| Relative risk | 1.0 | 1.1 | 1.2 | 1.3 | 0.38 |
| 95% CI | — | 0.6–2.0 | 0.7–2.3 | 0.7–2.4 | |
| P value | — | 0.78 | 0.51 | 0.42 | |

*CI denotes confidence interval.

**TABLE 4.** RELATIVE RISK OF FIRST MYOCARDIAL INFARCTION ASSOCIATED WITH THE HIGHEST QUARTILE OF BASE-LINE PLASMA C-REACTIVE PROTEIN CONCENTRATIONS AS COMPARED WITH THE LOWEST QUARTILE, ACCORDING TO THE YEAR OF STUDY FOLLOW-UP.

| GROUP* | FOLLOW-UP (YR) | | | |
|---|---|---|---|---|
| | 0–2 | 2–4 | 4–6 | ≥6 |
| Total cohort | | | | |
| Relative risk | 2.4 | 2.9 | 2.8 | 3.2 |
| 95% CI | 0.9–6.8 | 1.1–7.6 | 1.1–6.9 | 1.2–8.5 |
| P value | 0.09 | 0.03 | 0.03 | 0.02 |
| Nonsmokers | | | | |
| Relative risk | 2.8 | 2.9 | 2.7 | 2.9 |
| 95% CI | 0.9–8.7 | 1.0–8.3 | 1.0–7.0 | 1.1–8.2 |
| P value | 0.07 | 0.05 | 0.05 | 0.04 |

*CI denotes confidence interval.

nificantly with each increasing quartile of C-reactive protein (P for trend, <0.001). Similarly, the long-term effects of the concentration of C-reactive protein on the risk of myocardial infarction were virtually identical among nonsmokers (Table 4). Moreover, the relation between the concentration of C-reactive protein and myocardial infarction was not significantly altered in analyses that adjusted for body-mass index; the presence or absence of diabetes, hypertension, or a family history of premature coronary artery disease; and the plasma concentrations of total cholesterol, HDL cholesterol, triglycerides, lipoprotein(a), t-PA antigen, D-dimer, fibrinogen, or homocysteine (Table 5).

Finally, to assess whether the beneficial effect of aspirin on the risk of myocardial infarction varied according to the base-line level of C-reactive protein, we repeated these analyses for events occurring before January 25, 1988, the date when randomized aspirin treatment was terminated.

The risk of future myocardial infarction increased with each increasing quartile of C-reactive protein values for men randomly assigned to either aspirin or placebo, and the rates of myocardial infarction were lower in the aspirin group for all quartiles of C-reactive protein (Fig. 2). However, the magnitude of the beneficial effect of aspirin in preventing myocardial infarction was directly related to base-line levels of C-reactive protein. Specifically, randomized aspirin assignment was associated with a large and statistically significant reduction in the risk of myocardial infarction among men with base-line levels of C-reactive protein in the highest quartile (risk reduction, 55.7 percent; P=0.02). Among those with base-line levels of C-reactive protein in the lowest quartile, however, the reduction in risk associated with aspirin was far smaller and no longer statistically significant (risk reduction, 13.9 percent; P=0.77). These effects were linear across quartiles, so that the apparent benefit of aspirin diminished in magnitude with each decreasing quartile of inflammatory risk (Fig. 2). This finding remained essentially unchanged after further adjustment for other coronary risk factors, and the interaction between assignment to the aspirin group and base-line levels of C-reactive protein (treated as a log-transformed continuous variable) was statistically significant (P=0.048).

## DISCUSSION

These prospective data indicate that the base-line plasma concentration of C-reactive protein in apparently healthy men can predict the risk of first myocardial infarction and ischemic stroke. In addition, the risk of arterial thrombosis associated with the level of C-reactive protein was stable over long periods and was not modified by other factors, including smoking status, body-mass index, blood pressure, or the plasma concentration of total or HDL cholesterol, tri-

glyceride, lipoprotein(a), t-PA antigen, D-dimer, fibrinogen, or homocysteine. In contrast, the benefits of aspirin in reducing the risk of a first myocardial infarction diminished significantly with decreasing concentrations of C-reactive protein — an intriguing finding, since this substance has antiinflammatory as well as antiplatelet properties. Finally, there was no significant association for venous thromboembolism, suggesting that the relation of inflammation to vascular risk may be limited to the arterial circulation.

Because blood samples were collected at base line, we can exclude the possibility that acute ischemia affected levels of C-reactive protein. Furthermore, the statistically significant associations observed were present among nonsmokers, indicating that the effect of C-reactive protein on vascular risk is not simply the result of cigarette smoking.[11,12] Thus, our prospective data relating base-line levels of C-reactive protein to future risks of myocardial infarction and stroke among apparently healthy men greatly

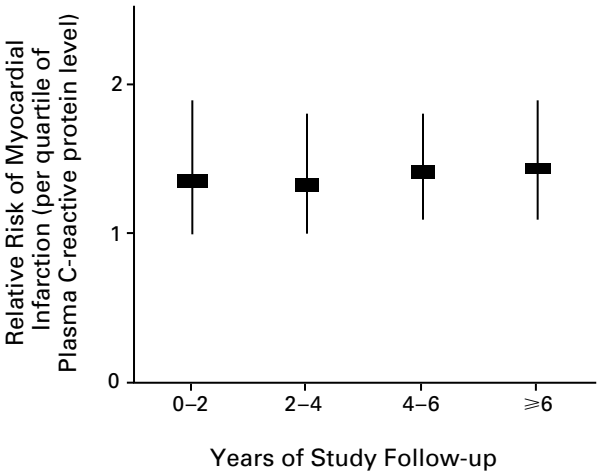


**Figure 1.** Relative Risk (and 95 Percent Confidence Intervals) of a First Myocardial Infarction Associated with Each Increasing Quartile of Base-Line C-Reactive Protein Values, According to the Year of Study Follow-up.

**TABLE 5.** RELATIVE RISK OF FUTURE MYOCARDIAL INFARCTION, ACCORDING TO BASE-LINE PLASMA CONCENTRATIONS OF C-REACTIVE PROTEIN, ADJUSTED FOR LIPID AND NONLIPID VARIABLES.*

| VARIABLES ADJUSTED FOR | QUARTILE OF C-REACTIVE PROTEIN CONCENTRATION (mg/liter) | | | | P FOR TREND |
|---|---|---|---|---|---|
| | ⩽0.55 | 0.56–1.14 | 1.15–2.10 | ⩾2.11 | |
| Total and HDL cholesterol | | | | | |
| Adjusted relative risk | 1.0 | 1.8 | 2.2 | 2.3 | 0.002 |
| 95% CI | — | 1.0–3.1 | 1.3–3.7 | 1.4–3.9 | |
| P value | — | 0.05 | 0.004 | 0.002 | |
| Triglycerides | | | | | |
| Adjusted relative risk | 1.0 | 1.8 | 2.1 | 2.8 | <0.001 |
| 95% CI | — | 1.0–3.2 | 1.2–3.7 | 1.6–4.9 | |
| P value | — | 0.06 | 0.008 | <0.001 | |
| Lipoprotein(a) | | | | | |
| Adjusted relative risk | 1.0 | 2.0 | 2.5 | 2.5 | <0.001 |
| 95% CI | — | 1.2–3.4 | 1.5–4.2 | 1.5–4.2 | |
| P value | — | 0.01 | <0.001 | <0.001 | |
| t-PA antigen | | | | | |
| Adjusted relative risk | 1.0 | 1.7 | 1.9 | 2.9 | 0.002 |
| 95% CI | — | 0.9–3.4 | 1.0–3.6 | 1.5–5.6 | |
| P value | — | 0.13 | 0.06 | 0.002 | |
| Total homocysteine | | | | | |
| Adjusted relative risk | 1.0 | 1.8 | 2.9 | 3.6 | <0.001 |
| 95% CI | — | 1.1–3.1 | 1.7–4.8 | 2.1–5.9 | |
| P value | — | 0.02 | <0.001 | <0.001 | |
| D-Dimer | | | | | |
| Adjusted relative risk | 1.0 | 2.2 | 2.4 | 2.7 | 0.001 |
| 95% CI | — | 1.2–4.1 | 1.3–4.2 | 1.5–4.7 | |
| P value | — | 0.007 | 0.003 | <0.001 | |
| Fibrinogen | | | | | |
| Adjusted relative risk | 1.0 | 2.2 | 2.2 | 2.9 | 0.01 |
| 95% CI | — | 1.1–4.7 | 1.0–4.4 | 1.4–5.9 | |
| P value | — | 0.04 | 0.04 | 0.005 | |
| Body-mass index, diabetes, history of hypertension, and family history of coronary artery disease | | | | | |
| Adjusted relative risk | 1.0 | 1.5 | 2.4 | 2.6 | <0.001 |
| 95% CI | — | 0.9–2.5 | 1.5–4.0 | 1.6–4.4 | |
| P value | — | 0.14 | <0.001 | <0.001 | |

*All models were further adjusted for random assignment of patients to receive aspirin and beta carotene. CI denotes confidence interval.

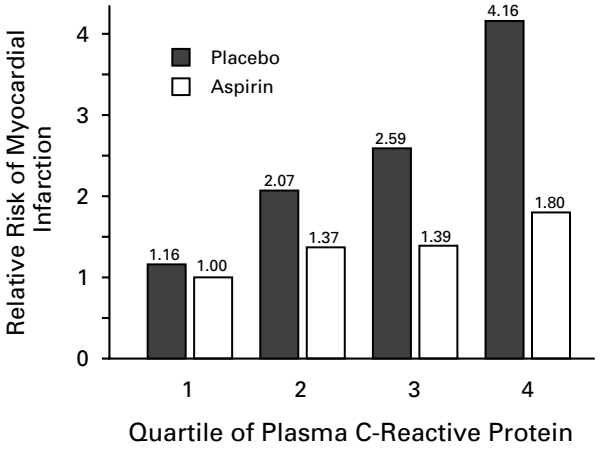


**Figure 2.** Relative Risk of a First Myocardial Infarction Associated with Base-Line Plasma Concentrations of C-Reactive Protein, Stratified According to Randomized Assignment to Aspirin or Placebo Therapy.

Analyses are limited to events occurring before the unblinding of the aspirin component of the Physicians' Health Study. The reduction in the risk of myocardial infarction associated with the use of aspirin was 13.9 percent in the first (lowest) quartile of C-reactive protein values, 33.4 percent in the second quartile, 46.3 percent in the third quartile, and 55.7 percent in the fourth (highest) quartile.

extend previous observations from studies of acutely ill patients,[9] patients with symptomatic coronary disease,[10] or those at high risk partly because of cigarette smoking.[11] Moreover, in these data, the effects of C-reactive protein were independent of a large number of lipid-related and non–lipid-related risk factors.

The mechanism that relates the level of C-reactive protein to atherothrombosis is unclear. Previous infection with *Chlamydia pneumoniae, Helicobacter pylori,* herpes simplex virus, or cytomegalovirus may be a source of the chronic inflammation detected by C-reactive protein.[21-27] It is also possible that C-reactive protein is a surrogate for interleukin-6,[28] a cellular cytokine associated with the recruitment of macrophages and monocytes into atherosclerotic plaques.[29] In addition, C-reactive protein can induce monocytes to express tissue factor, a membrane glycoprotein important in initiating coagulation.[30] Finally, it had been hypothesized that bronchial inflammation due to smoking was responsible for associations seen in previous studies relating C-reactive protein to vascular risk.[11] In this regard, our observation that the effect of C-reactive protein is present among nonsmokers makes bronchial inflammation a less likely mechanism. Furthermore, the finding that the effects are stable over long periods suggests that short-term effects on clotting are unlikely.

Our data regarding the interrelation of C-reactive protein and aspirin merit careful consideration. In the Physicians' Health Study, aspirin reduced the risk of a first myocardial infarction by 44 percent.[13] The present findings indicate that the effect of aspirin in preventing a first myocardial infarction was greatest among the men with the highest base-line C-reactive protein concentrations and that the benefit diminished significantly with decreasing concentrations of this inflammatory marker. Thus, although the antiplatelet effects of aspirin may be modified by underlying inflammation, these data also suggest the possibility that the benefit of aspirin may have been due, at least in part, to antiinflammatory effects.[31] Alternatively, patients with large inflammatory burdens may have a distinct vascular mechanism leading to thrombosis that is affected differently by aspirin therapy. For example, the protective effect of aspirin may differ in the setting of plaque rupture as compared with focal endothelial erosion.[32,33]

The potential limitations of these data also merit consideration. First, our analyses are based on a single base-line determination that may not accurately reflect inflammatory status over long periods. Furthermore, although coefficients of variation were low, misclassification due to laboratory error cannot be ruled out. It is important to note, however, that neither of these sources of variability can account for the observed associations, since any random misclassification would bias results toward the null hypothesis. Since our study was limited to measures of C-reactive protein, other prospective studies evaluating specific cytokines, cellular adhesion molecules, and chronic infectious agents will be required to further elucidate the role of inflammation in the initiation and progression of atherosclerosis.

We draw four main conclusions from these data. First, among apparently healthy men, the base-line level of inflammation as assessed by the plasma concentration of C-reactive protein predicts the risk of a first myocardial infarction and ischemic stroke, independently of other risk factors. Second, the base-line concentration of C-reactive protein is not associated with the risk of venous thrombosis, a vascular event generally not associated with atherosclerosis. Third, C-reactive protein is not simply a short-term marker of risk, as has previously been demonstrated in patients with unstable angina,[9] but is also a long-term marker of risk, even for events occurring six or more years later. This observation suggests that the effects of inflammation are probably mediated through a chronic process and excludes the possibility that undetected acute illness at base line is responsible for the observed effects. Finally, the benefits of aspirin appear to be modified by underlying inflammation — an observation that raises the possibility of antiinflammatory as well as antiplatelet effects of this agent. The latter observation also suggests the possibility that other antiinflammatory agents may have a role in preventing cardiovascular

disease. Moreover, these data suggest that inflammatory markers such as C-reactive protein may provide a method of identifying people for whom aspirin is likely to be more or less effective — a hypothesis requiring direct testing in randomized trials.

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**CORRECTION**

## Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men

Inflammation, Aspirin, and the Risk of Cardiovascular Disease in Apparently Healthy Men . On page 974, the sentence that begins in line 13 under the heading ``Laboratory Analysis´´ should have read, ``The C-reactive protein assay was standardized according to the WHO First International Reference Standard and had a sensitivity of 0.08 µg per *milliliter,´´* not ``0.08 µg per *microliter,´´* as printed.

ORIGINAL ARTICLE

# Inflammatory Markers and the Risk of Coronary Heart Disease in Men and Women

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## ABSTRACT

### BACKGROUND

Few studies have simultaneously investigated the role of soluble tumor necrosis factor $\alpha$ (TNF-$\alpha$) receptors types 1 and 2 (sTNF-R1 and sTNF-R2), C-reactive protein, and interleukin-6 as predictors of cardiovascular events. The value of these inflammatory markers as independent predictors remains controversial.

### METHODS

We examined plasma levels of sTNF-R1, sTNF-R2, interleukin-6, and C-reactive protein as markers of risk for coronary heart disease among women participating in the Nurses' Health Study and men participating in the Health Professionals Follow-up Study in nested case–control analyses. Among participants who provided a blood sample and who were free of cardiovascular disease at baseline, 239 women and 265 men had a nonfatal myocardial infarction or fatal coronary heart disease during eight years and six years of follow-up, respectively. Using risk-set sampling, we selected controls in a 2:1 ratio with matching for age, smoking status, and date of blood sampling.

### RESULTS

After adjustment for matching factors, high levels of interleukin-6 and C-reactive protein were significantly related to an increased risk of coronary heart disease in both sexes, whereas high levels of soluble TNF-$\alpha$ receptors were significant only among women. Further adjustment for lipid and nonlipid factors attenuated all associations; only C-reactive protein levels remained significant. The relative risk among all participants was 1.79 for those with C-reactive protein levels of at least 3.0 mg per liter, as compared with those with levels of less than 1.0 mg per liter (95 percent confidence interval, 1.27 to 2.51; P for trend <0.001). Additional adjustment for the presence or absence of diabetes and hypertension moderately attenuated the relative risk to 1.68 (95 percent confidence interval, 1.18 to 2.38; P for trend=0.008).

### CONCLUSIONS

Elevated levels of inflammatory markers, particularly C-reactive protein, indicate an increased risk of coronary heart disease. Although plasma lipid levels were more strongly associated with an increased risk than were inflammatory markers, the level of C-reactive protein remained a significant contributor to the prediction of coronary heart disease.

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Inflammation plays an essential role in the development of insulin resistance and type 2 diabetes mellitus, the initiation and progression of atherosclerotic lesions, and plaque disruption.[1,2] Interleukin-6 and tumor necrosis factor $\alpha$ (TNF-$\alpha$) are inflammatory cytokines and the main inducers of the secretion of C-reactive protein in the liver.[3] C-reactive protein is a marker of low-grade inflammation, and recent studies suggest that this protein has a role in the pathogenesis of atherosclerotic lesions in humans.[4] The effects of TNF-$\alpha$ are mediated by two receptors, type 1 and type 2 (TNF-R1 and TNF-R2), which circulate in soluble forms (sTNF-R1 and sTNF-R2, respectively) and can be measured with greater sensitivity and reliability than can TNF-$\alpha$ itself.[5] The soluble receptors may attenuate the bioactivity of TNF-$\alpha$ but may also serve as slow-release reservoirs and promote inflammation in the absence of free TNF ligand.[6]

Nonetheless, only a few studies have examined the relationship between levels of sTNF-R1, sTNF-R2, and interleukin-6 and the risk of coronary heart disease.[7-10] The predictive value of C-reactive protein for screening and its causal relationship to coronary heart disease remain matters of controversy.[11-17] We prospectively examined the association between inflammatory markers and the risk of coronary heart disease and the role of potential mediators among men and women in a nested case–control analysis.

## METHODS

### STUDY POPULATION

The Nurses' Health Study (NHS) and the Health Professionals Follow-up Study (HPFS) are prospective cohort investigations respectively involving 121,700 female U.S. registered nurses who were 30 to 55 years old at baseline in 1976 and 51,529 U.S. male health professionals who were 40 to 75 years old at baseline in 1986. Information about health and disease is assessed biennially, and information about diet is obtained every four years by means of self-administered questionnaires.[18,19] From 1989 through 1990, a blood sample was requested from all participants in the NHS, and 32,826 women provided one. Similarly, between 1993 and 1995, a blood sample was provided as requested by 18,225 men in the HPFS. Participants who provided blood samples were similar to those who did not, albeit the men who provided samples were somewhat younger than those who did not. In the NHS, among women without cardiovascular disease or cancer before 1990, we identified 249 women who had a nonfatal myocardial infarction or fatal coronary heart disease between the date of blood drawing and June 1998. In the HPFS, we identified 266 men who had a nonfatal myocardial infarction or fatal coronary heart disease between the date of blood drawing and the return of the 2000 questionnaire. Using risk-set sampling,[20] we randomly selected controls in a 2:1 ratio who were matched for age, smoking status, and date of blood sampling from the subgroup of participants who were free of cardiovascular disease at the time coronary disease was diagnosed in the case patients. Within the NHS cohort, an additional matching criterion was fasting status at the time of blood sampling.

### ASSESSMENT OF CORONARY HEART DISEASE

Study physicians who were unaware of the participant's exposure status confirmed the diagnosis of myocardial infarction on the basis of the criteria of the World Health Organization (symptoms plus either diagnostic electrocardiographic changes or elevated levels of cardiac enzymes). Deaths were identified from state vital records and the National Death Index or reported by the participant's next of kin or the postal system. Fatal coronary heart disease was confirmed by an examination of hospital or autopsy records, by the listing of coronary heart disease as the cause of death on the death certificate, if coronary heart disease was the underlying and most plausible cause, and if evidence of previous coronary heart disease was available.

### ASSESSMENT OF OTHER FACTORS

Anthropometric, lifestyle, and dietary data were derived from the questionnaire administered in 1990 to women and 1994 to men, with missing information substituted from previous questionnaires. Body-mass index was calculated as the weight in kilograms divided by the square of the height in meters. Average nutrient intake was computed with the use of a semiquantitative food-frequency questionnaire. Physical activity was expressed in terms of metabolic equivalent (MET)–hours. The questionnaires and the validity and reproducibility of measurements have been described previously.[18,21]

### MEASUREMENT OF BIOCHEMICAL VARIABLES

Blood samples from women were collected in tubes treated with liquid sodium heparin, and those from men were collected in EDTA-treated tubes. The

tubes were then placed on ice packs, stored in Styrofoam containers, returned to our laboratory by overnight courier, centrifuged, and divided into aliquots for storage in liquid-nitrogen freezers (–130°C or colder).

The levels of C-reactive protein were determined by means of a highly sensitive immunoturbidimetric assay with the use of reagents and calibrators from Denka Seiken; this assay has a day-to-day variability of 1 to 2 percent. Levels of sTNF-R1, sTNF-R2, and interleukin-6 were measured by means of enzyme-linked immunosorbent assays (R&D Systems), which have a day-to-day variability of 3.5 to 9.0 percent. Levels of inflammatory markers were largely unaffected by transport conditions and reproducible within subjects over time.[22,23] Total, high-density lipoprotein (HDL), and directly obtained low-density lipoprotein (LDL) cholesterol and triglycerides were measured according to standard methods with the use of reagents from Roche Diagnostics and Genzyme. Study samples were sent to the laboratory for analysis in randomly ordered batches, and the laboratory personnel were unaware of a sample's case–control status.

The study protocol was approved by the institutional review board of the Brigham and Women's Hospital and the Human Subjects Committee Review Board of Harvard School of Public Health.

### EXCLUSIONS

After the exclusion of participants with missing data on biomarker levels, our data sets consisted of 708 women (239 patients and 469 controls) and 794 men (265 patients and 529 controls). The assay for interleukin-6 required slightly more plasma than we originally reserved for this assay among women. Therefore, analyses involving interleukin-6 were restricted to the subgroup of 676 women for whom interleukin-6 levels were available.

### STATISTICAL ANALYSIS

We analyzed the two cohorts separately. Inflammatory markers were divided into quintiles, from the lowest to highest levels, on the basis of the sex-specific distributions among the controls. With risk-set sampling, the odds ratio derived from the logistic regression directly estimates the hazard ratio and, thus, the relative risk.[20] We analyzed the association between biomarker levels and the risk of coronary heart disease using both conditional and unconditional logistic regression, with adjustment for matching factors. Because both analyses provided essentially the same results, we present the results of unconditional logistic regression, which parallel the results in the subgroup analyses.

In our multivariable model, we further adjusted for parental history of coronary heart disease before the age of 60 years (yes vs. no), alcohol intake (nondrinker, 0.1 to 4.9 g per day, 5.0 to 14.9 g per day, 15.0 to 29.9 g per day, or at least 30.0 g per day), body-mass index (less than 20, 20 to 24, 25 to 29, 30 to 34, or 35 or more), physical activity (in quintiles from lowest to highest level), ratio of total to HDL cholesterol (in quintiles from lowest to highest ratio), and use of postmenopausal hormone therapy (yes vs. no — for women only). Finally, we also added a history of diabetes (yes vs. no) and hypertension (yes vs. no) at baseline to the model to assess the effect of these potential mediators. Baseline was defined as the year blood was drawn.

Correlation coefficients were calculated with the use of age-adjusted Spearman partial-correlation coefficients. To test for linear trend, we used the median levels of inflammatory markers in the control categories as a continuous variable. To pool the estimates of relative risk for men and women, we used the weighted average of estimates according to the random-effects model of DerSimonian and Laird.[24]

All P values are two-tailed, and P values below 0.05 were considered to indicate statistical significance. All analyses were performed with the use of SAS software, version 8.2 (SAS Institute).

## RESULTS

### BASELINE CHARACTERISTICS

Women in whom coronary heart disease developed during follow-up had significantly higher baseline levels of sTNF-R1 and sTNF-R2 than did control women; however, the levels did not differ significantly between men in whom coronary heart disease developed during follow-up and men in the control group (Table 1). In the case of both men and women, patients had significantly higher baseline levels of interleukin-6 and C-reactive protein than controls.

The levels of sTNF-R1 and sTNF-R2 showed a high degree of correlation with each other (Table 2). The correlation with and between the other inflammatory markers was moderate and ranged from 0.27 for sTNF-R1 and C-reactive protein to 0.45 for interleukin-6 and C-reactive protein. The levels of inflammatory markers were moderately inversely associated with HDL cholesterol levels.

**Table 1. Baseline Characteristics of Women and Men in Whom Coronary Heart Disease Developed during Follow-up and Matched Controls.***

| Characteristic | Women | | | Men | | |
|---|---|---|---|---|---|---|
| | Patients (N=239) | Controls (N=469) | P Value† | Patients (N=265) | Controls (N=529) | P Value† |
| Age (yr) | 60.4±6.5 | 60.2±6.5 | — | 65.2±8.3 | 65.1±8.3 | — |
| Current smoker (%) | 31.4 | 31.8 | — | 12.4 | 11.5 | — |
| Body-mass index | 26.9±5.7 | 25.3±4.3 | <0.001 | 26.2±3.5 | 25.7±3.5 | 0.05 |
| Parental history of CHD before 60 yr of age (%) | 21.3 | 12.4 | 0.002 | 15.1 | 11.0 | 0.10 |
| Postmenopausal (%) | 89.9 | 87.3 | 0.31 | — | — | — |
| Postmenopausal hormone therapy among postmenopausal women (%) | 31.7 | 41.0 | 0.03 | — | — | — |
| Medications (%) | | | | | | |
| Aspirin‡ | 15.1 | 21.3 | 0.05 | 39.1 | 34.9 | 0.25 |
| Cholesterol-lowering drug | 4.2 | 2.6 | 0.24 | 8.8 | 6.9 | 0.32 |
| History of hypertension (%) | 57.7 | 28.8 | <0.001 | 42.3 | 30.6 | 0.001 |
| History of diabetes (%) | 19.7 | 6.4 | <0.001 | 9.4 | 4.4 | 0.005 |
| Metabolic syndrome (%)§ | 43.9 | 18.3 | <0.001 | 40.4 | 26.1 | <0.001 |
| Total fat intake (% of energy) | 31.8±5.8 | 31.7±6.1 | 0.82 | 31.0±6.7 | 30.3±7.0 | 0.23 |
| Saturated fat intake (% of energy) | 10.8±2.5 | 10.7±2.7 | 0.84 | 10.4±2.7 | 10.1±2.9 | 0.12 |
| Alcohol consumption (g/day) | | | | | | |
| Median | 0.9 | 1.8 | <0.001 | 5.5 | 7.0 | 0.11 |
| Interquartile range | 0.0–3.7 | 0.0–8.6 | | 0.9–15.4 | 0.9–18.3 | |
| Physical activity (MET-hr/wk) | | | | | | |
| Median | 11.0 | 11.5 | 0.26 | 22.8 | 27.3 | 0.06 |
| Interquartile range | 3.9–22.7 | 5.1–23.0 | | 8.5–44.7 | 11.8–48.9 | |
| sTNF-R1 (pg/ml) | 1438±585 | 1267±354 | <0.001 | 1513±502 | 1506±541 | 0.86 |
| sTNF-R2 (pg/ml) | 2777±987 | 2489±710 | <0.001 | 2991±869 | 2945±870 | 0.48 |
| Interleukin-6 (pg/ml)¶ | | | | | | |
| Median | 1.99 | 1.65 | 0.001 | 1.86 | 1.53 | 0.01 |
| Interquartile range | 1.30–3.05 | 1.15–2.65 | | 1.10–3.07 | 0.98–2.88 | |
| C-reactive protein (mg/liter) | | | | | | |
| Median | 3.10 | 2.20 | <0.001 | 1.68 | 1.08 | <0.001 |
| Interquartile range | 1.30–7.50 | 1.00–5.10 | | 0.76–3.15 | 0.52–2.38 | |
| Cholesterol (mg/dl) | | | | | | |
| Total | 235.4±40.1 | 225.7±38.7 | 0.002 | 214.7±39.9 | 204.7±36.7 | <0.001 |
| LDL | 142.9±34.1 | 132.2±36.4 | <0.001 | 135.6±36.4 | 127.0±31.1 | 0.001 |
| HDL | 51.5±14.7 | 60.5±17.4 | <0.001 | 42.1±11.3 | 45.9±12.5 | <0.001 |
| Total-to-HDL cholesterol ratio | 4.91±1.55 | 4.02±1.31 | <0.001 | 5.37±1.41 | 4.74±1.40 | <0.001 |
| Triglycerides (mg/dl) | 157.6±96.7 | 126.3±76.3 | <0.001 | 181.8±116.7 | 153.8±121.1 | 0.002 |

* Data on women are from the Nurses' Health Study and include eight years of follow-up, and data on men are from the Health Professionals Follow-up Study and include six years of follow-up. Matching criteria were age, smoking status, and date of blood sampling; among women, additional matching criteria included fasting status at the time of blood sampling. Plus–minus values are means ±SD. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for triglycerides to millimoles per liter, multiply by 0.01129. sTNF-R1 and sTNF-R2 denote soluble tumor necrosis factor receptor types 1 and 2, CHD coronary heart disease, and MET-hr metabolic equivalent–hours. The body-mass index is the weight in kilograms divided by the square of the height in meters.

† P values for the difference between patients and controls (unadjusted) were determined by Student's t-test for variables expressed as means ±SD, by Wilcoxon's rank-sum test for variables expressed as medians, and by the chi-square test for variables expressed as percentages.

‡ Current aspirin use was defined as every one to four days for women and as two or more times per week for men.

§ The metabolic syndrome is defined by the presence of at least three of the following five abnormalities: a body-mass index of at least 25, a triglyceride level of at least 150 mg per deciliter (1.7 mmol per liter), an HDL cholesterol level of less than 50 mg per deciliter for women or less than 40 mg per deciliter for men, a history of hypertension or a history of diabetes or the development of diabetes during follow-up, or a glycosylated hemoglobin level of at least 7 percent at baseline.

¶ Data on interleukin-6 levels were missing for 32 women.

**Table 2. Age-Adjusted Spearman Partial-Correlation Coefficients between Selected Cardiovascular Risk Factors among 469 Control Women and 529 Control Men.***

| Sex and Risk Factor | Risk Factor | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | sTNF-R1 | sTNF-R2 | Interleukin-6† | CRP | TC | LDL | HDL | TC:HDL | BMI |
| **Women** | | | | | | | | | |
| sTNF-R1 | — | | | | | | | | |
| sTNF-R2 | 0.77‡ | — | | | | | | | |
| Interleukin-6 | 0.31‡ | 0.28‡ | — | | | | | | |
| CRP | 0.29‡ | 0.28‡ | 0.44‡ | — | | | | | |
| TC | –0.07 | –0.09§ | –0.05 | 0.03 | — | | | | |
| LDL | 0.02 | <0.01 | –0.03 | 0.04 | 0.87‡ | — | | | |
| HDL | –0.30‡ | –0.36‡ | –0.15¶ | –0.17‡ | 0.08 | –0.22‡ | — | | |
| TC:HDL | 0.22‡ | 0.27‡ | 0.09 | 0.15‡ | 0.45‡ | 0.67‡ | –0.83‡ | — | |
| BMI | 0.30‡ | 0.27‡ | 0.26‡ | 0.37‡ | 0.12§ | 0.18‡ | –0.33‡ | 0.37‡ | — |
| **Men** | | | | | | | | | |
| sTNF-R1 | — | | | | | | | | |
| sTNF-R2 | 0.67‡ | — | | | | | | | |
| Interleukin-6 | 0.32‡ | 0.28‡ | — | | | | | | |
| CRP | 0.27‡ | 0.28‡ | 0.45‡ | — | | | | | |
| TC | –0.16‡ | –0.13‡ | –0.17‡ | 0.03 | — | | | | |
| LDL | –0.16‡ | –0.11§ | –0.16‡ | –0.003 | 0.86‡ | — | | | |
| HDL | –0.25‡ | –0.21‡ | –0.20‡ | –0.24‡ | 0.20‡ | 0.13¶ | — | | |
| TC:HDL | 0.15‡ | 0.12¶ | 0.10§ | 0.25‡ | 0.39‡ | 0.39‡ | –0.80‡ | — | |
| BMI | 0.16‡ | 0.14‡ | 0.23‡ | 0.40‡ | 0.04 | 0.01 | –0.28‡ | 0.31‡ | — |

* sTNF-R1 and sTNF-R2 denote soluble tumor necrosis factor receptor types 1 and 2, CRP C-reactive protein, TC total cholesterol, LDL low-density lipoprotein cholesterol, HDL high-density lipoprotein cholesterol, and BMI body-mass index.
† Seventeen women were excluded from the analysis of interleukin-6 because they had missing values.
‡ P<0.001.
§ P<0.05.
¶ P<0.01.

### MAIN EFFECTS

After adjustment for matching factors, women in the highest quintile of each inflammatory marker, as compared with women in the lowest quintile, had a significantly increased risk of coronary heart disease — by a factor of 1.95 to 2.57 — with significant trends across quintiles (Table 3). After additional adjustment for the presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, level of physical activity, the ratio of total to HDL cholesterol, body-mass index, and the use or nonuse of postmenopausal hormone therapy, these associations were attenuated and no longer significant, except for C-reactive protein (model 2 in Table 3). Additional adjustment for the presence or absence of diabetes and hypertension, which are potentially in the causal pathway, further reduced the association for all inflammatory markers.

Among men, we did not find an association between the levels of soluble TNF-$\alpha$ receptors and the risk of coronary heart disease (Table 3). Men in the highest quintile of interleukin-6 had a 57 percent increase in the risk of coronary heart disease, as compared with men in the lowest quintile, after adjustment for matching factors, although this association was not significant and was further attenuated after multivariable adjustment. However, we found a significant association between C-reactive protein levels and the risk of coronary heart disease. Multivariable adjustment and adjustment for the presence or absence of hypertension and diabetes

**Table 3. Relative Risks of Coronary Heart Disease during Follow-up, According to the Quintile of Plasma Levels of Inflammatory Markers at Baseline.***

| Variable† | Quintile of Plasma Level | | | | | P for Trend‡ |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| | *relative risk (95 percent confidence interval)* | | | | | |
| **Women** | | | | | | |
| **sTNF-R1** | | | | | | |
| Median — pg/ml | 880 | 1083 | 1221 | 1379 | 1744 | |
| Quintile value — pg/ml | <928 | 928–1146 | 1147–1296 | 1297–1508 | ≥1509 | |
| Model 1 (matching factors) | 1.0 | 1.21 (0.69–2.11) | 1.20 (0.68–2.09) | 1.56 (0.90–2.70) | 2.57 (1.50–4.39) | <0.001 |
| Model 2 (multivariable) | 1.0 | 1.08 (0.60–1.97) | 0.91 (0.50–1.67) | 1.14 (0.63–2.08) | 1.50 (0.82–2.74) | 0.12 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 1.06 (0.57–1.97) | 0.90 (0.48–1.69) | 1.02 (0.54–1.90) | 1.24 (0.66–2.34) | 0.43 |
| **sTNF-R2** | | | | | | |
| Median — pg/ml | 1718 | 2060 | 2365 | 2724 | 3405 | |
| Quintile value — pg/ml | <1892 | 1892–2223 | 2224–2549 | 2550–3019 | ≥3020 | |
| Model 1 (matching factors) | 1.0 | 1.72 (0.97–3.04) | 1.92 (1.09–3.39) | 2.19 (1.24–3.88) | 2.51 (1.41–4.45) | 0.003 |
| Model 2 (multivariable) | 1.0 | 1.39 (0.75–2.56) | 1.48 (0.80–2.74) | 1.41 (0.76–2.60) | 1.36 (0.72–2.58) | 0.59 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 1.40 (0.74–2.65) | 1.38 (0.73–2.62) | 1.30 (0.69–2.46) | 1.20 (0.62–2.33) | 0.96 |
| **Interleukin-6§** | | | | | | |
| Median — pg/ml | 0.82 | 1.23 | 1.65 | 2.37 | 4.15 | |
| Quintile value — pg/ml | <1.08 | 1.08–1.44 | 1.45–1.91 | 1.92–2.91 | ≥2.92 | |
| Model 1 (matching factors) | 1.0 | 1.42 (0.81–2.51) | 1.15 (0.65–2.05) | 1.98 (1.16–3.40) | 1.92 (1.11–3.31) | 0.01 |
| Model 2 (multivariable) | 1.0 | 1.16 (0.63–2.13) | 0.96 (0.51–1.79) | 1.32 (0.72–2.40) | 1.33 (0.73–2.43) | 0.30 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 1.08 (0.58–2.03) | 0.81 (0.42–1.55) | 1.01 (0.54–1.89) | 1.05 (0.56–1.97) | 0.79 |
| **C-reactive protein** | | | | | | |
| Median — mg/liter | 0.50 | 1.18 | 2.20 | 4.02 | 9.14 | |
| Quintile value — mg/liter | <0.80 | 0.80–1.70 | 1.71–2.91 | 2.92–5.96 | ≥5.97 | |
| Model 1 (matching factors) | 1.0 | 1.28 (0.74–2.23) | 1.03 (0.59–1.81) | 1.54 (0.91–2.63) | 2.18 (1.30–3.64) | <0.001 |
| Model 2 (multivariable) | 1.0 | 1.17 (0.64–2.14) | 0.81 (0.43–1.52) | 1.17 (0.64–2.14) | 1.86 (1.00–3.46) | 0.008 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 1.23 (0.66–2.32) | 0.89 (0.46–1.72) | 1.22 (0.65–2.30) | 1.61 (0.84–3.07) | 0.08 |

moderately attenuated this relationship; after accounting for these variables, men in the highest quintile of C-reactive protein, as compared with those in the lowest quintile, had a relative risk of coronary heart disease of 2.55 (95 percent confidence interval, 1.40 to 4.65; P for trend=0.02).

For comparison, in the final multivariable-adjusted model (including the presence or absence of diabetes and hypertension and C-reactive protein levels), the relative risk of coronary heart disease for the highest quintile of the ratio of total to HDL cholesterol, as compared with the lowest quintile, was 4.33 (95 percent confidence interval, 2.11 to 8.90; P for trend <0.001) in women and 3.29 (95 percent confidence interval, 1.84 to 5.90; P for trend <0.001) in men.

### SUBGROUP ANALYSES

Overall, we found no significant interactions between various low and high cardiovascular risk groups and the association of biomarkers with the risk of coronary heart disease, although the association of C-reactive protein was generally stronger in low-risk subgroups. For example, in the multivari-

**Table 3. (Continued.)**

| Variable† | Quintile of Plasma Level | | | | | P for Trend‡ |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| | *relative risk (95 percent confidence interval)* | | | | | |
| **Men** | | | | | | |
| **sTNF-R1** | | | | | | |
| Median — pg/ml | 1005 | 1205 | 1391 | 1627 | 2124 | |
| Quintile value — pg/ml | <1111 | 1111–1301 | 1302–1510 | 1511–1793 | ≥1794 | |
| Model 1 (matching factors) | 1.0 | 1.01 (0.63–1.63) | 1.13 (0.70–1.82) | 0.96 (0.58–1.57) | 1.06 (0.64–1.77) | 0.90 |
| Model 2 (multivariable) | 1.0 | 0.95 (0.57–1.58) | 1.00 (0.60–1.65) | 0.84 (0.49–1.42) | 0.85 (0.49–1.46) | 0.48 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 0.94 (0.56–1.56) | 0.99 (0.60–1.65) | 0.82 (0.48–1.40) | 0.78 (0.45–1.36) | 0.32 |
| **sTNF-R2** | | | | | | |
| Median — pg/ml | 1969 | 2421 | 2812 | 3209 | 4090 | |
| Quintile value — pg/ml | <2242 | 2242–2614 | 2615–2966 | 2967–3564 | ≥3565 | |
| Model 1 (matching factors) | 1.0 | 0.80 (0.49–1.31) | 0.90 (0.55–1.47) | 1.12 (0.69–1.82) | 1.12 (0.68–1.86) | 0.33 |
| Model 2 (multivariable) | 1.0 | 0.68 (0.40–1.15) | 0.81 (0.48–1.36) | 0.94 (0.56–1.57) | 0.91 (0.54–1.56) | 0.78 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 0.72 (0.42–1.21) | 0.81 (0.48–1.37) | 0.98 (0.59–1.65) | 0.92 (0.53–1.58) | 0.80 |
| **Interleukin-6** | | | | | | |
| Median — pg/ml | 0.69 | 1.09 | 1.53 | 2.43 | 5.73 | |
| Quintile value — pg/ml | <0.88 | 0.88–1.29 | 1.30–1.89 | 1.90–3.15 | ≥3.16 | |
| Model 1 (matching factors) | 1.0 | 1.09 (0.66–1.81) | 1.19 (0.72–1.98) | 1.52 (0.93–2.48) | 1.57 (0.95–2.57) | 0.06 |
| Model 2 (multivariable) | 1.0 | 0.94 (0.55–1.60) | 0.99 (0.59–1.69) | 1.25 (0.74–2.10) | 1.31 (0.78–2.21) | 0.17 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 0.97 (0.57–1.65) | 0.98 (0.58–1.68) | 1.24 (0.73–2.09) | 1.31 (0.77–2.22) | 0.19 |
| **C-reactive protein** | | | | | | |
| Median — mg/liter | 0.27 | 0.60 | 1.08 | 2.05 | 5.24 | |
| Quintile value — mg/liter | <0.44 | 0.44–0.80 | 0.81–1.49 | 1.50–2.78 | ≥2.79 | |
| Model 1 (matching factors) | 1.0 | 1.81 (1.04–3.17) | 2.00 (1.15–3.50) | 2.74 (1.59–4.71) | 3.29 (1.91–5.65) | <0.001 |
| Model 2 (multivariable) | 1.0 | 1.75 (0.97–3.14) | 1.83 (1.02–3.30) | 2.27 (1.26–4.09) | 2.73 (1.51–4.96) | 0.007 |
| Model 3 (model 2+diabetes and hypertension) | 1.0 | 1.75 (0.97–3.16) | 1.74 (0.96–3.15) | 2.14 (1.18–3.88) | 2.55 (1.40–4.65) | 0.02 |

* The group of women included 239 patients and 469 controls with eight years of follow-up. The group of men included 265 patients and 529 controls with six years of follow-up. sTNF denotes soluble tumor necrosis factor receptor. Quintiles and median values of plasma inflammatory markers are based on values in controls. For each relative risk, quintile 1 served as the reference group.

† Model 1 was adjusted for matching factors (age, smoking status, and the month of blood sampling). Among women, data were also adjusted for fasting status at the time of blood sampling. Model 2 was adjusted for matching factors, presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, level of physical activity, ratio of total cholesterol to HDL cholesterol, and body-mass index. Among women, the multivariable model was also adjusted for the use or nonuse of postmenopausal hormone therapy.

‡ P values for trend are based on the median levels of inflammatory markers in quintiles of the controls.

§ A total of 32 women were excluded from the analyses for interleukin-6 owing to missing values for interleukin; 224 patients and 452 controls were analyzed.

able-adjusted model (excluding the presence or absence of hypertension and diabetes), the relative risk in the highest as compared with the lowest quintile of C-reactive protein was 2.53 among women with a body-mass index of less than 25 (95 percent confidence interval, 1.04 to 6.18; P for trend=0.02) and 6.25 among men with a body-mass index of less than 25 (95 percent confidence interval, 2.28 to 17.1; P for trend=0.005). Similarly, among participants with LDL cholesterol levels of less than 130 mg per

deciliter (3.4 mmol per liter), the corresponding relative risks were 3.54 (95 percent confidence interval, 1.19 to 10.5; P for trend=0.01) for women and 2.52 (95 percent confidence interval, 1.09 to 5.83; P for trend= 0.04) for men. Among participants without hypertension, the corresponding relative risks were 1.87 (95 percent confidence interval, 0.77 to 4.56; P for trend=0.02) for women and 3.01 (95 percent confidence interval, 1.41 to 6.44; P for trend=0.02) for men.

### CLINICAL CUTOFF POINTS FOR C-REACTIVE PROTEIN

We further categorized the study participants, on the basis of recently proposed cutoff points for C-reactive protein, as having low levels (less than 1.0 mg per liter), moderate levels (1.0 to 2.9 mg per liter), and high levels (at least 3.0 mg per liter).[25] In these analyses, participants with high levels of C-reactive protein, as compared with those with low levels, had a relative risk of coronary heart disease of approximately 1.8 after adjustment for covariates (including body-mass index and lipid levels) (Table 4). When we pooled the risk estimates for men and women, the final multivariable-adjusted relative risk (including adjustment for the presence or absence of diabetes and hypertension) was 1.68 in the group with high levels of C-reactive protein, as compared with the group with low levels (95 percent confidence interval, 1.18 to 2.38; P for trend= 0.008) (Table 4). This is similar to the pooled estimate (relative risk, 1.48; 95 percent confidence interval, 1.08 to 2.04; P for trend=0.03) after we controlled for covariates from the Framingham risk score,[26] including age, presence or absence of hypertension and diabetes, ratio of total to HDL cholesterol, and smoking status.

We found a gradient of risk of coronary heart disease within each increasing category of C-reactive protein and ratio of total to HDL cholesterol (Fig. 1). This finding supports the hypothesis that the levels of C-reactive protein may predict risk beyond the information afforded by lipid levels. However, despite the independent associations, the gradient of risk associated with lipid levels was greater than that for C-reactive protein levels.

### ADDITIONAL ANALYSES

When we stratified our analysis according to the time to an event in two-year intervals, the relative risk of coronary heart disease associated with C-reactive protein levels remained relatively stable over time (data not shown). When we repeated our main analyses after excluding participants with C-reactive protein levels of at least 10.0 mg per liter, we found essentially the same results. C-reactive protein levels may be affected by hormone therapy.[10] However, results were similar when we used quintiles of C-reactive protein based on levels in women in the control group who reported never using hormones.

## DISCUSSION

In these two nested case–control studies, we found that high plasma levels of C-reactive protein were associated with an increased risk of coronary heart disease among women and men without previous cardiovascular disease. Elevated plasma levels of sTNF-R1 and sTNF-R2 were related to an increased risk among women, but not men. We found only a moderate suggestion of increased risk associated with elevated levels of interleukin-6. For all markers, associations were substantially attenuated and — with the exception of C-reactive protein — no longer significant after adjustment for cardiovascular risk factors, particularly body-mass index and the presence or absence of diabetes and hypertension. These findings are consistent with a role of these inflammatory markers in the elevated risk of cardiovascular events that is associated with type 2 diabetes and hypertension.

TNF-$\alpha$ and interleukin-6 are the main inducers of hepatic production of acute-phase proteins, including C-reactive protein.[3] These inflammatory markers are associated with biologic and environmental risk factors for cardiovascular events, including components of the metabolic syndrome (obesity, insulin resistance, diabetes, hypertension, and low HDL cholesterol levels), and lifestyle factors, such as smoking, abstinence from alcohol, and physical inactivity.[27-29]

Compelling evidence suggests that inflammation causally contributes to several precursors of cardiovascular disease. TNF-$\alpha$ and interleukin-6 can cause insulin resistance in animal models, and plasma levels of C-reactive protein and interleukin-6 have been shown to predict type 2 diabetes in humans.[30,31] The increased cytokine synthesis in obesity may promote insulin resistance and impaired glucose uptake, type 2 diabetes, and ultimately, coronary heart disease.[30] In line with these hypotheses, we found that plasma levels of interleukin-6 and C-reactive protein, in particular, were related to

**Table 4. Relative Risks of Coronary Heart Disease during Follow-up According to the Baseline Level of C-Reactive Protein.***

| Variable† | CRP <1.0 mg/liter | CRP 1.0–2.9 mg/liter | CRP ≥3.0 mg/liter | P for Trend‡ |
|---|---|---|---|---|
| | *relative risk (95 percent confidence interval)* | | | |
| **Women** | | | | |
| No. of patients | 41 | 73 | 125 | |
| No. of controls | 114 | 170 | 185 | |
| Model 1 (matching factors) | 1.0 | 1.22 (0.77–1.93) | 1.93 (1.25–2.99) | <0.001 |
| Model 2 (multivariable) | 1.0 | 1.21 (0.75–1.96) | 1.94 (1.21–3.10) | 0.002 |
| Model 3 (model 2+body-mass index) | 1.0 | 1.16 (0.71–1.90) | 1.71 (1.04–2.80) | 0.02 |
| Model 4 (model 3+TC:HDL) | 1.0 | 1.09 (0.66–1.82) | 1.64 (0.98–2.75) | 0.02 |
| Model 5 (model 4+diabetes and hypertension) | 1.0 | 1.17 (0.69–2.00) | 1.53 (0.89–2.62) | 0.09 |
| **Men** | | | | |
| No. of patients | 86 | 108 | 71 | |
| No. of controls | 254 | 175 | 100 | |
| Model 1 (matching factors) | 1.0 | 1.90 (1.34–2.71) | 2.20 (1.46–3.32) | <0.001 |
| Model 2 (multivariable) | 1.0 | 1.88 (1.31–2.69) | 2.17 (1.43–3.31) | 0.002 |
| Model 3 (model 2+body-mass index) | 1.0 | 1.85 (1.28 –2.68) | 2.08 (1.34–3.23) | 0.006 |
| Model 4 (model 3+TC:HDL) | 1.0 | 1.71 (1.17–2.49) | 1.91 (1.22–3.00) | 0.02 |
| Model 5 (model 4+diabetes and hypertension) | 1.0 | 1.60 (1.09–2.34) | 1.79 (1.14–2.83) | 0.03 |
| **Men and Women** | | | | |
| Model 1 (matching factors) | 1.0 | 1.61 (1.22–2.14) | 2.07 (1.54–2.79) | <0.001 |
| Model 2 (multivariable) | 1.0 | 1.61 (1.20–2.14) | 2.06 (1.51–2.82) | <0.001 |
| Model 3 (model 2+body-mass index) | 1.0 | 1.57 (1.17–2.11) | 1.90 (1.37–2.65) | <0.001 |
| Model 4 (model 3+TC:HDL) | 1.0 | 1.46 (1.08–1.98) | 1.79 (1.27–2.51) | <0.001 |
| Model 5 (model 4+diabetes and hypertension) | 1.0 | 1.44 (1.05–1.96) | 1.68 (1.18–2.38) | 0.008 |

* Data on women are from the Nurses' Health Study and include eight years of follow-up, and data on men are from the Health Professionals Follow-up Study and include six years of follow-up. The subjects with the lowest level of C-reactive protein (CRP) served as the reference group. TC:HDL denotes the ratio of total cholesterol to high-density lipoprotein cholesterol.

† Model 1 was adjusted for matching factors (age, smoking status, and month of blood sampling); data for women were also adjusted for fasting status at the time of blood sampling. Model 2 was adjusted for matching factors, as well as the presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, level of physical activity, and use or nonuse of hormone therapy among postmenopausal women. Model 5 was adjusted for everything listed in model 4 as well as the presence or absence of diabetes and hypertension.

‡ P values for trend are based on median levels in the three C-reactive protein groups in the controls.

the risk of coronary heart disease and that the risks were attenuated after adjustment for the presence or absence of diabetes and hypertension.

TNF-$\alpha$ has a limited half-life and is difficult to measure in large-scale epidemiologic studies.[5,6] In a nested case–control study, Ridker et al. reported a multivariable-adjusted relative risk of recurrent coronary events of 2.5 (95 percent confidence interval, 1.3 to 5.1) among men whose TNF-$\alpha$ levels exceeded the 95th percentile, as compared with men with lower levels.[32] Cesari et al. reported a relative risk of of coronary events of 1.79 (95 percent confidence interval, 1.18 to 2.71) among elderly participants without cardiovascular disease who had the highest of three levels of TNF-$\alpha$, as compared with those who had the lowest levels.[8] The value of as-

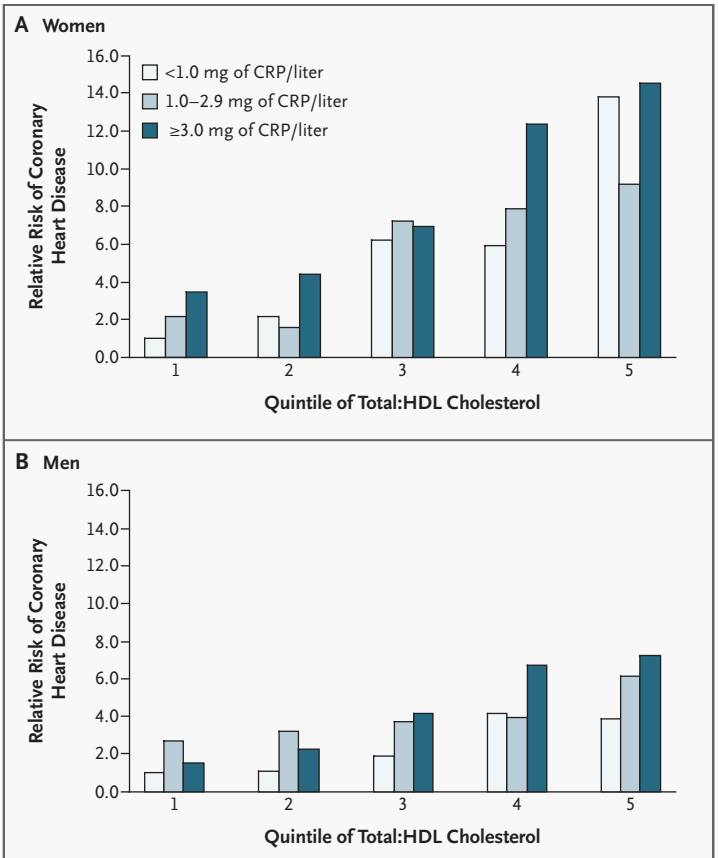


**Figure 1. Multivariable-Adjusted Relative Risk of Coronary Heart Disease among Women (Panel A) and Men (Panel B), According to the Baseline Level of C-Reactive Protein (CRP) and the Quintile of the Ratio of Total to HDL Cholesterol.**

Data on women are from the Nurses' Health Study and include eight years of follow-up, and data on men are from the Health Professionals Follow-up Study and include six years of follow-up. The model was adjusted for age, smoking status, date of blood sampling, presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, level of physical activity, and body-mass index. Among women, the multivariable model was also adjusted for fasting status at the time of blood sampling and the use or nonuse of postmenopausal hormone therapy. In each panel, the subjects in quintile 1 who had a CRP level of less than 1.0 mg per liter served as the reference group.

sessing circulating levels of TNF-$\alpha$ is unknown, since such levels can be very low and unstable. The levels of soluble TNF-$\alpha$ receptors may be more stable and may better reflect longer-term average circulating levels of TNF-$\alpha$, although data on the role of soluble TNF-$\alpha$ receptors in coronary heart disease are scarce.[7,33] It is unclear why we found a difference in risk between men and women associated with elevated levels of soluble TNF-$\alpha$ receptors; however, others also have found differences between women and men with respect to lipids[34] and in the overall prediction of risk.[35] Similarly, mechanisms of insulin sensitivity, rather than inflammation, may contribute more to the risk of coronary heart disease in women than men.

Findings of an association between interleukin-6 levels and the risk of coronary heart disease have been inconsistent.[8,10,36] In our study, this association was substantially reduced and no longer significant after multivariable adjustment.

C-reactive protein is the most extensively studied inflammatory marker in prospective settings. In an early meta-analysis of 11 prospective studies, the relative risk of coronary heart disease in subjects with the highest of three C-reactive protein levels, as compared with those with the lowest levels, was 2.0 (95 percent confidence interval, 1.6 to 2.5) among population-based studies.[37] Eleven other prospective studies have since been published. In an updated meta-analysis, Danesh et al. reported an overall odds ratio of 1.58 (95 percent confidence interval, 1.48 to 1.68) among subjects with the highest of three levels of C-reactive protein, as compared with subjects with the lowest level.[16] This risk estimate is similar to that in our comparisons of C-reactive protein levels of at least 3.0 mg per liter with those of less than 1.0 mg per liter. However, the degree of adjustment for traditional cardiovascular risk factors differed markedly among the studies included in the meta-analysis.

An important question is whether knowing the level of C-reactive protein adds materially to risk prediction. In the Women's Health Study, Ridker et al. reported that the level of C-reactive protein was a stronger predictor than the LDL cholesterol level and that it added to the information provided by the Framingham risk score.[12,38] Comparing C-reactive protein levels of at least 3.0 mg per liter with those of less than 1.0 mg per liter, they reported a relative risk of 1.5 (95 percent confidence interval, 1.2 to 1.9) after adjustment for the Framingham risk score and the presence or absence of diabetes.[38]

In the Atherosclerosis Risk in Communities Study, Ballantyne et al. reported a relative risk of coronary heart disease of 1.72 (95 percent confidence interval, 1.24 to 2.39) among subjects with a C-reactive protein level of at least 3.0 mg per liter, as compared with subjects with a level of less than 1.0 mg per liter (adjusted for components of the Framingham risk score, including the presence or ab-

sence of diabetes).[14] In the Monitoring Trends and Determinants in Cardiovascular Disease (MONICA) study, comparing C-reactive protein levels of at least 3.0 mg per liter with those of less than 1.0 mg per liter, Koenig et al. reported a hazard ratio of 2.21 (95 percent confidence interval, 1.49 to 3.27), adjusted for the Framingham risk score.[13] In contrast, in the Rotterdam Study, measuring the level of C-reactive protein did not improve the prediction of coronary events beyond that afforded by the Framingham risk score, with an odds ratio of 1.2 (95 percent confidence interval, 0.6 to 2.2) among participants in the highest quartile of C-reactive protein, as compared with those in the lowest quartile.[39]

In our analysis, the pooled relative risk among men and women classified according to clinical cutoff points for the levels of C-reactive protein was 1.48 (95 percent confidence interval, 1.08 to 2.04; P for trend=0.03) after we accounted for covariates in the Framingham risk score, including the presence or absence of diabetes. Our results are similar to those of Ridker et al.[38] and Ballantyne et al.,[14] as well as those of the recent meta-analysis by Danesh et al.,[16] a fact that suggests that after adjustment for the Framingham risk score, the relative risk associated with a clinical cutoff point of at least 3.0 mg per liter, as compared with a cutoff of less than 1.0 mg per liter, is probably moderately less than previously suggested in the guidelines for the clinical assessment of inflammatory markers issued by the American Heart Association and the Centers for Disease Control and Prevention (relative risk, 1.5 vs. approximately 2.0).[25] Nevertheless, our findings support the theory that the level of C-reactive protein provides an additional measure of the risk of coronary heart disease beyond that afforded by the Framingham risk score.

Our study has some limitations. As with any observational study design, there is the possibility of unmeasured confounding. However, we controlled for most known cardiovascular risk factors. Though we obtained only a single blood sample at baseline, previous studies have shown the levels of biomarkers to be relatively stable over time.[22,23] Since the ranges of anthropometric variables in our cohorts were quite broad, the biologic relationships found should be widely generalizable. Though we excluded men and women with missing data on blood levels, generalizability should be minimally affected because the participants were similar to those who did not provide blood samples.

Although the Framingham risk score is a tool for estimating the 10-year risk of coronary heart disease among healthy subjects,[26] it does not include other well-established risk factors, such as body-mass index, alcohol intake, level of physical activity, or the presence or absence of a parental history of coronary heart disease.[40] Therefore, to examine the role of inflammatory markers in coronary heart disease, we used an etiologic approach in our main analyses, to take into account the pathophysiology of coronary heart disease and include the major cardiovascular risk factors, beyond those included in the Framingham risk score, for comparison.

Our questionnaires did not include questions on the use of hydroxymethylglutarylcoenzyme A reductase inhibitors (statins) because these drugs were not widely used at time of blood sampling. However, the reported use of cholesterol-lowering drugs was generally low in both cohorts.

In conclusion, our findings suggest that high levels of C-reactive protein are associated with an increased risk of coronary heart disease among men and women and that the level of C-reactive protein is a significant marker of the risk of coronary heart disease, even after careful multivariable adjustment. Though all other associations were attenuated after multivariable adjustment, high levels of sTNF-R1 and sTNF-R2 may be also associated with an increased risk and deserve further exploration in other populations. From a clinical standpoint, although the ratio of total to HDL cholesterol was more strongly associated with the risk of coronary heart disease than were the levels of inflammatory markers, the level of C-reactive protein was still a significant contributor to the prediction of coronary heart disease.

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# C-Reactive Protein and Other Circulating Markers of Inflammation in the Prediction of Coronary Heart Disease

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## ABSTRACT

### BACKGROUND

C-reactive protein is an inflammatory marker believed to be of value in the prediction of coronary events. We report data from a large study of C-reactive protein and other circulating inflammatory markers, as well as updated meta-analyses, to evaluate their relevance to the prediction of coronary heart disease.

### METHODS

Measurements were made in samples obtained at base line from up to 2459 patients who had a nonfatal myocardial infarction or died of coronary heart disease during the study and from up to 3969 controls without a coronary heart disease event in the Reykjavik prospective study of 18,569 participants. Measurements were made in paired samples obtained an average of 12 years apart from 379 of these participants in order to quantify within-person fluctuations in inflammatory marker levels.

### RESULTS

The long-term stability of C-reactive protein values (within-person correlation coefficient, 0.59; 95 percent confidence interval, 0.52 to 0.66) was similar to that of both blood pressure and total serum cholesterol. After adjustment for base-line values for established risk factors, the odds ratio for coronary heart disease was 1.45 (95 percent confidence interval, 1.25 to 1.68) in a comparison of participants in the top third of the group with respect to base-line C-reactive protein values with those in the bottom third, and similar overall findings were observed in an updated meta-analysis involving a total of 7068 patients with coronary heart disease. By comparison, the odds ratios in the Reykjavik Study for coronary heart disease were somewhat weaker for the erythrocyte sedimentation rate (1.30; 95 percent confidence interval, 1.13 to 1.51) and the von Willebrand factor concentration (1.11; 95 percent confidence interval, 0.97 to 1.27) but generally stronger for established risk factors, such as an increased total cholesterol concentration (2.35; 95 percent confidence interval, 2.03 to 2.74) and cigarette smoking (1.87; 95 percent confidence interval, 1.62 to 2.16).

### CONCLUSIONS

C-reactive protein is a relatively moderate predictor of coronary heart disease. Recommendations regarding its use in predicting the likelihood of coronary heart disease may need to be reviewed.

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Since atherosclerosis may, in part, be an inflammatory disease,[1] circulating factors related to inflammation may be predictors of cardiovascular disease in general populations.[2] A recent statement from the Centers for Disease Control and Prevention and the American Heart Association concluded that it is reasonable to measure C-reactive protein, a sensitive circulating marker of inflammation, as an adjunct to the measurement of established risk factors in order to assess the risk of coronary heart disease.[3] The report acknowledged, however, that the epidemiologic data to support this view were not entirely consistent and recommended that larger prospective studies be conducted to improve the reliability of the evidence.

We measured C-reactive protein concentrations in approximately 2400 patients with coronary heart disease diagnosed since their enrollment in the cohort and approximately 4000 controls nested within the Reykjavik Study, a prospective cohort study of about 19,000 middle-aged men and women without a history of myocardial infarction. The number of cases of coronary heart disease in this cohort was about four times as great as in the largest previous study[4] and should reduce the scope for random error in our estimates. We also assessed the effect of within-person variation in the concentrations of inflammatory markers[5] in serial blood samples obtained over a period of several years in several hundred participants. To compare the predictive value of the C-reactive protein concentration with that of some other inflammatory markers studied in coronary heart disease, we also analyzed the erythrocyte sedimentation rate and circulating concentrations of von Willebrand factor, each of which can also fluctuate considerably in acute-phase inflammatory responses.[6,7] To help put the new data in context, we updated meta-analyses of previous relevant studies of each of these inflammatory markers.

## METHODS

### PATIENTS AND CONTROLS

The Reykjavik Study, initiated in 1967 as a prospective study of cardiovascular disease, has been described in detail previously.[8] All men born between 1907 and 1934 and all women born between 1908 and 1935 who were residents of Reykjavik, Iceland, and its adjacent communities on December 1, 1966, were identified in the national population register and then invited to participate in the study during five stages of recruitment between 1967 and 1991. A total of 8888 men and 9681 women without a history of myocardial infarction were enrolled, reflecting a response rate of 72 percent.[9]

Nurses administered questionnaires, made physical measurements, performed spirometry and electrocardiography, and collected venous blood samples after an overnight fast for the measurement of the erythrocyte sedimentation rate and to prepare aliquots of serum, which were stored at −20°C for subsequent analysis. All participants have subsequently been monitored with respect to death from any cause and the occurrence of major cardiovascular conditions, with a total loss to follow-up of only about 0.6 percent of participants.[9]

A total of 2459 men and women with available serum samples had major coronary events between the beginning of follow-up and December 31, 1995, for a mean (±SD) duration of follow-up of 17.5±8.7 years, as compared with 20.6±8.2 years among controls. Among the men, 1073 deaths from coronary heart disease and 701 nonfatal myocardial infarctions were recorded (564 confirmed and 137 possible myocardial infarctions), and among the women, 385 died of coronary heart disease and 300 had a nonfatal myocardial infarction (237 confirmed and 63 possible myocardial infarctions). Deaths from coronary heart disease were ascertained from central registers on the basis of a death certificate listing an *International Classification of Diseases* code of 410 through 414, and the diagnosis of nonfatal myocardial infarction was based on the criteria of the Monitoring Trends and Determinants in Cardiovascular Disease study.

We selected 3969 control subjects from among the participants who had survived to the end of the study period without having a myocardial infarction. The controls were frequency-matched to the patients with respect to the calendar year of recruitment, sex, and age (in five-year increments).[10]

The National Bioethics Committee and the Data Protection Authority of Iceland approved the study protocol. All participants provided informed consent.

### LABORATORY METHODS

Laboratory measurements were made without knowledge of the participants' disease status, and thus samples from patients and controls were randomly distributed among assay plates. Concentrations of C-reactive protein were measured by latex-enhanced immunoturbidimetry, with a lower limit of detection of 0.02 mg per liter (Roche Diagnostics).[11] The variation in C-reactive protein values within runs was less than 1 percent, and the be-

tween-day variability was 1 percent at a concentration of 14 mg per liter and 3.7 percent at a concentration of 3.8 mg per liter. The concentration of von Willebrand factor was determined by means of a sensitive enzyme immunoassay. We also determined the concentration of von Willebrand factor in paired plasma and serum samples from 56 healthy persons from another study and found close agreement between plasma and serum values (correlation coefficient, 0.94).[7] The Wintrobe method was used to measure the erythrocyte sedimentation rate in fresh blood samples obtained at the time of base-line venesection.[6] Other biochemical and hematologic measurements involved the use of standard assays, as previously described.[8] Measurements were made in pairs of samples obtained from 379 participants a mean of about 12 years apart. Data on erythrocyte sedimentation rate from the Reykjavik Study have been reported previously.[12]

### STATISTICAL ANALYSIS

Comparisons between patients and controls were made by means of unmatched stratified logistic regression fitted according to the unconditional maximum likelihood (Stata software, version 7). To maximize the ability to compare our results with those of previous reports, primary analyses of values of C-reactive protein, erythrocyte sedimentation rate, and von Willebrand factor were prespecified to compare extreme thirds of patients and controls with respect to the distribution of values in the controls. Subsidiary analyses involved other cutoff values. Odds ratios were sequentially adjusted for the following variables: age, sex, calendar year of enrollment, smoking status, systolic blood pressure, total cholesterol level, triglyceride level, body-mass index (the weight in kilograms divided by the square of the height in meters), forced expiratory volume in one second, presence or absence of diabetes, socioeconomic status, and the concentrations of other markers of inflammation.

To estimate the discriminative value of predictive models, we calculated the areas under the receiver-operating-characteristic curve, in order to determine whether the sequential addition of data on inflammatory markers increased the predictive value of major established coronary risk factors, as described previously.[13] We performed meta-analyses of studies published before January 2003 that included essentially general populations (i.e., cohorts not selected on the basis of preexisting disease) with more than a year of follow-up, using search, abstraction, and data-synthesis methods that have been described previously and using nonfatal myocardial infarction or death from coronary heart disease as end points.[6,7,14] We combined the results of the studies by using inverse variance-weighted averages of logarithmic odds ratios. Heterogeneity was assessed by means of standard $\chi^2$ tests. Odds ratios are given with 95 percent confidence intervals, and two-sided P values are reported. Since previous studies have reported on the predictive values of single base-line measurements of inflammatory markers with respect to coronary heart disease, odds ratios have not been corrected for regression dilution in the present study, so as to allow direct comparisons with previous work.[5]

## RESULTS

The mean age at the time of the coronary heart disease event was 70.2±9.7 years. There were significant differences between patients and controls with respect to established coronary risk factors, such as smoking status, body-mass index, blood pressure, and serum lipid concentrations (Table 1).

### BASE-LINE ASSOCIATIONS AND LONG-TERM STABILITY OF INFLAMMATORY MARKERS

The partial correlation coefficients (adjusted for age, sex, calendar year of recruitment, and smoking status) for C-reactive protein, on the one hand, and the erythrocyte sedimentation rate and von Willebrand factor, on the other, were 0.38 and 0.18, respectively (P<0.001 for each comparison), and the partial correlation coefficient for the erythrocyte sedimentation rate and the von Willebrand factor concentration was 0.17 (P<0.001). A higher C-reactive protein concentration was significantly associated with cigarette smoking (P<0.001), an increased body-mass index (P<0.001), a low forced expiratory volume in one second (P<0.001), and an increased triglyceride concentration (P<0.001) (data not shown). Higher values for the erythrocyte sedimentation rate were significantly associated with older age (P<0.001), female sex (P<0.001), a low hemoglobin value (P<0.001), a low hematocrit (P<0.001), an elevated serum uric acid concentration (P<0.001), a low forced expiratory volume in one second (P<0.001), and smoking (P<0.001). A higher von Willebrand factor concentration was significantly associated with older age (P<0.001) and smoking (P<0.001).

Among 379 participants who provided paired blood samples, the within-person correlation coefficients for C-reactive protein, erythrocyte sedimen-

**Table 1. Base-Line Characteristics of the Patients with Coronary Heart Disease and Controls.***

| Characteristic | Patients (N=2459) | Controls (N=3969) | P Value |
|---|---|---|---|
| Age — yr | 55.8±9.3 | 55.7±9.1 | — |
| Male sex — no. (%) | 1774 (72) | 2743 (69) | — |
| Current smoker (including cigarettes, cigars, pipes) — no. (%) | 1417 (58) | 1941 (49) | <0.001 |
| Current cigarette smoker — no. (%) | 962 (39) | 1266 (32) | <0.001 |
| History of diabetes — no. (%) | 83 (3) | 63 (2) | <0.001 |
| Nonmanual occupation — no. (%)† | 703 (40) | 1227 (42) | 0.15 |
| Education beyond high school — no. (%)‡ | 354 (27) | 645 (30) | 0.12 |
| Home owner — no. (%)§ | 1962 (84) | 3201 (85) | 0.39 |
| Lives in apartment block — no. (%)¶ | 1186 (53) | 1833 (50) | 0.09 |
| Height — m | 1.71±0.087 | 1.72±0.087 | 0.07 |
| Weight — kg | 76±14 | 75±14 | <0.001 |
| Body-mass index | 26±3.9 | 25±3.7 | <0.001 |
| Systolic blood pressure — mm Hg | 146±22 | 141±20 | <0.001 |
| Diastolic blood pressure — mm Hg | 89±11 | 87±11 | <0.001 |
| Forced expiratory volume in 1 sec — liters | 2.8±0.85 | 2.9±0.86 | 0.002 |
| Protein or glucose in urine — no. (%) | 112 (5) | 102 (3) | <0.001 |
| Blood value‖ | | | |
| Total serum cholesterol — mmol/liter | 6.82±1.18 | 6.40±1.14 | <0.001 |
| Serum triglycerides — mmol/liter** | 1.19±0.79 | 1.03±0.62 | <0.001 |
| Fasting glucose — mmol/liter | 4.6±1.1 | 4.5±0.8 | <0.001 |
| Serum creatinine — μmol/liter | 77±14 | 75±13 | <0.001 |
| Serum uric acid — μmol/liter | 312±73 | 300±66 | <0.001 |
| Hemoglobin — mmol/liter | 9.2±0.80 | 9.1±0.81 | <0.001 |
| Hematocrit — % | 44.8±3.6 | 44.2±3.5 | <0.001 |
| Inflammatory marker†† | | | |
| C-reactive protein — mg/liter** | 1.75±5.3 | 1.28±5.2 | <0.001 |
| Erythrocyte sedimentation rate — mm/hr** | 7.4±10.6 | 6.3±9.7 | <0.001 |
| von Willebrand factor — IU/dl** | 107.4±48.1 | 103.2±46.2 | <0.001 |

* Plus–minus values are means ±SD. The body-mass index is the weight in kilograms divided by the square of the height in meters.
† Information on occupation was available for 1742 patients and 2888 controls.
‡ Information on education was available for 1292 patients and 2157 controls.
§ Information on home ownership was available for 2323 patients and 3754 controls.
¶ Information on the type of residence was available for 2258 patients and 3646 controls. Other categories included "duplex" and "villa."
‖ To convert values for cholesterol to milligrams per deciliter, divide by 0.02586. To convert values for triglycerides to milligrams per deciliter, divide by 0.01129. To convert values for glucose to milligrams per deciliter, divide by 0.05551. To convert values for creatinine to milligrams per deciliter, divide by 88.4. To convert values for uric acid to milligrams per deciliter, divide by 59.48. To convert values for hemoglobin to grams per deciliter, divide by 0.6206.
** Values were log-transformed for analysis and presented as geometric means ±SD.
†† Information on C-reactive protein, erythrocyte sedimentation rate, and von Willebrand factor was available for 2406, 2440, and 2445 patients, respectively, and 3891, 3942, and 3948 controls, respectively.

tation rate, and von Willebrand factor were 0.59 (95 percent confidence interval, 0.52 to 0.66), 0.67 (95 percent confidence interval, 0.61 to 0.73), and 0.57 (95 percent confidence interval, 0.50 to 0.64), respectively. These values were similar with respect to long-term consistency to the values for systolic blood pressure (correlation coefficient, 0.66; 95 percent confidence interval, 0.60 to 0.72), diastolic blood pressure (correlation coefficient, 0.53; 95 percent confidence interval, 0.46 to 0.60), and total serum cholesterol (correlation coefficient, 0.60; 95 percent confidence interval, 0.54 to 0.66).

**Table 2. Relative Odds of Coronary Heart Disease (CHD) among Patients Who Had Levels of Inflammatory Markers in the Top Third of the Distribution of Values for Controls, as Compared with Those Who Had Values in the Bottom Third of This Distribution.**

| Factor* | Patients | | | Controls | | | Odds Ratio (95% Confidence Interval)† | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | Top Third | Middle Third | Bottom Third | Top Third | Middle Third | Bottom Third | Adjusted for Age, Sex, and Period | Adjusted for Age, Sex, Period, and Smoking Status | Adjusted for Age, Sex, Period, and CHD Risk Factors | Adjusted for Age, Sex, Period, CHD Risk Factors, and Socioeconomic Status | Adjusted for Age, Sex, Period, CHD Risk Factors, Socioeconomic Status, and Levels of Other Inflammatory Markers |
| | *no. of participants* | | | | | | | | | | |
| **Inflammatory markers** | | | | | | | | | | | |
| Up to 2459 patients and 3969 controls | | | | | | | | | | | |
| C-reactive protein level (2.0 and 0.78 mg/liter) | 1090 | 742 | 574 | 1294 | 1300 | 1297 | 1.92 (1.68–2.18) | 1.76 (1.54–2.01) | 1.45 (1.25–1.69)‡ | 1.45 (1.25–1.68) | 1.36 (1.16–1.58) |
| Erythrocyte sedimentation rate (10 and 4 mm during 1st hr) | 922 | 733 | 785 | 1220 | 1201 | 1521 | 1.64 (1.44–1.87) | 1.55 (1.36–1.78) | 1.31 (1.13–1.51) | 1.30 (1.13–1.51) | 1.18 (1.01–1.38) |
| von Willebrand factor level (124 and 88 IU/dl) | 913 | 789 | 743 | 1310 | 1316 | 1322 | 1.23 (1.09–1.40) | 1.19 (1.05–1.35) | 1.12 (0.98–1.28) | 1.11 (0.97–1.27) | 1.06 (0.92–1.22) |
| Up to 2083 patients without evidence of CHD at base line and 3969 controls§ | | | | | | | | | | | |
| C-reactive protein level (2.0 and 0.78 mg/liter) | 887 | 635 | 520 | 1294 | 1300 | 1297 | 1.81 (1.58–2.07) | 1.65 (1.43–1.89) | 1.37 (1.17–1.60) | 1.37 (1.17–1.59) | 1.30 (1.10–1.52) |
| Erythrocyte sedimentation rate (10 and 4 mm during 1st hr) | 766 | 633 | 671 | 1220 | 1201 | 1521 | 1.53 (1.34–1.77) | 1.45 (1.26–1.68) | 1.22 (1.05–1.43) | 1.22 (1.04–1.42) | 1.10 (0.95–1.28) |
| von Willebrand factor level (124 and 88 IU/dl) | 752 | 675 | 643 | 1310 | 1316 | 1322 | 1.25 (1.09–1.43) | 1.20 (1.05–1.37) | 1.15 (0.99–1.33) | 1.14 (0.98–1.31) | 1.11 (0.94–1.31) |
| **Some established CHD risk factors** | | | | | | | | | | | |
| Up to 2459 patients and 3969 controls | | | | | | | | | | | |
| Total cholesterol level (6.80 and 5.85 mmol/liter) | 1150 | 826 | 482 | 1309 | 1320 | 1334 | 2.62 (2.29–3.00) | 2.68 (2.30–3.08) | 2.33 (2.01–2.70) | 2.35 (2.03–2.74) | 2.38 (2.05–2.77) |
| Current smoking (vs. never)¶ | 1417 | 544 | 498 | 1941 | 851 | 1177 | 1.74 (1.53–1.98) | 1.74 (1.53–1.98) | 1.87 (1.63–2.22) | 1.87 (1.62–2.16) | 1.75 (1.51–2.03) |
| Systolic blood pressure (147 and 131 mm Hg) | 1041 | 742 | 670 | 1308 | 1240 | 1402 | 1.72 (1.51–1.96) | 1.85 (1.62–2.10) | 1.51 (1.32–1.74) | 1.50 (1.30–1.73) | 1.50 (1.30–1.74) |

* Values in parentheses refer to cutoffs of values for the top and bottom thirds, respectively, of the distribution of values in controls. To convert values for cholesterol to milligrams per deciliter, divide by 88.4.

† "Period" refers to the calendar year of recruitment. Markers of socioeconomic status were nonmanual occupation, education beyond high school, home ownership, and type of residence.

‡ The adjusted odds ratios for coronary heart disease for C-reactive protein with the use of alternative comparisons were as follows: 1.55 (95 percent confidence interval, 1.31 to 1.84) for the top quarter as compared with the bottom quarter of distribution, 1.65 (95 percent confidence interval, 1.36 to 2.00) for the top fifth as compared with the bottom fifth of distribution, and 1.20 (95 percent confidence interval, 1.12 to 1.27) per standard deviation (log scale).

§ These analyses exclude patients with electrocardiographic evidence of coronary heart disease or a history of angina. As described in the Methods section, persons with a history of myocardial infarction were excluded from the base-line survey of the Reykjavik Study.

¶ Smoking status does not reflect thirds of a continuous distribution: frequencies among patients and controls are tabulated for current smokers (top), former smokers (middle), and nonsmokers (bottom), and odds ratios compare current smokers with those who never smoked.

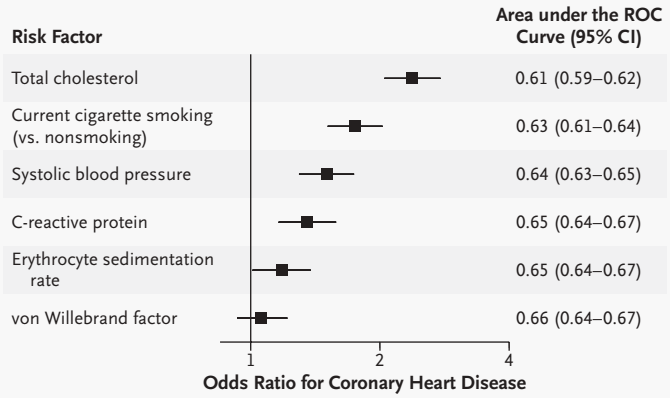


**Figure 1. Odds Ratios for Coronary Heart Disease among 2459 Patients with Coronary Heart Disease and 3969 Controls.**

Comparisons are between patients and controls with values in the top third and those in the bottom third of the distribution of values for controls, except for comparisons involving smoking status. Squares denote odds ratios, and horizontal lines represent 95 percent confidence intervals. The information plotted in this figure is based on odds ratios listed in the next-to-last column of Table 2. Logistic-regression analysis was used to calculate the areas under the receiver-operating-characteristic (ROC) curve after adjustment for age, sex, and period, with data on major established risk factors and inflammatory markers added to the model in the order of the strength of each variable's association with coronary heart disease.

### INFLAMMATORY MARKERS AND INCIDENT CORONARY HEART DISEASE

The odds ratio for coronary heart disease was 1.92 (95 percent confidence interval, 1.68 to 2.18; $\chi^2$= 105, with 1 df) among patients with values in the top third (cutoff value, 2.0 mg per liter), as compared with the bottom third (cutoff value, 0.78 mg per liter), of base-line C-reactive protein concentrations in the control group. The odds ratio fell to 1.45 (95 percent confidence interval, 1.25 to 1.68; $\chi^2$= 28, with 1 df) after adjustment for smoking status, other established coronary risk factors, and indicators of socioeconomic status (Table 2). Comparisons between the top and bottom thirds of patients and controls with respect to the other markers gave the following adjusted odds ratios for coronary heart disease: for erythrocyte sedimentation rate (cutoff value of 10 mm in first hour of measurement for the top third and 4 mm in first hour for the bottom third), 1.30 (95 percent confidence interval, 1.13 to 1.51; $\chi^2$=13, with 1 df), and for von Willebrand factor (cutoff value of 124 IU per deciliter for the top third and 88 IU per deciliter for the bottom third), 1.11 (95 percent confidence interval, 0.97 to 1.27; $\chi^2$=26, with 1 df) (Table 2 and Fig. 1). The calculated areas under receiver-operating-characteristic curves indicate that information on the C-reactive protein concentration (and the other inflammatory markers that were assessed) provided comparatively little additional predictive value over that provided by assessment of major established risk factors (Fig. 1).

These findings were not materially changed in analyses restricted to the 2083 patients without evidence of coronary heart disease at base line (Table 2), to the 2206 patients with C-reactive protein values who had a confirmed myocardial infarction or died of coronary heart disease, or to the participants without evidence of acute-phase reactions at the base-line examination (i.e., this analysis excluded 132 patients and 152 controls with a C-reactive protein concentration of more than 10 mg per liter[15] or an erythrocyte sedimentation rate of more than 30 mm during the first hour). The findings were also unaffected by changes in the cutoff values (e.g., analyses of quarters or fifths, or according to increases of 1 SD) (Table 2).

Associations between the C-reactive protein concentration and the risk of coronary heart disease did not vary significantly according to established risk factors, such as smoking or increased blood lipid concentrations, blood pressure, or body-mass index (data not shown). An exploratory analysis suggested the possibility of more extreme odds ratios among the 1049 patients who died of coronary heart disease or had a nonfatal myocardial infarction within 10 years after enrollment (odds ratio, 1.84; 95 percent confidence interval, 1.49 to 2.28), as compared with the 1357 patients who had such an event after the first decade (odds ratio, 1.26; 95 percent confidence interval, 1.05 to 1.51). Such a trend, however, was not observed in the updated meta-analysis, described below, which was based on published data from 22 studies[2,4,13,14,16-33] (Fig. 2). Therefore, it requires further examination involving larger numbers of participants with individual data. Such analysis is also required for a reliable characterization of the shape of the association between C-reactive protein and coronary heart disease.

### UPDATED META-ANALYSIS

Twenty-two prospective studies of C-reactive protein (including the present study) have involved a total of 7068 patients, with a weighted mean age at entry of 57 years and a weighted mean follow-up of 12 years[2,4,13,14,16-33] (Table 3). All studies used high-sensitivity assays, and all but two[19,32] report-

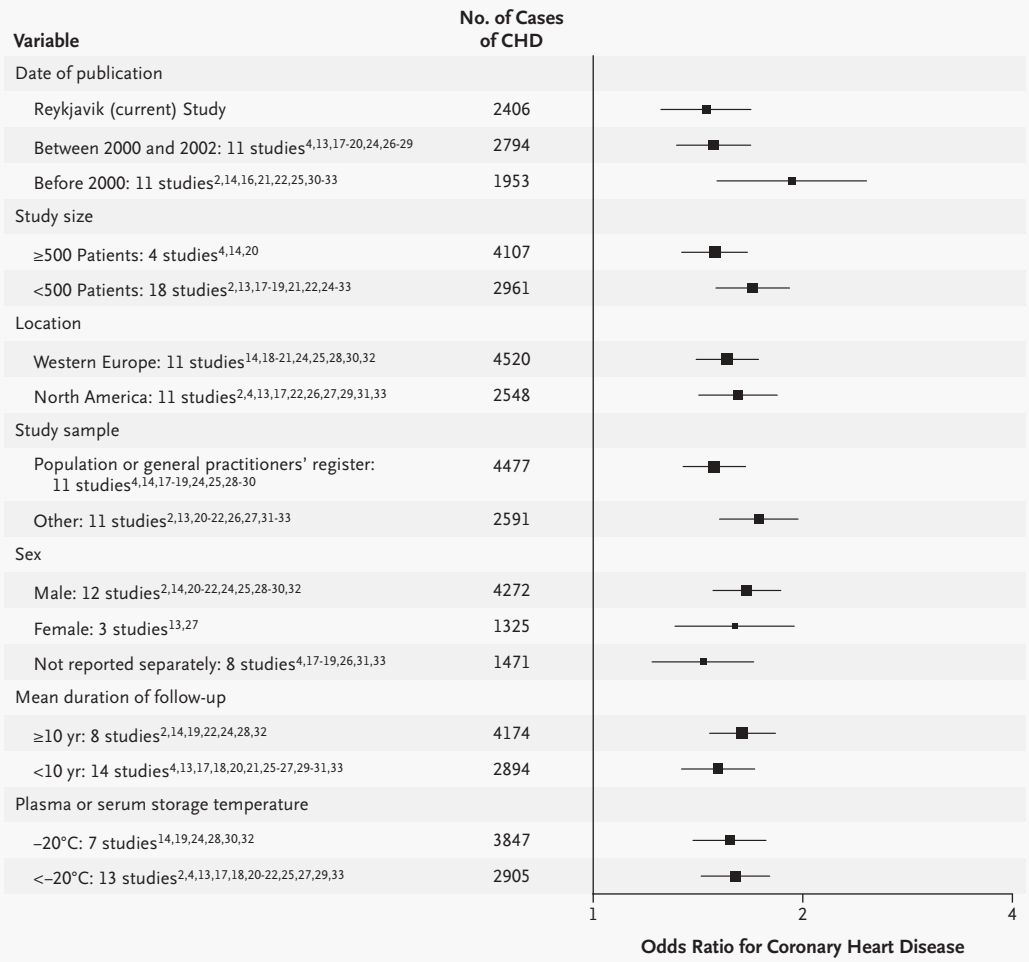


**Figure 2. Twenty-Two Prospective Studies of the Association of C-Reactive Protein Concentrations with the Risk of Coronary Heart Disease (CHD) in Essentially General Populations, Grouped According to Several Study Characteristics.**

One of the 11 studies published before 2000 was updated in 2002[13,16]; hence, data on 85 cases from this study contributed to two subtotals, but we did not double-count these cases in estimating the overall odds ratio. Two studies[17,18] published in 1999 (comprising a total of 98 cases) were not included in a previous meta-analysis of studies published before March 2000[14]; they have been included in the 11 studies published between 2000 and 2002. Although three studies published after 2000,[17-19] involving a total of 245 cases of coronary heart disease, reported results for deaths from cardiovascular causes rather than specifically from coronary heart disease, the majority of these deaths were likely to have been due to coronary heart disease. It was not possible to separate results for 77 cases of coronary revascularization from results for nonfatal myocardial infarction and death from coronary heart disease in another study.[20] The odds ratios used were those reported in studies that had adjusted for age, sex, smoking status, and other established risk factors for coronary heart disease (such as blood lipid levels, blood pressure, body-mass index, and diabetes status). The "Other" category in "Sample" includes participants selected according to various criteria (e.g., the absence of a history of coronary disease in randomized trials). The Reykjavik Study provided separate estimates for men (732 cases with C-reactive protein values) and women (674 cases with C-reactive protein values). Information on the storage temperature used for samples was unavailable for two studies involving a total of 316 cases.[26,31] Odds ratios involve comparisons of patients in the top third versus those in the bottom third of C-reactive protein concentrations. The horizontal lines represent 99 percent confidence intervals.

**Table 3. Comparison of Characteristics of Prospective Studies of C-Reactive Protein and Coronary Heart Disease (CHD) in Essentially General Populations.***

| Study | Location | Population/Sampling Method† | Time of Base-Line Survey | No. of Cases of CHD | Total No. of Partici-pants | Age Range | Male Sex | Mean Duration of Follow-up | C-Reactive Protein Assay | | | Plasma or Serum Storage Temperature |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Source | Type‡ | Standard | |
| | | | | | | *yr* | % | *yr* | | | | °C |
| Reykjavik | Iceland | Population register/complete birth cohorts | 1967–1991 | 2406 | 18,569 | 33–59 | 48 | 20 | Roche | LEIA | WHO 85/506 | –20 |
| ARIC[4] | U.S. | Listing of households/random | 1987–1989 | 615 | 15,792 | 45–64 | 43 | 8 | United Biotech | ELISA | WHO 85/506 | –70 |
| WOSCOPS[20] | Scotland | Coronary screening clinic/complete | 1989–1995 | 580 | 6,595 | 45–64 | 100 | 6 | In-house | ELISA | IFCC CRM470 | –70 |
| BRHS[14] | U.K. | General practitioners' list/random | 1978–1980 | 506 | 5,661 | 40–59 | 100 | 16 | In-house or Abbott | MEIA | WHO 85/506 | –20 |
| Women's Health Study[13] | U.S. | Female health professionals/complete | 1992–1995 | 371 | 28,345 | >45 | 0 | 8 | In-house | NS | NS | –70 |
| WHIOS[27] | U.S. | Trial screenees/complete | 1994–1998 | 280 | 93,724 | 50–79 | 0 | 3 | In-house | NS | NS | –70 |
| Caerphilly[28] | Wales | Electoral rolls/random | 1979–1983 | 249 | 2,512 | 45–59 | 100 | 14 | In-house | ELISA | NS | –20 |
| MRFIT[2] | U.S. | Industry and government employees/complete | 1973–1976 | 246 | 12,866 | 35–57 | 100 | 10 | In-house | ELISA | WHO 85/506 | –50 to –70 |
| Physicians' Health[22] | U.S. | Physicians' register/complete | 1982 | 246 | 22,071 | 40–84 | 100 | 14 | In-house | ELISA | WHO 85/506 | –80 |
| Helsinki Heart[32] | Finland | Industry employees/complete | 1981–1982 | 241 | 4,081 | 40–55 | 100 | 10 | Eucardio Laboratory | ELISA | NS | –20 |
| AFCAPS/TEXCAPS[26] | U.S. | Civilian and military clinics/complete | 1990–1993 | 216 | 6,605 | 45–73 | NS | 5 | Dade Behring | LEIA | WHO 85/506 | NS |
| Speedwell[23,30] | U.K. | General practitioners' list/complete | 1979–1982 | 165 | 1,690 | 47–67 | 100 | 6 | Dade Behring | LEIA | WHO 85/506 | –20 |
| CHS[33] | U.S. | Medicine eligibility lists/complete | 1989–1990 | 150 | 5,201 | ≥65 | 43 | 3 | In-house | ELISA | NS | –70 |
| Helsinki Aging[19] | Finland | Population register/random from birth cohorts | 1989 | 147 | 651 | 75–85 | 28 | 10 | Medix Diacor | EIA (unspecified) | WHO 85/506 | –20 |
| RHPP[33] | U.S. | Medicare beneficiaries/complete | 1995 | 145 | 3,884 | 65–79 | 43 | 3 | In-house | ELISA | WHO 85/506 | –70 |
| Glostrup[24] | Denmark | Population register/random from birth cohorts | 1976–1984 | 133 | 5,637 | 30–50 | 100 | 12 | In-house | ELISA | Commercial (Behring) | –20 |
| Quebec[29] | Canada | Population register/random | 1985 | 105 | 2,100 | 45–77 | 100 | 5 | Dade Behring | LEIA | WHO 85/506 | –80 |
| Kaiser Permanente[31] | U.S. | Insurance-plan enrollees/random | 1967–1979 | 100 | 261 | NS | NS | 5 | In-house | ELISA | NS | NS |
| Iowa 65+[17] | U.S. | Population register/complete for those >65 yr of age | 1982 | 74 | 3,673 | >65 | 41 | 4 | In-house | ELISA | WHO 85/506 | –70 |

| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MONICA Augsburg[25] | Germany | Population register/random | 1984–1992 | 53 | 5,069 | 25–74 | 100 | 7 | In-house | IRMA | WHO 85/506 | –70 |
| Hoorn[18] | Netherlands | Population register/random | 1989–1992 | 24 | 2,484 | 50–75 | 48 | 5 | In-house | ELISA | Commercial (Behring) | –70 |
| Göteborg Intervention[21] | Sweden | Hypertension screening clinic/random | 1993 | 16 | 508 | 50–72 | 100 | 3 | Dade Behring | LEIA | WHO 85/506 | –80 |

* EIA denotes enzyme immunoassay, ELISA enzyme-linked immunosorbent assay, IRMA immunoradiometric assay, LEIA latex-enhanced immunoassay, MEIA microparticle capture enzyme immunoassay, NS not specified, ARIC Atherosclerosis Risk in Communities, WOSCOPS West of Scotland Coronary Prevention Study, BRHS British Regional Heart Study, U.K. United Kingdom, WHIOS Women's Health Initiative Observational Study, MRFIT Multiple Risk Factor Intervention Trial, AFCAPS/TEXCAPS Air Force/Texas Coronary Atherosclerosis Prevention Study, CHS Cardiovascular Health Study, RHPP Rural Health Promotion Project, MONICA Monitoring Trends and Determinants in Cardiovascular Disease.

† A random sampling method was one in which a randomly selected subgroup of eligible persons was invited to participate. A complete sampling method was one in which all eligible persons in the study population were invited to participate.

‡ Eligibility was based on more restrictive criteria in the following studies: WOSCOPS (persons with a low-density lipoprotein level of 174 to 232 mg per deciliter [4.5 to 6.0 μmol per liter]), WHIOS (women ineligible for a randomized trial of hormone-replacement therapy and dietary modification), MRFIT (persons with a high risk of coronary heart disease on the basis of blood pressure, cholesterol concentration, and smoking status), Helsinki Heart Study (persons with high levels of non–high-density lipoprotein cholesterol), AFCAPS/TEXCAPS (persons with below-average levels of high-density lipoprotein cholesterol), and the Göteborg Intervention Study (persons with hypertension).

ed adjustment for at least smoking status and some other established risk factors for coronary heart disease. There was evidence of heterogeneity between these studies ($\chi^2$=46, with 21 df; P=0.001), but with the exception of the date of publication ($\chi^2$=15, with 2 df; P<0.001), characteristics such as sample size ($\chi^2$=4.0, with 1 df; P=0.04), location ($\chi^2$=0.3, with 1 df; P=0.58), sampling method ($\chi^2$=5.2, with 1 df; P=0.02), sex of participants ($\chi^2$=3.4, with 2 df; P=0.18), mean duration of follow-up ($\chi^2$=1.6, with 1 df; P=0.20), and sample storage temperature ($\chi^2$=0.1, with 1 df; P=0.77) did not account for much of the overall heterogeneity (Fig. 2).

The tendency toward more extreme findings in studies published before 2000 is consistent with the preferential publication of positive results in earlier studies. Restriction of analyses to the four studies involving more than 500 patients,[4,14,20] comprising 4107 cases of coronary heart disease, should limit any such bias, and yielded a combined odds ratio of 1.49 (95 percent confidence interval, 1.37 to 1.62; $\chi^2$=10.6, with 3 df; P=0.01). This value is somewhat smaller than the overall odds ratio of 1.58 (95 percent confidence interval, 1.48 to 1.68) derived from combining all 22 studies.

A previous meta-analysis[6] of prospective studies of the effect of the erythrocyte sedimentation rate (based on 1703 cases of coronary heart disease) reported an odds ratio for coronary heart disease of about 1.3 (95 percent confidence interval, 1.2 to 1.5), and this estimate is reinforced by the odds ratio of 1.33 (95 percent confidence interval, 1.22 to 1.44) that we calculated in our updated meta-analysis (which involved an additional 2683 cases from a further two studies[34]). The present updated meta-analysis of prospective studies of von Willebrand factor (which adds 2445 cases of coronary heart disease to the previous total of 1524 cases) yielded an odds ratio of 1.23 (95 percent confidence interval, 1.14 to 1.33), which is probably weaker than the previous estimate of about 1.5 (95 percent confidence interval, 1.1 to 2.0).[7]

## DISCUSSION

We found that the decade-to-decade consistency of values for C-reactive protein, the erythrocyte sedimentation rate, and von Willebrand factor is similar to that of values for blood pressure and total serum cholesterol concentration, suggesting that these inflammatory markers are sufficiently stable for

potential use in the long-term prediction of coronary heart disease. Our findings — reinforced by an updated meta-analysis — indicate, however, that the odds ratio for coronary heart disease in people with elevated C-reactive protein values is lower than that reported recently. Whereas a previous meta-analysis[14] of studies published before 2000 (based on 1953 cases of coronary heart disease) reported an odds ratio for coronary heart disease of about 2.0 (95 percent confidence interval, 1.6 to 2.5), our updated meta-analysis, which adds 5115 cases of coronary heart disease from a further 12 studies, yielded an odds ratio of about 1.5 in a comparison of people with base-line values in the top third with those with base-line values in the bottom third for the population. Moreover, in comparison with major established risk factors (such as an increased total serum cholesterol concentration and cigarette smoking), the C-reactive protein concentration was a relatively moderate predictor of the risk of coronary heart disease and added only marginally to the predictive value of established risk factors for coronary heart disease. These findings suggest that recent recommendations regarding the use of measurements of C-reactive protein in the prediction of coronary heart disease may need to be reviewed.[3]

The potential limitations of our study merit careful consideration. The validity of our measurements is demonstrated by the reasonably high decade-to-decade consistency of C-reactive protein values recorded in paired samples from 379 participants (a level of stability that was at least as high as those recorded in previous studies with sampling intervals of just one to five years[35-38]). Further validation is suggested by the finding of the expected base-line associations of C-reactive protein with other inflammatory markers and with established coronary risk factors.

The mean values and the distributions of several established coronary risk factors (and the strength of their associations with the risk of coronary heart disease) in our study were generally similar to those reported in other western European populations.[8] Therefore, although the relative homogeneity of the Reykjavik population should have minimized certain residual biases (such as that due to differences in socioeconomic status), the present findings should have wider relevance. Only total serum cholesterol concentrations were measured in the present study (rather than those of its subfractions, which have opposing effects on the risk of coronary heart disease), thereby underestimating the predictive ability of lipid concentrations (and potentially overestimating the adjusted predictive value of the C-reactive protein concentration).

No information was recorded on the use of aspirin and statins, which, like hormone-replacement treatment, may alter C-reactive protein values. However, fewer than 5 percent of the women in this study reported the use of such hormonal treatment during recruitment, and the use of aspirin and of statins was similarly uncommon in the general middle-aged population of Reykjavik between 1967 and 1991. We did not address the separate issues of the predictive value of inflammatory markers with respect to the risk of cardiac complications among patients recently hospitalized for acute coronary syndromes[39] or the long-term risk of coronary heart disease in patients with a history of cardiovascular disease.[14]

As suggested by the statement of the Centers for Disease Control and Prevention and the American Heart Association,[3] further clarification of the predictive value of C-reactive protein in coronary heart disease in general populations will require the pooling of studies on the basis of data for individual participants from each of the available prospective studies. Such a strategy will permit more complete adjustment for other risk factors and for within-person fluctuations of C-reactive protein levels, more precise quantification of the associations in particular subgroups (such as age-, sex-, and duration-specific associations as well as assessments of combinations of inflammatory markers), more reliable characterization of the shape of any dose–response relation, and more detailed investigation of potential sources of heterogeneity.

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# C-Reactive Protein Adds to the Predictive Value of Total and HDL Cholesterol in Determining Risk of First Myocardial Infarction

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# C-Reactive Protein Adds to the Predictive Value of Total and HDL Cholesterol in Determining Risk of First Myocardial Infarction

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***Background***—C-reactive protein (CRP) is a sensitive marker of inflammation, and elevated levels have been associated with future risk of myocardial infarction (MI). However, whether measurement of CRP adds to the predictive value of total cholesterol (TC) and HDL cholesterol (HDL-C) in determining risk is uncertain.

***Methods and Results***—Among 14 916 apparently healthy men participating in the Physicians' Health Study, baseline levels of CRP, TC, and HDL-C were measured among 245 study subjects who subsequently developed a first MI (cases) and among 372 subjects who remained free of cardiovascular disease during an average follow-up period of 9 years (controls). In univariate analyses, high baseline levels of CRP, TC, and TC:HDL-C ratio were each associated with significantly increased risks of future MI (all $P$ values $<0.001$). In multivariate analyses, models incorporating CRP and lipid parameters provided a significantly better method to predict risk than did models using lipids alone (all likelihood ratio test $P$ values $<0.003$). For example, relative risks of future MI among those with high levels of both CRP and TC (RR=5.0, $P=0.0001$) were greater than the product of the individual risks associated with isolated elevations of either CRP (RR=1.5) or TC (RR=2.3). In stratified analyses, baseline CRP level was predictive of risk for those with low as well as high levels of TC and the TC:HDL-C ratio. These findings were virtually identical in analyses limited to nonsmokers and after control for other cardiovascular risk factors.

***Conclusions***—In prospective data from a large cohort of apparently healthy men, baseline CRP level added to the predictive value of lipid parameters in determining risk of first MI. **(*Circulation*. 1998;97:2007-2011.)**

**Key Words:** myocardial infarction ■ epidemiology ■ C-reactive protein ■ risk factors ■ cholesterol

C-reactive protein is a sensitive marker of systemic inflammation, and prospective data from a population of apparently healthy men indicate that baseline levels predict risk of first MI.[1] Specifically, among men free of prior cardiovascular disease participating in the Physicians' Health Study, we recently reported that those with baseline levels of CRP in the highest quartile had a threefold increase in risk of developing future MI compared with those with levels in the lowest quartile (relative risk, 2.9; $P<0.001$).[1] In this population, risk estimates were stable over long periods of time, were significant among the subgroup of nonsmokers, and were independent of a number of other risk factors for cardiovascular disease. As such, these data demonstrate that CRP is a marker of cardiovascular risk not only among those with stable and unstable angina,[2–4] the elderly,[5] and selected high-risk patients[6] but also among individuals with no current evidence of cardiovascular disease.[1]

**See p 2000**

From a clinical perspective, the question has been raised as to whether CRP adds to the ability to predict atherothrombotic risk with more confidence than currently achievable with standard lipid screening. We therefore reexamined data from the Physicians' Health Study to determine whether measuring CRP added to the predictive value of TC and HDL-C in determining subsequent risk of first MI. In addition, we sought to determine whether the risks of future MI associated with CRP were present among those with low-risk as well as high-risk profiles as assessed by baseline lipid status.

## Methods

In the US Physicians' Health Study,[7] 14 916 men initially free of reported cardiovascular disease, cancer, or other chronic illness provided a baseline plasma sample before randomization and were prospectively followed up for the first occurrence of MI. Details of the Physicians' Health Study, a randomized, double-blind, placebo-controlled trial of aspirin and $\beta$-carotene in the primary prevention of cardiovascular disease and cancer, have been described elsewhere, as have the methods used to collect, store, and process baseline blood specimens.[1,7] Morbidity follow-up was >99% complete and mortality follow-up was 100% over the ≈9 years of follow-up in the present analysis. Reported MI that occurred during the study follow-up period was confirmed if medical record review demonstrated symptoms consistent with MI and the presence of either diagnostic

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**Selected Abbreviations and Acronyms**

CRP = C-reactive protein
HDL-C = HDL cholesterol
MI = myocardial infarction
TC = total cholesterol

ECG changes or cardiac enzymes. Silent MIs were not included because they could not be accurately dated. Deaths due to MI were confirmed when autopsy reports, symptoms, circumstances of death, and a history of coronary disease were consistent with this diagnosis.

In our original description of CRP in the Physicians' Health Study, we reported data from 246 initially healthy study participants who subsequently developed a first MI (cases) and from a group of 543 age- and smoking-matched study participants who remained free of cardiovascular disease during study follow-up (controls).[1] For each of these case and control subjects, blood collected at enrollment was thawed and assayed for CRP by methods described elsewhere.[1] In addition, baseline blood samples of 245 cases (99%) and 372 controls (69%) were successfully analyzed for TC and HDL-C.[8] These 617 initially healthy participants in the Physicians' Health Study form the basis for this report.

Means or proportions for baseline clinical characteristics and measured risk factors were computed for the case and control groups and compared by Student's $t$ test or the $\chi^2$ statistic. Univariate logistic regression analyses were used to determine whether baseline levels of CRP, TC, and the TC:HDL-C ratio were predictive of future risk of MI. In these analyses, baseline levels were divided into quartiles based on the distribution of the control values.

On an a priori basis, we evaluated the combined role of hypercholesterolemia and elevations of CRP in predicting risk of MI in three stages, which allowed us to explore from a clinical perspective the sensitivity and robustness of any findings to the choice of alternative cut points. Thus, we first used the likelihood ratio test to determine whether logistic regression models that included lipid parameters and CRP provided a significantly better fit than did logistic regression models limited to lipid parameters alone. In these analyses, lipid parameters and log-normalized CRP levels were both treated as continuous variables.

Second, logistic regression analyses were performed in which the referent group was those individuals with both TC and CRP levels below the 75th percentile cut point for each of these parameters (TC−, CRP−). In this analysis, relative risks of developing a first MI were computed for individuals with hypercholesterolemia alone (TC+, CRP−), for individuals with elevations of CRP alone (TC−, CRP+), and for individuals with both hypercholesterolemia and elevations of CRP (TC+, CRP+).

Third, we divided case and control subjects into nine groups according to tertile of TC and CRP level. In this analysis, logistic regression was used to simultaneously evaluate the risks of first MI in each of these groups, with those with the lowest tertile of both TC and CRP used as the referent group. Similar analyses were performed after case and control subjects were divided into nine groups according to tertile of the TC:HDL-C ratio.

Finally, to evaluate whether increasing levels of CRP were a predictor of risk for first MI among those with low as well as high lipid parameters, we performed stratified analyses in which tests for trends across increasing quartiles of CRP were computed separately for those with levels of TC and the TC:HDL-C ratio above or below the approximate median value for the study group.

All analyses were repeated for the subgroup of nonsmokers, and additional multivariate analyses were used to control for the presence or absence of other cardiovascular risk factors. $P$ values are two-tailed, and 95% CIs were computed.

## Results

Table 1 presents the baseline clinical characteristics of the subjects evaluated. Because study participants in our original report were matched on smoking and age, these variables were similar among those who subsequently developed a first MI (cases) and among those who remained free of reported cardiovascular disease over the follow-up period (controls). As expected, case subjects had less favorable lipid profiles than did control subjects.

**TABLE 1. Baseline Characteristics of Study Participants Who Subsequently Developed First MI (Cases) and Those Who Remained Free of Reported Vascular Disease During the Average 8-Year Follow-up Period (Controls)**

| | Cases (N=245) | Controls (N=372) | $P$ |
|---|---|---|---|
| Age, y±SD | 58.2±8.6 | 59.4±8.9 | ... |
| Smoking status, % | | | |
| Never | 44.3 | 41.8 | ... |
| Past | 40.6 | 39.9 | |
| Current | 15.2 | 18.3 | |
| Diabetes, % | 5.3 | 3.2 | 0.2 |
| Body mass index, kg/m$^2$ | 25.6 | 24.9 | 0.01 |
| Systolic blood pressure, mm Hg | 128.5 | 127.3 | 0.3 |
| Diastolic blood pressure, mm Hg | 80.1 | 79.0 | 0.08 |
| TC level, mg/dL | 229.4 | 210.9 | 0.001 |
| HDL-C level, mg/dL | 46.4 | 49.3 | 0.008 |
| TC:HDL-C ratio | 5.3 | 4.5 | 0.001 |

Correlations between log-normalized CRP and TC ($r$=0.15) and between log-normalized CRP and HDL-C ($r$=−0.15) were small in magnitude. Thus, <3% of the variance in CRP levels in these data was explained by the lipid parameters.

In univariate analyses, baseline levels of CRP, TC, and the TC:HDL-C ratio were each associated with increased risk of future MI (all $P$ values <0.001). As shown in Table 2, the relative risk of future MI increased 38% with each increasing quartile of CRP (95% CI, 19% to 61%; $P$<0.001), 62% for each increasing quartile of TC (95% CI, 39% to 90%; $P$<0.001), and 59% for each increasing quartile of the TC:HDL-C ratio (95% CI, 37% to 86%; $P$<0.001). The 95% CIs for these risk estimates overlap and are consistent with prior reports from the entire cohort.[1,4]

To evaluate whether CRP added to the predictive value of lipids on risk of first MI, likelihood ratio tests were used to compare the fit of prediction models using CRP and lipids to the fit of models using lipids alone. In these analyses, the assessment of both parameters provided a significantly improved ability to predict risk. For example, models including both CRP and TC provided a significant improvement in prediction ($P$=0.003) compared with models including only

**TABLE 2. Relative Risks of First MI Associated With Each Quartile Increase of CRP, TC, and TC:HDL-C Ratio**

| Parameter | RR | 95% CI | $P$ |
|---|---|---|---|
| CRP | 1.38 | 1.19–1.61 | 0.0001 |
| TC | 1.62 | 1.39–1.90 | 0.0001 |
| TC:HDL-C | 1.59 | 1.37–1.86 | 0.0001 |

**TABLE 3. Relative Risks of First MI According to the Presence (TC+) or Absence (TC−) of TC Levels in Excess of the 75th Percentile of the Control Distribution (234 mg/dL) and/or the Presence (CRP+) or Absence (CRP−) of CRP Levels in Excess of the 75th Percentile of the Control Distribution (2.11 mg/L)**

| | CRP−, TC− | CRP+, TC− | CRP−, TC+ | CRP+, TC+ |
|---|---|---|---|---|
| Crude relative risk | 1.0 | 1.5 | 2.3 | 5.0 |
| 95% CI | ... | 0.9–2.4 | 1.5–3.7 | 2.5–9.8 |
| *P* | ... | 0.1 | 0.0003 | 0.0001 |
| Adjusted relative risk* | 1.0 | 1.4 | 2.1 | 5.2 |
| 95% CI | ... | 0.8–2.3 | 1.3–3.4 | 2.5–10.5 |
| *P* | ... | 0.02 | 0.002 | 0.0001 |

*Adjusted for family history of coronary artery disease, history of hypertension, body mass index, diabetes, age, and smoking status.

TC, whereas models involving CRP significantly improved prediction compared with models based solely on the TC:HDL-C ratio ($P=0.002$) or on TC and HDL-C entered as separate variables ($P=0.002$). These relationships were not significantly altered in models limited to nonsmokers or that further controlled for the effects of other cardiovascular risk factors.

Table 3 presents the relative risks of first MI in analyses in which study subjects were categorized as being above or below the 75th percentile cut point for TC and CRP. As shown, compared with those with levels of TC and CRP less than the 75th percentile cut point for each parameter (TC−, CRP−), those with elevations of TC alone (TC+, CRP−) had a 2.3-fold increase in risk, whereas those with elevations of CRP alone (TC−, CRP+) had a 1.5-fold increase in risk. In contrast, the risk of first MI associated with elevations of both TC and CRP (TC+, CRP+) was increased 5-fold (RR=5.0; 95% CI, 2.5 to 9.8; $P=0.0001$). As shown in Table 3 and in Fig 1, these effects were not significantly altered in analyses controlling for other risk factors.

Fig 2 illustrates the relative risks of first MI in analyses in which study participants were stratified into nine groups according to tertile of TC as well as tertile of CRP. As shown, risks of future MI increased with each of these parameters such that those in the highest tertile of both TC and CRP had a relative risk of first MI 5.3 times that of individuals in the lowest tertile of both parameters (95% CI, 2.4 to 11.7; $P=0.0001$). Similarly, Fig 3 illustrates the relative risks of first MI in analyses in which study participants were stratified into nine groups according to tertile of the TC:HDL-C ratio as well as tertile of CRP.

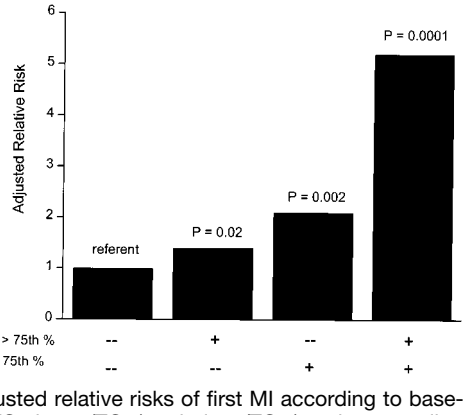


**Figure 1.** Adjusted relative risks of first MI according to baseline levels of TC above (TC+) or below (TC−) 75th percentile of control group (234 mg/dL) and baseline CRP levels above (CRP+) or below (CRP−) 75th percentile of control group (2.11 mg/L).

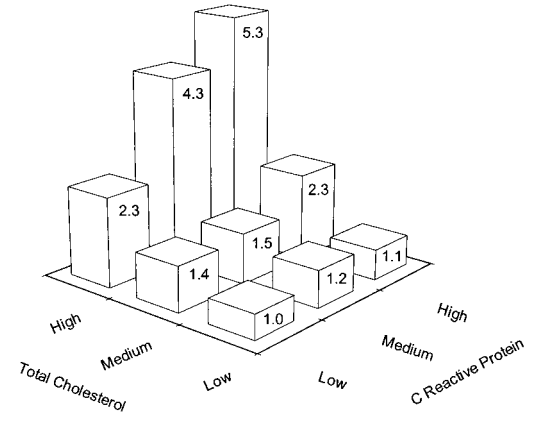


**Figure 2.** Relative risks of first MI among apparently healthy men associated with high (>223 mg/dL), middle (191 to 223 mg/dL), and low (<191 mg/dL) tertiles of TC and high (>1.69 mg/L), middle (0.72 to 1.69 mg/L), and low (<0.72 mg/L) tertiles of CRP.

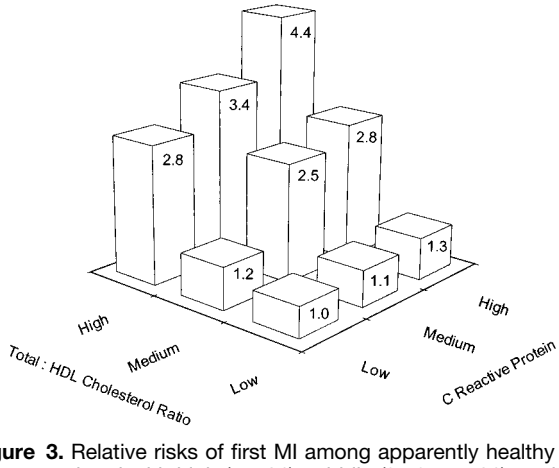


**Figure 3.** Relative risks of first MI among apparently healthy men associated with high (>5.01), middle (3.78 to 5.01), and low (<3.78) tertiles of the TC:HDL-C ratio and high (>1.69 mg/L), middle (0.72 to 1.69 mg/L), and low (<0.72 mg/L) tertiles of CRP.

**TABLE 4. Relative Risks of First MI According to Baseline Levels of CRP, Stratified by Baseline Lipid and Lipoprotein Levels**

| | Quartile of CRP (range, mg/L) | | | | |
|---|---|---|---|---|---|
| | 1 (≤0.55) | 2 (0.56–1.14) | 3 (1.15–2.10) | 4 (≥2.11) | *P* for Trend |
| TC ≤210 | | | | | |
| All subjects | | | | | |
| RR | 1.0 | 1.5 | 1.6 | 2.5 | .03 |
| 95% CI | ... | 0.6–3.5 | 0.7–3.6 | 1.1–5.6 | |
| *P* | ... | 0.4 | 0.3 | 0.03 | |
| Nonsmokers | | | | | |
| RR | 1.0 | 1.4 | 1.2 | 2.1 | .1 |
| 95% CI | ... | 0.6–3.3 | 0.5–3.0 | 0.9–5.0 | |
| *P* | ... | 0.5 | 0.6 | 0.09 | |
| TC >210 | | | | | |
| All subjects | | | | | |
| RR | 1.0 | 2.4 | 3.1 | 3.1 | .001 |
| 95% CI | ... | 1.2–4.9 | 1.6–5.9 | 1.6–6.0 | |
| *P* | ... | 0.01 | 0.001 | 0.001 | |
| Nonsmokers | | | | | |
| RR | 1.0 | 2.2 | 3.1 | 3.6 | .001 |
| 95% CI | ... | 1.1–4.7 | 1.5–6.2 | 1.7–7.6 | |
| *P* | ... | 0.03 | 0.002 | 0.001 | |
| TC:HDL-C ≤4.0 | | | | | |
| All subjects | | | | | |
| RR | 1.0 | 2.0 | 2.2 | 3.0 | .03 |
| 95% CI | ... | 0.7–5.4 | 0.8–5.7 | 1.1–7.9 | |
| *P* | ... | 0.2 | 0.1 | 0.03 | |
| Nonsmokers | | | | | |
| RR | 1.0 | 2.0 | 1.7 | 2.7 | .08 |
| 95% CI | ... | 0.7–5.6 | 0.6–4.5 | 1.0–7.5 | |
| *P* | ... | 0.2 | 0.4 | 0.05 | |
| TC:HDL-C >4.0 | | | | | |
| All subjects | | | | | |
| RR | 1.0 | 1.6 | 2.2 | 2.3 | .008 |
| 95% CI | ... | 0.8–3.1 | 1.2–4.2 | 1.2–4.2 | |
| *P* | ... | 0.2 | 0.01 | 0.01 | |
| Nonsmokers | | | | | |
| RR | 1.0 | 1.5 | 2.2 | 2.6 | .004 |
| 95% CI | ... | 0.8–3.0 | 1.1–4.2 | 1.3–5.1 | |
| *P* | ... | 0.2 | 0.02 | 0.007 | |

Table 4 presents the relative risks of first MI according to baseline levels of CRP in analyses in which the study population was stratified according to baseline lipid profile. As shown, statistically significant associations were found between baseline level of CRP and risk of first MI for study participants with low as well as high levels of TC and the TC:HDL-C ratio. Similar relationships were found in analyses limited to nonsmokers.

## Discussion

In these prospective data deriving from a large cohort of apparently healthy men, baseline CRP level added to the predictive value of TC and HDL-C in determining risk of first MI. Indeed, interactive models evaluating elevations of CRP and lipids raise the possibility that the joint effects of both risk factors may be slightly greater than the product of the individual effects of each risk factor considered separately. Moreover, baseline level of CRP is a predictor of risk of first MI for men at low as well as high risk as determined by their lipid profiles. These relationships were minimally altered in analyses either limited to nonsmokers or adjusted for other risk factors, including hypertension, body mass, diabetes, and family history of coronary disease. Finally, these results were

robust to the choice of several cut points for both CRP and lipid parameters.

The present data describing at least additive relationships between CRP and lipids in terms of risk prediction extend prior findings relating CRP to cardiovascular disease.[1–6] Specifically, elevated levels of CRP are associated with increased risks of MI or sudden death among those with stable and unstable angina pectoris,[2–4] as well as coronary heart disease in the elderly[5] and coronary mortality among high-risk patients.[6] However, because CRP levels increase in response to acute ischemia and are chronically elevated among smokers,[9] it had been uncertain whether the inflammation detected by CRP in these studies is causal or due to the effects of other factors, such as ischemia or cigarette consumption. Moreover, these prior studies did not evaluate whether the effects of CRP were present among those with high- as well as low-risk lipid profiles or whether the risks associated with CRP were additive to those determined by standard lipid analysis.

All the apparently healthy men in the Physicians' Health Study were free of any history of cardiovascular disease when blood samples were obtained. Thus, the potential for confounding by the presence of symptomatic ischemia in these data is unlikely. Moreover, the risks of future MI associated with CRP in the Physicians' Health Study were present for nonsmokers, providing evidence against the possibility that observed effects are simply the result of cigarette consumption.[9]

The fact that lipid parameters and CRP levels were measured only once at baseline in our study is a potential limitation, because random fluctuation in these parameters over time would tend to increase the variance in our data. However, if random, such variation would most likely bias our findings toward a null result and lead to an underestimation of true predictive values. Conversely, because assays for CRP as well as all lipid parameters were performed on the same baseline plasma sample, these data are compatible with the potential utility of simultaneous assessment of inflammatory markers and lipid parameters as a method of risk detection.

It is currently estimated that up to half of all MIs in the United States occur among individuals with moderate to low risk as determined by assessment of TC and HDL-C levels.[10] The present data raise the possibility that assessment of CRP may provide a method of determining risk of future MI among apparently low-risk individuals, including nonsmokers. Because relatively simple interventions such as exercise, weight loss, and diet restriction can lead to substantial reductions in risk of first MI,[10] assessment of CRP might have clinical utility if improved risk stratification leads to improved compliance with lifestyle modification. Confirmation of these data in other prospective cohorts is thus of critical importance, as are studies in women, for whom data are lacking on the predictive value of inflammatory markers.

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# C-Reactive Protein and the Future Risk of Thromboembolic Stroke in Healthy Men

J. David Curb, Robert D. Abbott, Beatriz L. Rodriguez, Pamela Sakkinen, Jordan S. Popper, Katsuhiko Yano and Russell P. Tracy

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# C-Reactive Protein and the Future Risk of Thromboembolic Stroke in Healthy Men

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***Background***—Evidence suggests that C-reactive protein (CRP) is related to thromboembolic (TE) stroke. Whether associations are altered in the presence of other risk factors is unclear. The purpose of this study was to additionally assess the relation between CRP and TE stroke.

***Methods and Results***—On the basis of 20 years of follow-up after CRP measurement, 259 cases of TE stroke were identified and compared with 1348 controls. Subjects were aged 48 to 70 years when CRP was measured. Levels of CRP were positively associated with TE stroke throughout the 20 years of follow-up. Although associations were modest within 5 years of CRP measurement, the odds of stroke in the top versus bottom CRP quartile increased over time to a 3.8-fold excess by 10 to 15 years into follow-up ($P<0.001$). For men without hypertension or diabetes, the overall corresponding odds were 1.6 to 1.7 ($P<0.05$). In men ≤55 years of age, the odds increased to a 3-fold excess ($P=0.006$), and in nonsmokers, there was a 5.8-fold excess ($P<0.001$). Associations in past and current smokers, in men >55 years of age, and in those with hypertension or diabetes were not significant.

***Conclusions***—Findings suggest that elevated CRP in middle adulthood and in men with healthier risk factor profiles may be important as a risk factor for TE stroke. Use of CRP levels as a clinical screen to identify an increased risk of cardiovascular disease in otherwise healthy men warrants consideration. **(*Circulation*. 2003;107:2016-2020.)**

**Key Words:** stroke ■ inflammation ■ epidemiology

C-reactive protein (CRP) is an acute-phase protein thought to be a measure of inflammatory processes in cardiovascular disease.[1–12] Although evidence suggests that CRP is associated with stroke,[7–10] it is uncertain whether the association has similar meaning in different age groups and in those at high and low risk of cardiovascular disease. The purpose of this study was to examine the relation between levels of CRP and the development of thromboembolic (TE) stroke over a 20-year period in largely middle-aged and healthy Japanese-American men in the Honolulu Heart Program. The association of CRP levels to early versus late disease will also be assessed, as will its relation with disease that may be poorly explained by age, hypertension, diabetes, and other cardiovascular risk factors. Effects will also be assessed in the absence of intervening coronary heart disease.

## Methods

### Baseline Examination

From 1965 to 1968, the Honolulu Heart Program began following 8006 men of Japanese ancestry through a series of physical examinations and comprehensive follow-up for cardiovascular disease.[13–15] At the time of study enrollment, subjects were aged 45 to 68 years. Procedures were in accordance with institutional guidelines and approved by an institutional review committee. Informed consent was obtained from the study participants.

Because the inventory of frozen sera from the time of study enrollment was largely depleted, measurement of CRP for this report is based on samples that were available at examinations given from 1967 to 1970. Other risk factors included diabetes, hypertension, total cholesterol, body mass index, and the use of cigarettes. Data on alcohol intake and physical activity were taken from examinations that were given at the time of study enrollment (1965 to 1968). Atrial fibrillation was not included among the risk factors, because it was rarely observed in this age group of Japanese-American men. Statistical adjustment for atrial fibrillation also failed to alter the findings in the present report.

Among the collected risk factors, diabetes was diagnosed on the basis of a medical history or the use of insulin or oral hypoglycemic therapy. Hypertension was defined as systolic or diastolic blood pressures ≥160 and 95 mm Hg, respectively, or when high blood pressure was being medically treated. Measurement of physical activity was based on the physical activity index, a commonly used index for quantifying overall metabolic output during a typical 24-hour period.[16,17]

### TE Stroke Cases and Controls

Assessment of the effects of CRP on the risk of future stroke is based on a nested case-control design. There were 7498 men (≈95% of the

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surviving cohort) who were available for follow-up at the time of CRP measurement. Among this group, cases of TE stroke and controls were selected from 5686 subjects in whom serum samples were available. Sera from 1812 participants were not obtained because of depletion from random selection for special substudies or, in a few instances, for use in small studies of rare diseases. After excluding cases of stroke and coronary heart disease that were identified in 20 years of follow-up, 1348 controls were randomly selected from the remaining 4145 men.

During the 20-year course of follow-up, information on cardiovascular events was obtained through a comprehensive system of surveillance of hospital discharges, death certificates, autopsy records, and repeat examinations. For this report, subjects were followed up for the first occurrence of a TE stroke (without distinction between an atherothrombotic infarction and embolic event), on the basis of medical records showing a neurological deficit of sudden or rapid onset that persisted for longer than 24 hours or resulted in death. A review of all suspected stroke outcomes by the Honolulu Heart Program Morbidity and Mortality Review Committee confirmed all diagnoses. There were 259 cases of stroke that were identified in the course of follow-up. All were selected without a history of coronary heart disease.

## CRP Determination

Serum specimens collected at the time of CRP measurement were stored at −20°C until 1980, at which time they were transferred to −70°C. Although specimens in the present study had previously been thawed and refrozen, measurement of CRP is known to be relatively stable under such conditions.[18] For each participant, determination of a CRP level was based on an enzyme-linked immunosorbent assay, a colorimetric competitive immunoassay that uses purified protein and polyclonal anti-CRP antibodies. The interassay coefficient of variation was ≈5%.

## Statistical Analysis

Estimated age-adjusted risk factor comparisons between the cases and controls were derived from analysis of covariance methods after log-transforming the CRP values.[18,19] Similar comparisons were made across CRP quartiles. Estimated age-adjusted percents of men with a TE stroke were also derived across CRP quartiles and within separate 5-year periods of follow-up after the baseline examination.[19,20] Such percents were not intended to represent the incidence of TE stroke but rather to show how the cases of TE stroke were distributed across the observed CRP quartiles. Beyond the first 5 years of follow-up, cases of TE stroke and deaths among the controls that preceded a follow-up period were deleted from the corresponding calculations. Cases of TE stroke that came after a 5-year follow-up period were treated as controls.

To assess the relation between levels of CRP and the odds of a TE stroke, statistical analysis relied on proportional hazards regression models.[21] Use of the proportional hazards regression model, which provides estimates of the relative odds of disease that are similar to those from logistic regression,[22] also allows for the modeling of the time to an event among the cases of TE stroke and the time to death among the controls who failed to survive the 20-year period of follow-up. To provide a test for trend, CRP was also modeled as a continuous variable. All reported *P* values were based on 2-sided tests of significance.

# Results

At the time of CRP measurement, the average ages of the cases and controls were 58.1 years (range, 49 to 69) and 55.8 years (range, 48 to 70), respectively ($P<0.001$). Among the cases, the average age when a TE stroke occurred was 69 years (range, 51 to 87), and the average time to an event was 10.5 years (range, 2 months to 20 years).

Baseline characteristics of the study participants according to case-control status are described in Table 1. As expected, at the time of CRP measurement, risk factor profiles were significantly less favorable in cases than in controls. Frequency of hypertension and diabetes were more than doubled in the TE stroke cases ($P<0.001$). Cases had higher levels of total cholesterol ($P<0.01$) and body mass index ($P<0.001$) as well. Cases were more likely to be current cigarette smokers ($P<0.001$) and less likely to have stopped smoking than controls ($P<0.05$). Cases also consumed more alcohol and were less physically active than controls, although differences were not statistically significant.

**TABLE 1. Distribution of CRP, Mean Age, and Age-Adjusted Mean Risk Factor Levels for Controls and Cases of Thromboembolic Stroke**

| Risk Factor | Controls (n=1348)* | Cases (n=259) |
|---|---|---|
| CRP, mg/L | | |
| Geometric mean† | 0.60 | 0.78‡ |
| Arithmetic mean† | 1.16 | 1.43 |
| 25th percentile | 0.32 | 0.44 |
| Median | 0.54 | 0.73 |
| 75th percentile | 1.00 | 1.39 |
| Range | 0.1 to 79.20 | 0.1 to 34.19 |
| Age, y | 55.8±5.4§ | 58.1±5.7‡ |
| Hypertension, % | 10.6 | 27.0‡ |
| Diabetes, % | 13.3 | 27.0‡ |
| Total cholesterol, mmol/L | 5.6±0.9 (215±35)‖ | 5.8±1.0 (222±40)¶ |
| Body mass index, kg/m$^2$ | 23.5±3.0 | 24.3±3.3‡ |
| Past cigarette smoker, % | 27.8 | 21.3# |
| Current cigarette smoker, % | 37.6 | 54.4‡ |
| Alcohol intake, oz/mo | 13.2±22.5 | 15.2±26.6 |
| Physical activity index | 33.0±4.5 | 32.7±4.6 |

*Sample size.
†Age-adjusted.
‡Significantly different from controls ($P<0.001$).
§Mean±SD.
‖Corresponding units in mg/dL.
¶Significantly different from controls ($P<0.01$).
#Significantly different from controls ($P<0.05$).

Risk factor differences across quartiles of CRP are additionally described in Table 2. Here, frequency of hypertension was more than doubled in the top versus bottom quartile of CRP (19.4 versus 8.4%, $P<0.001$). Although not statistically significant, the percent of men with diabetes also seemed positively related to CRP concentrations. Differences in total cholesterol across quartile strata were modest. Smoking, however, increased with rising CRP levels ($P<0.001$), with a jump in the use of cigarettes between the third and top CRP quartiles. Although less clear, the percent of men who were past smokers tended to decline with increasing CRP levels ($P<0.05$). Although body mass index increased consistently with increasing CRP levels, amounts of physical activity declined ($P<0.001$). There was no clear association between the intake of alcohol and levels of CRP.

Table 3 provides the age-adjusted percent of men with a TE stroke within each quartile of CRP and within separate 5-year periods of follow-up after CRP measurement. Although associations were modest within 5 years of CRP measurement, the relationship increased over time up to 10 to 15 years into

**TABLE 2. Mean Age and Age-Adjusted Risk Factor Levels by Quartile of CRP**

| | Quartile* of CRP (Range, mg/L) | | | |
|---|---|---|---|---|
| Risk Factor | 1st (0.10 to 0.32)† | 2nd (0.33 to 0.54) | 3rd (0.55 to 1.00) | 4th (1.01 to 79.2) |
| Sample size | 386 | 384 | 412 | 425 |
| Age, y‡ | 55.8±5.2§ | 55.9±5.4 | 56.1±5.6 | 56.8±5.8 |
| Hypertension, %‡ | 8.4 | 12.1 | 12.9 | 19.4 |
| Diabetes, % | 13.1 | 15.5 | 15.1 | 18.5 |
| Total cholesterol, mmol/L | 5.5±0.9 (214±36)‖ | 5.6±0.9 (217±34) | 5.6±1.0 (218±37) | 5.6±0.9 (217±36) |
| Body mass index, kg/m$^2$¶ | 22.7±2.7 | 23.4±2.9 | 24.1±3.1 | 24.3±3.3 |
| Past cigarette smoker, %¶ | 28.9 | 28.2 | 28.4 | 21.7 |
| Current cigarette smoker, %‡ | 32.3 | 36.2 | 37.6 | 54.1 |
| Alcohol intake, oz/mo | 12.5±22.7 | 11.4±18.9 | 15.3±26.0 | 14.6±24.1 |
| Physical activity index# | 33.4±4.6 | 33.2±4.7 | 32.9±4.6 | 32.3±4.1 |

*Quartiles are based on the distribution of CRP among the controls.
†Range in C-reactive protein.
‡Significant increase with increasing CRP ($P<0.001$).
§Mean±SD.
‖Corresponding units in mg/dL.
¶Significant decline with increasing CRP ($P<0.05$).
#Significant decline with increasing CRP ($P<0.001$).

follow-up. During this time, 8.3% of the men in the top CRP quartile had a stroke compared with 2.3% in the bottom quartile ($P<0.001$). Although changes in the relation between CRP and the risk of TE stroke seemed to occur with time, differences were not statistically significant.

The estimated relative odds of a TE stroke for men in the top versus bottom quartile of CRP, after age and risk factor adjustment, is additionally described for each 5-year period of follow-up in Table 4. Relative odds of a TE stroke are also provided within risk factor strata across the entire 20-year period.

After age and risk factor adjustment, the estimated relative odds of a TE stroke in men in the top versus bottom quartile of CRP were no longer statistically significant within the 5- to 10- and 15- to 20-year periods of follow-up. For the 10- to 15-year period, however, the odds of a TE stroke rose significantly with increasing CRP level when CRP was modeled as a continuous risk factor ($P=0.010$). There was also a 2.6-fold excess in the odds of a TE stroke in the top versus bottom quartile of CRP during this period of follow-up ($P<0.05$).

Although sample sizes were reduced within risk factor strata, the estimated odds of a TE stroke continued to increase significantly with rising CRP levels for middle-aged men (≤55 years, $P=0.018$), in men without hypertension or diabetes ($P=0.019$ and $P=0.006$, respectively), and in those who were never smokers of cigarettes ($P<0.001$). For men without hypertension or diabetes, there was a 1.6- to 1.7-fold excess ($P<0.05$) in the odds of a TE stroke in the top versus bottom CRP quartile. In middle-aged men, the corresponding odds increased to a 3-fold excess ($P=0.006$), and in nonsmokers, there was a 5.8-fold excess ($P<0.001$). Effects were also independent of total cholesterol, body mass index, alcohol intake, physical activity index, and the other risk factors in Table 4. Although associations were weaker in men with hypertension or diabetes, differences in associations from those when either condition was absent were not statistically significant. In contrast, the effect of CRP on the risk of TE stroke was significantly stronger in never smokers compared with past smokers ($P=0.005$) and current smokers ($P=0.010$).

**TABLE 3. Age-Adjusted Percent of Men Who Suffered a Thromboembolic Stroke by Quartile of CRP and by Period of Follow-Up After Blood Draw**

| | Quartile* of CRP | | | |
|---|---|---|---|---|
| Follow-Up Period | 1st | 2nd | 3rd | 4th |
| 0 to 5 y | 3.8 (14 of 386)† | 4.3 (16 of 384) | 4.1 (17 of 412) | 4.0 (18 of 425) |
| 5 to 10 y‡ | 2.0 (7 of 367) | 1.7 (6 of 360) | 4.4 (17 of 390) | 4.8 (20 of 397)§ |
| 10 to 15 y‖ | 2.3 (8 of 356) | 2.6 (9 of 346) | 6.2 (22 of 359)§ | 8.3 (31 of 361)¶ |
| 15 to 20 y‡ | 3.7 (12 of 333) | 5.8 (19 of 322) | 6.1 (19 of 314) | 7.9 (24 of 301)§ |
| Overall‖ | 10.9 (41 of 386) | 13.2 (50 of 384) | 18.2 (75 of 412)# | 21.1 (93 of 425)¶ |

*Quartiles are based on the distribution of CRP among the controls.
†Cases of thromboembolic stroke/sample size.
‡Significant increase with increasing CRP ($P<0.05$).
§Significantly different from the 1st quartile ($P<0.05$).
‖Significant increase with increasing CRP ($P<0.001$).
¶Significantly different from the 1st quartile ($P<0.001$).
#Significantly different from the 1st quartile ($P<0.01$).

**TABLE 4. Estimated Age-Adjusted and Risk Factor–Adjusted Relative Odds of Thromboembolic Stroke for Men in the Top vs Bottom Quartiles of CRP**

| | Age-Adjusted | | Risk Factor–Adjusted* | |
|---|---|---|---|---|
| Risk Strata | Relative Odds | Test for Trend (*P*) | Relative Odds | Test for Trend (*P*) |
| Follow-up period, y | | | | |
| 0 to 5 | 1.1 (0.5 to 2.2)† | 0.573 | 0.8 (0.4 to 1.7) | 0.861 |
| 5 to 10 | 2.5 (1.1 to 5.9) | 0.021 | 1.9 (0.8 to 4.6) | 0.124 |
| 10 to 15 | 3.8 (1.7 to 8.3) | <0.001 | 2.6 (1.1 to 5.8) | 0.010 |
| 15 to 20 | 2.5 (1.2 to 5.1) | 0.010 | 1.8 (0.9 to 3.8) | 0.144 |
| Overall | 2.3 (1.6 to 3.3) | <0.001 | 1.6 (1.1 to 2.4) | 0.008 |
| Age, y | | | | |
| 48 to 55 | 3.8 (1.8 to 8.0) | <0.001 | 3.0 (1.4 to 6.4) | 0.018 |
| 56 to 70 | 1.8 (1.2 to 2.9) | <0.001 | 1.3 (0.8 to 2.0) | 0.076 |
| Hypertension | | | | |
| Absent | 2.1 (1.4 to 3.3) | <0.001 | 1.6 (1.0 to 2.6) | 0.019 |
| Present | 1.3 (0.6 to 2.7) | 0.128 | 1.3 (0.6 to 2.7) | 0.221 |
| Diabetes | | | | |
| Absent | 2.5 (1.6 to 3.9) | <0.001 | 1.7 (1.0 to 2.7) | 0.006 |
| Present | 1.5 (0.8 to 3.0) | 0.258 | 1.4 (0.7 to 2.8) | 0.580 |
| Smoking status | | | | |
| Never | 6.1 (2.5 to 14.8) | <0.001 | 5.8 (2.3 to 14.4) | <0.001 |
| Past | 1.2 (0.5 to 2.6) | 0.508 | 0.8 (0.3 to 1.8) | 0.520 |
| Current | 1.6 (1.0 to 2.6) | 0.052 | 1.3 (0.8 to 2.2) | 0.152 |

*Adjusted for total cholesterol, body mass index, alcohol intake, physical activity index, and the other risk factors in this table.

†95% confidence interval.

## Discussion

Although the longitudinal data in this report provide additional evidence for an association between CRP and the incidence of TE stroke, specific strengths of the present study are worth noting. To date, the Honolulu Heart Program offers the longest and most complete follow-up of the association between CRP and stroke.[7–10] Follow-up also includes a well-defined acute event without intervening coronary heart disease. Although others have also reported on an association between CRP and cardiovascular disease, documentation of an association with stroke has been equivocal. Earlier reports have combined stroke with other forms of cardiovascular disease as a single event. Recent findings from the Framingham Study are also based on the combination of stroke with poorly defined transient ischemic attacks as a pooled event in an elderly sample that comprised half of surviving cohort members.[9] Although the Physician's Health Study also showed an increase in the risk of ischemic stroke with increasing levels of CRP, possible differences in the importance of the association between age groups, smoking strata, and groups at high and low risk of cardiovascular disease were not addressed.[7] Although an association between CRP and the risk of stroke was observed in the Third National Health and Nutrition Examination Survey, stroke cases were self-reported, and the timing of events was not recorded.[10] Levels of CRP were also undetectable in 71% of the study participants.

Findings of a stronger relation between CRP levels and the risk of stroke in low- versus high-risk groups in the Honolulu Heart Program are also consistent with other reports. As with stroke, similar effects of CRP on myocardial infarction were also observed in the Honolulu Heart Program.[23] Here, adverse effects were stronger in middle-aged men, in men without hypertension or diabetes, and in those who were nonsmokers. Although small sample sizes could have weakened the capacity to detect effects in higher-risk individuals, others have described strong relations between CRP and cardiovascular disease in nonsmoking women and in women without hypertension or diabetes as well.[8] Findings from the Framingham Study also describe stronger associations in women than in men for the combined events of ischemic stroke and transient ischemic attack.[9]

The weaker association in older or less healthy men could have several explanations. Although inflammation may still be important, in the presence of other risk factors, a high risk of stroke could mask any residual (and perhaps weaker) effects of inflammation. Even in the absence of important cardiovascular conditions, stroke can still occur.[24] In such cases, effects of isolated processes, such as inflammation, may be more apparent in disease progression.

Regardless of susceptibility, mechanistic derangements by which CRP is associated with the long-term incidence of atherosclerotic disease are not clear. Inflammatory mediators and products have been associated with cellular proliferation, lipid accumulation, and thrombosis. Markers of low-level inflammation, such as CRP, may reflect activity in any of these systems.[25,26] Levels of CRP may also be directly related to processes of pathophysiology through complement activation

and tissue factor expression. Although reports are conflicting, it is possible that CRP reflects reactions to infectious agents that have been associated with atherosclerotic diseases.[27] Observations in the Honolulu Heart Program that show a greater frequency of smoking cessation in men in the bottom quartile of CRP (where the risk of stroke is low) versus the top quartile additionally suggest that inflammation may be reversible. Smoking cessation in individuals with an elevated CRP may also be an effective strategy for improving CRP levels and reducing the adverse effects of inflammation.

Among the strengths of the present study, there are also limitations. For example, the Honolulu Heart Program is entirely composed of men of Japanese ancestry. Extensions to other groups could be important. Findings in Framingham and the Women's Health Study suggest that associations in men are likely to apply to women.[8,9] In general, the relative risk of stroke that is associated with most risk factors in Honolulu is similar to the relative risk observed in other population-based samples.[28,29] Levels of CRP also fall within the range of values reported elsewhere. In some instances, average values are higher,[9,10] whereas in others they are lower.[3,5,7,8] Overall increases in the risk of stroke and transient ischemic attacks in Framingham men and in men in the Physician's Health Study in the top versus bottom quartiles of CRP are also similar to those found in Hawaii.[7,9]

Although elevated levels of CRP seem to be related to an increased risk of TE stroke in middle-aged men and in those free of important cardiovascular risk factors, it remains to be determined if the measurement of CRP in cigarette smokers or in those with hypertension or diabetes can provide additional prognostic information. Whether CRP levels can be used as a clinical screen to identify an increased risk of cardiovascular disease in otherwise healthy adults also warrants additional consideration.

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# Elevated C-Reactive Protein Levels in Overweight and Obese Adults

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**Context** Human adipose tissue expresses and releases the proinflammatory cytokine interleukin 6, potentially inducing low-grade systemic inflammation in persons with excess body fat.

**Objective** To test whether overweight and obesity are associated with low-grade systemic inflammation as measured by serum C-reactive protein (CRP) level.

**Design and Setting** The Third National Health and Nutrition Examination Survey, representative of the US population from 1988 to 1994.

**Participants** A total of 16 616 men and nonpregnant women aged 17 years or older.

**Main Outcome Measures** Elevated CRP level of 0.22 mg/dL or more and a more stringent clinically raised CRP level of more than 1.00 mg/dL.

**Results** Elevated CRP levels and clinically raised CRP levels were present in 27.6% and 6.7% of the population, respectively. Both overweight (body mass index [BMI], 25-29.9 kg/m$^2$) and obese (BMI, ≥30 kg/m$^2$) persons were more likely to have elevated CRP levels than their normal-weight counterparts (BMI, <25 kg/m$^2$). After adjustment for potential confounders, including smoking and health status, the odds ratio (OR) for elevated CRP was 2.13 (95% confidence interval [CI], 1.56-2.91) for obese men and 6.21 (95% CI, 4.94-7.81) for obese women. In addition, BMI was associated with clinically raised CRP levels in women, with an OR of 4.76 (95% CI, 3.42-6.61) for obese women. Waist-to-hip ratio was positively associated with both elevated and clinically raised CRP levels, independent of BMI. Restricting the analyses to young adults (aged 17-39 years) and excluding smokers, persons with inflammatory disease, cardiovascular disease, or diabetes mellitus and estrogen users did not change the main findings.

**Conclusion** Higher BMI is associated with higher CRP concentrations, even among young adults aged 17 to 39 years. These findings suggest a state of low-grade systemic inflammation in overweight and obese persons.

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ADIPOSE TISSUE PREVIOUSLY WAS considered a passive storage depot for fat but is now known to play an active role in metabolism.[1,2] Among the recently discovered compounds expressed in human adipose tissue is the proinflammatory cytokine interleukin 6 (IL-6).[3,4] Moreover, IL-6 produced in the adipose tissue of healthy humans is released into the circulation.[4,5] Adipose tissue is estimated to produce about 25% of the systemic IL-6 in vivo.[4] Because of the inflammatory properties of IL-6, including the stimulation of acute-phase protein production in the liver,[6,7] the release of IL-6 from adipose tissue may induce low-grade systemic inflammation in persons with excess body fat.

A sensitive marker for systemic inflammation is the acute-phase C-reactive protein (CRP). In a meta-analysis of 7 prospective studies, elevated serum CRP concentration was shown to predict future risk of coronary heart disease.[8] C-reactive protein levels well below the conventional clinical upper limit of normal of 1 mg/dL have been associated with a 2- to 3-fold increase in risk of myocardial infarction, ischemic stroke, peripheral arterial disease, and coronary heart disease mortality in healthy men and women.[9-13]

This study tested whether overweight and obesity are associated with low-grade systemic inflammation as measured by serum CRP concentration.

**For editorial comment see p 2169.**

## METHODS

### Survey Design and Data Sources

The study included 16 616 adult participants of the Third National Health and Nutrition Examination Survey (NHANES III), 1988-1994. NHANES III was conducted by the National Center for Health Statistics of the Centers for Disease Control and Prevention.[14] The survey had a complex, stratified, multistage probability-cluster design for selecting a sample of approximately 40 000 persons representative of the noninstitutionalized civilian US population. Children younger than 5 years, persons aged 60 years or older, Mexican American persons, and non-Hispanic blacks were sampled at higher rates than others. Eighty-one percent

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of all eligible adults consented to an initial interview in their household. Of the 20 050 persons aged 17 years or older who were interviewed, 18 162 were subsequently examined in a mobile examination center or in their homes. Persons with missing data on height, body weight, or serum CRP level (n = 1239) and pregnant women (n = 307, validated by urine pregnancy test) were excluded, leaving 16 616 persons (7938 men and 8678 women) available for the statistical analyses.

Body weight and height were measured using standardized procedures.[15] Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters and used as an indicator of body fat.[16,17] The 1998 clinical guidelines[18] were used to define overweight (BMI, 25-29.9 kg/m$^2$) and obesity (BMI ≥30 kg/m$^2$).

Waist circumference was measured at the level of the high point of the iliac crest and the circumference at the level of maximum extension of the buttocks.[15] The waist-to-hip ratio, calculated as waist circumference divided by hip circumference, was used as an indicator of abdominal visceral fat.[19]

Serum specimens for the measurement of CRP were stored at –70°C and analyzed within 2 months after phlebotomy. C-reactive protein was analyzed using a modification of the Behring Latex-Enhanced CRP assay on the Behring Nephelometer Analyzer System (Behring Diagnostics, Westwood, Mass) (M.H.W., Phyllis R. Daum, MT [ASCP], G.M.M., unpublished data, 1999). Both within- and between-assay quality control procedures were used and the coefficient of variation of the method was 3.2% to 16.1% through the period of data collection. The assay could detect a minimal CRP concentration of 0.22 mg/dL, and values below this level were classified as undetectable. The assay was designed primarily to detect inflammation and was included as part of the NHANES III cohort to help detect inflammation as a confounding variable for interpretation of nutrition markers. Because most individuals had values less than the minimum detectable concentration, CRP is treated as a categorical rather than a continuous variable.

Race was defined by self-report as non-Hispanic white, non-Hispanic black, or Mexican American. People outside these categories were classified as other. Smoking status was based on self-report and categorized as never, former, or current smoking. All persons with a serum cotinine concentration of more than 57 nmol/L (10 ng/mL)[20] as measured by high-performance liquid chromatography and atmospheric-pressure chemical ionization tandem mass spectroscopy[21] were categorized as current smokers, irrespective of self-report. Inflammatory disease prevalence was determined through self-report of physician-diagnosed conditions (chronic bronchitis, asthma, emphysema, and rheumatoid arthritis) and self-report of "having a cold" in the past few days. A serum tube dilution latex fixation test for rheumatoid factor was assessed in persons aged 60 years or older.[22] All persons with a positive test result (≥1:40 titer) were categorized as having rheumatoid arthritis or a related inflammatory disorder, irrespective of self-report. Cardiovascular disease included self-reported physician-diagnosed myocardial infarction and stroke and angina as assessed by the Rose Angina Questionnaire.[23] Diabetes mellitus was defined as self-reported physician-diagnosed diabetes mellitus with insulin use or, in the case of undiagnosed diabetes mellitus, a fasting plasma glucose level of at least 6.99 mmol/L (126 mg/dL).[24,25] Estrogen use was based on self-report, categorized as contraceptive medications (oral or implant) or estrogen replacement therapy.

### Statistical Analyses

The study population was divided into 2 categories based on CRP concentration, undetectable (<0.22 mg/dL) and elevated (≥0.22 mg/dL). The population was also divided into 2 categories based on the conventional clinical cut point for inflammation, a CRP concentration of more than 1.00 mg/dL. Two outcome variables were defined: elevated CRP level (≥0.22 mg/dL), which was compared with undetectable CRP, and clinically raised CRP level (>1.00 mg/dL), which was compared with CRP level of no more than 1.00 mg/dL. Within each sex, the relationship between BMI and CRP concentration category was examined by multiple logistic regression analysis. We calculated odds ratios (ORs) and 95% confidence intervals (CIs) for BMI as a categorical variable according to the clinical guidelines, with normal weight (BMI <25 kg/m$^2$) as the reference category, and for BMI as a continuous variable, expressed per 5-kg/m$^2$ (about 1 SD) increment. Moreover, ORs per SD increment of waist-to-hip ratio (0.1 units) were calculated. Adjustments were made for potential confounders, including age, race, smoking status, estrogen use, inflammatory disease, and other diseases associated with low-grade inflammation, including cardiovascular disease[8,26,27] and diabetes mellitus.[28] To assess potential effect modification by age, smoking status, disease status, or estrogen use, the analyses were repeated, restricted to young (aged 17-39 years), healthy non–estrogen-using nonsmokers. Odds ratios do not approximate risk ratios when the prevalence of the outcome variable in the study population is greater than 10%.[29] The calculated OR for elevated CRP concentration therefore should not be interpreted as a risk ratio. Analyses were performed using SAS (SAS Institute Inc, Cary, NC) and SUDAAN (Research Triangle Institute, Research Triangle Park, NC) and incorporated sampling weights to account for oversampling and nonresponse to the household interview and examination.[30] Variance estimates were calculated with SUDAAN, incorporating the complex sampling design of NHANES III.[30]

## RESULTS

Elevated CRP levels (≥0.22 mg/dL) were present in 21.8% of men and 33.1% of women, and clinically raised CRP levels (>1.00 mg/dL) in 4.4% and 8.9%, respectively. Other characteristics of the study population are shown in TABLE 1.

With increasing BMI, the prevalence of elevated CRP level increased

in both men and women (FIGURE). However, with increasing BMI the prevalence of clinically raised CRP level increased among women only; the prevalence was 4.0% (95% CI, 3.3%-4.8%) in normal-weight women, 7.7% (95% CI, 6.4%-9.4%) in overweight women, and 20.2% (95% CI, 18.1%-22.5%) in obese women.

Obese men were 2.13 times more likely and obese women 6.21 times more likely to have elevated CRP levels compared with their normal-weight counterparts (TABLE 2). Per 1-SD increase in BMI, men were 1.38 and women were 2.04 times more likely to have elevated CRP levels. Among women, BMI was also associated with clinically raised CRP levels. Obese women were 4.76 times more likely to have clinically raised CRP levels compared with normal-weight women. Per 1-SD increment in BMI, women were 1.69 times more likely to have clinically raised CRP levels.

The waist-to-hip ratio was independently associated with both elevated and clinically raised CRP levels in men and women. Per 1-SD increase in waist-to-hip ratio, men were 1.41 and women were 1.21 times more likely to have elevated CRP levels (Table 2). The OR for clinically raised CRP levels per 1-SD increase in waist-to-hip ratio was 1.36 in men and 1.28 in women.

The association between BMI and CRP was also investigated after stratification by age group (young = 17-39 years; middle-aged = 40-59 years; old = ≥60 years). Among women, the association between BMI and CRP categories was influenced by age group. Older obese women were less likely to have elevated or clinically raised CRP levels than young obese women. A similar effect modification by age group in women was observed using BMI as a categorical variable. No effect modification by age group was observed in men.

To avoid any potential effect modification by age, inflammatory disease, cardiovascular disease, diabetes mellitus, current smoking, or estrogen use, the analyses were repeated restricted to healthy, nonsmoking, non–estrogen-using persons aged 17 to 39 years. The positive association between BMI category and elevated CRP level remained statistically significant after adjustment for age, race, smoking status (never and former smoking only), and waist-to-hip ratio (TABLE 3). In this restricted analysis, BMI also remained positively associated with clinically raised CRP levels among women.

**Table 1.** Characteristics of Study Population*

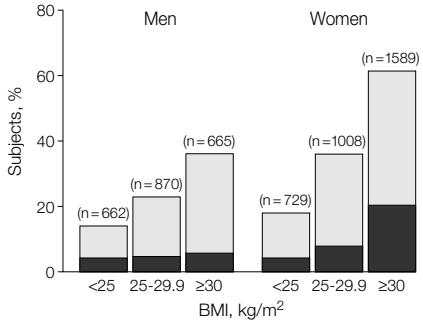
| | Men | Women |
|---|---|---|
| Sample size, No. | 7938 | 8678 |
| Age, y | | |
| 17-39 | 50.4 | 45.6 |
| 40-59 | 30.6 | 30.4 |
| ≥60 | 19.0 | 24.0 |
| Race | | |
| Non-Hispanic white | 76.7 | 76.1 |
| Non-Hispanic black | 9.9 | 11.3 |
| Mexican American | 5.7 | 4.6 |
| Other | 7.7 | 8.0 |
| Body mass index, kg/m$^2$ | | |
| <25 (normal) | 41.9 | 50.1 |
| 25.0-29.9 (overweight) | 38.8 | 25.6 |
| ≥30 (obese) | 19.3 | 24.3 |
| C-reactive protein level, mg/dL | | |
| ≤0.21 (undetectable) | 78.2 | 66.9 |
| 0.22-1.00 (elevated) | 17.4 | 24.2 |
| >1.00 (clinically raised) | 4.4 | 8.9 |
| Smoking status | | |
| Never | 37.5 | 54.9 |
| Former | 27.9 | 18.9 |
| Current | 34.6 | 26.2 |
| Disease | | |
| Inflammatory disease† | 23.6 | 29.6 |
| Cardiovascular disease‡ | 7.4 | 7.3 |
| Diabetes mellitus | 6.1 | 5.8 |
| Estrogen use | | |
| None | . . . | 80.3 |
| Contraceptive | . . . | 11.0 |
| Hormone replacement | . . . | 8.7 |
| Waist-to-hip ratio, mean (SE) | 0.95 (0.00) | 0.86 (0.00) |

*All data are expressed as percentages unless noted. Ellipses indicate data not applicable.
†Includes current cold, asthma, emphysema, chronic bronchitis, and rheumatoid arthritis.
‡Includes angina, myocardial infarction, and stroke.

## COMMENT

Previous studies in middle-aged and elderly persons have reported a positive association between BMI and CRP concentration.[12,26,27] However, in these age groups, the association may have been confounded by disease. Rheumatoid arthritis, diabetes mellitus, and cardiovascular disease are prevalent diseases

**Figure.** Prevalence of Elevated (≥0.22 mg/dL) Serum C-Reactive Protein Concentration by BMI Category in Men and Women Aged 17 Years or Older




Normal weight was considered a body mass index (BMI) of less than 25 kg/m$^2$; overweight, 25 to 29.9 kg/m$^2$; and obese, 30 kg/m$^2$ or more. The prevalence of clinically raised (>1.00 mg/dL) serum C-reactive protein concentration is indicated in black.

in older persons and are associated with both obesity[31-33] and increased CRP concentrations.[8,26-28,34] We carefully controlled for inflammatory disease and other factors known to influence CRP concentrations. A higher prevalence of low-grade systemic inflammation was observed in overweight and obese persons compared with normal-weight persons. Most importantly, our study extends these findings to young adults aged 17 to 39 years, in whom the prevalence of any confounding subclinical disease is generally very low. Of interest is our observation that the distribution of body fat is associated with CRP concentration independent of BMI. A high waist-to-hip ratio, indicative of a large amount of abdominal visceral fat, was associated with low-grade systemic inflammation in men and women.

Our results, together with the evidence of previous studies, have important implications for the health risks of overweight and obese individuals, including those at young ages. Based on NHANES III data, we estimated that 53.9% of US adults aged 17 years or older are overweight or obese. Overweight, obesity, and a large waist-to-hip ratio pose a considerable health risk, including cardiovascular health.[33,35-37] Low-grade systemic inflammation has been shown to increase the risk for cardiovascular disease.[9-13] Some of the increased risk for cardiovascular disease in overweight and obese persons may be explained by our observation that increased CRP concentrations are more prevalent in these persons.

C-reactive protein concentrations well below the conventional clinical upper limit of normal of 1 mg/dL have been associated with a 2- to 3-fold increase in risk of myocardial infarction, ischemic stroke, and peripheral arterial disease in healthy men and women.[9-13] In addition, elevated CRP levels are predictive of cardiac complications in patients with unstable angina or myocardial infarction[38,39] and CRP induces the production of tissue factor, a potent procoagulant, in monocytes.[40] Moreover, elevated CRP concentrations are associated with increased coronary heart disease mortality and total mortality.[9,41]

Approximately 25% of circulating IL-6 is estimated to be released by human subcutaneous adipose tissue in vivo,[2] and IL-6 stimulates the production of acute-phase proteins in the liver.[6,7] This might explain the observed associations between BMI and CRP. In vitro, human abdominal visceral adipose tissue releases more IL-6 compared with subcutaneous adipose tissue,[5] possibly explaining our observation that a higher waist-to-hip ratio, after adjustment for BMI and several confounders, was independently associated with elevated CRP level.

Body mass index is an important clinical indicator of overweight and obesity,[18] but its use as an indicator of body fatness has limitations. At a similar BMI, women have more body fat than men.[42] This difference was reflected in our data, showing a higher prevalence of elevated and clinically raised CRP levels in women compared with men in overweight and obese persons (Figure). The higher prevalence of elevated and clinically raised CRP levels among obese women compared with obese men could also be due to by the fact that women were more likely to be extremely obese: a BMI of 35 to 40 kg/m$^2$ was prevalent among 3.4% of men and 6.4% of women, and a BMI of 40 kg/m$^2$ or more was present among 1.7% of men and 3.6% of women. Both phenomena might also explain why BMI was associated with clinically raised CRP levels in women but not men.

Persons with a normal body weight (BMI <25 kg/m$^2$) were used as the reference group. However, this group included a small percentage (1.3% of men and 3.8% of women) of underweight persons (BMI <18.5 kg/m$^2$) who might be more likely to be in poor health, with associated higher CRP concentrations. However, when the analyses were

**Table 2.** Adjusted Odds Ratios (95% Confidence Intervals) for Elevated and Clinically Raised Serum C-Reactive Protein (CRP) Concentrations in 16 616 Men and Women*

| | Elevated CRP Level (≥0.22 mg/dL)† | | Clinically Raised CRP Level (>1.00 mg/dL)‡ | |
|---|---|---|---|---|
| | Men | Women | Men | Women |
| Body mass index, kg/m$^2$ | | | | |
| <25 (normal weight) | 1.0 (referent) | 1.0 (referent) | 1.0 (referent) | 1.0 (referent) |
| 25-29.9 (overweight) | 1.41 (1.09-1.81) | 2.23 (1.86-2.67) | 0.90 (0.54-1.51) | 1.65 (1.19-2.28) |
| ≥30 (obese) | 2.13 (1.56-2.91) | 6.21 (4.94-7.81) | 0.84 (0.49-1.41) | 4.76 (3.42-6.61) |
| Per SD increment | 1.38 (1.22-1.55) | 2.04 (1.89-2.20) | 1.08 (0.88-1.33) | 1.69 (1.49-1.92) |
| Waist-to-hip ratio per SD increment | 1.41 (1.17-1.69) | 1.21 (1.07-1.37) | 1.36 (1.01-1.84) | 1.28 (1.07-1.54) |

*Data are adjusted for race, age, smoking status, inflammatory disease, cardiovascular disease, diabetes mellitus, estrogen use (women only), and each other.
†Compared with a CRP level of less than 0.22 mg/dL.
‡Compared with a CRP level of no more than 1.00 mg/dL.

**Table 3.** Adjusted Odds Ratios (95% Confidence Interval) for Elevated and Clinically Raised Serum C-Reactive Protein (CRP) Concentrations in 3303 Young (Aged 17-39 Years), Nonsmoking, Non–Estrogen-Using Men and Women Without Inflammatory Disease, Cardiovascular Disease, or Diabetes Mellitus*

| | Elevated CRP Level (≥0.22 mg/dL)† | | Clinically Raised CRP Level (>1.00 mg/dL)‡ | |
|---|---|---|---|---|
| | Men | Women | Men | Women |
| Body mass index, kg/m$^2$ | | | | |
| <25 (normal weight) | 1.0 (referent) | 1.0 (referent) | 1.0 (referent) | 1.0 (referent) |
| 25-29.9 (overweight) | 1.35 (0.59-3.11) | 2.87 (1.47-5.58) | 0.11 (0.01-1.03) | 1.42 (0.36-5.64) |
| ≥30 (obese) | 2.85 (1.33-6.10) | 12.90 (5.61-29.65) | 0.64 (0.09-4.68) | 8.56 (2.09-34.95) |
| Per SD increment | 1.61 (1.20-2.16) | 2.46 (1.83-3.32) | 1.17 (0.58-2.37) | 2.26 (1.49-3.41) |
| Waist-to-hip ratio per SD increment | 1.59 (1.06-2.38) | 1.76 (1.13-2.72) | 2.26 (0.89-5.74) | 1.43 (0.75-2.71) |

*Data are adjusted for race, age, smoking status (never and former smoking), and each other.
†Compared with a CRP level of less than 0.22 mg/dL.
‡Compared with a CRP level of less than 1.00 mg/dL.

repeated after exclusion of underweight people in the reference group, similar results were obtained.

Because the lower detection limit of the CRP assay was 0.22 mg/dL, serum CRP level was used as a categorical variable. It is unlikely that the use of a more sensitive assay would have changed the conclusions of the study. The association between obesity and CRP concentration was observed regardless of the CRP cut point that was used (≥0.22 or >1.00 mg/dL). Second, although the cut point of 1.0 mg/dL has been used in clinical studies, more recent epidemiological studies have shown an increased risk for cardiovascular disease at CRP levels of 0.2 mg/dL and higher.[9-13]

We used a single CRP measurement that may not accurately reflect long-term inflammation status. The biological variability of CRP is substantial, with reported values ranging from 10.6% to 63.0%.[43-46] However, because random misclassification due to biological variability will lead to underestimation of true associations, this limitation is unlikely to explain our findings.

Measurements of the serum concentration of IL-6 were not available in the present study. Although the results support the hypothesis that IL-6 produced by the adipocytes increase CRP concentration, direct assessment of IL-6 concentration is needed in future studies to further test this hypothesis.

In conclusion, the results of this large-scale cross-sectional study show that higher BMI is associated with higher CRP concentrations that could not be explained by inflammatory disease or other factors or diseases known to increase CRP concentrations. Because these associations also were observed among young adults aged 17 to 39 years, subclinical disease is unlikely to explain our findings. These data suggest that a state of low-grade systemic inflammation is present in overweight and obese persons.

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# Rosuvastatin in the Primary Prevention of Cardiovascular Disease Among Patients With Low Levels of Low-Density Lipoprotein Cholesterol and Elevated High-Sensitivity C-Reactive Protein: Rationale and Design of the JUPITER Trial*

Paul M Ridker and on behalf of the JUPITER Study Group

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# Rosuvastatin in the Primary Prevention of Cardiovascular Disease Among Patients With Low Levels of Low-Density Lipoprotein Cholesterol and Elevated High-Sensitivity C-Reactive Protein

## Rationale and Design of the JUPITER Trial*

Paul M Ridker, MD, MPH; on behalf of the JUPITER Study Group

Completed randomized trials of statin therapy demonstrate that 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors reduce the risk of myocardial infarction, stroke, and other cardiovascular events among individuals with established coronary disease and overt hyperlipidemia.[1–6] In aggregate, use of statin therapy in these trials has been associated with an approximate 30% reduction in cardiovascular event rates. Largely on the basis of these cholesterol reduction trials, current treatment algorithms from the National Cholesterol Education Program (NCEP) Adult Treatment Panel III endorse the use of statins in secondary prevention and encourage increased use of statins in primary prevention among those with hyperlipidemia and diabetes.[7]

Unfortunately, despite evidence provided by the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS[2]) and the West of Scotland Coronary Prevention Study (WOSCOPS[3]), use of statins for the primary prevention of cardiovascular disease has not been widely adopted in a cost-effective manner. From a clinical perspective, there are several reasons for this slow adoption.

First, almost half of all cardiovascular events occur among apparently healthy men and women who have normal or even low levels of LDL cholesterol (LDL-C). Thus, better screening methods are needed in primary prevention to detect high-risk individuals for whom the number needed to treat (NNT) is small enough to make prophylactic statin therapy cost effective. Second, there has been controversy within the completed clinical trials suggesting that the benefits of statins may extend beyond LDL-C reduction alone. In both the Heart Protection Study of stable high-risk patients[6] and the MIR-ACL (Myocardial Ischemia Reduction with Aggressive Cholesterol Lowering) study of patients with acute coronary syndromes,[8] the risk reduction associated with statin therapy was almost identical among those with low as well those with as high levels of LDL-C. Further, statin therapy reduces the risk of stroke, yet LDL-C is not an important risk factor for this disease.[9,10]

### The Role of High-Sensitivity C-Reactive Protein (hsCRP) in Cardiovascular Disease

In an effort to improve vascular risk detection, many physicians screen for hsCRP, an inflammatory biomarker associated with a markedly increased risk of myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death, even among apparently healthy individuals with low levels of LDL-C.[11] To date, more than a dozen large-scale studies demonstrate in aggregate that hsCRP levels are a strong, independent predictor of future vascular events[12–20] and that hsCRP adds prognostic information on risk at all levels of LDL-C, at all levels of the Framingham Risk Score, and at all levels of the metabolic syndrome[15,21–23] (Figure 1). Moreover, hsCRP predicts risk of recurrent coronary events and has important prognostic value in acute coronary ischemia and after coronary interventions.[24–30]

As our understanding that atherothrombosis is fundamentally an inflammatory disease has developed,[31] so too has evidence regarding CRP as a direct participant both in the early initiation of atherosclerotic lesions and in the conversion of stable to unstable plaques. In particular, evidence has recently accumulated that shows CRP to be a direct participant in the atherothrombotic process capable of augmenting the innate inflammatory response, triggering expression of adhesion molecules and monocyte chemoattractant protein-1, attenuating expression of endothelial NO synthase, inducing plasminogen activator inhibitor-1, and having a direct effect on arterial thrombosis[32–37] (Figure 2).

On the basis of these data, an expert panel assembled by the Centers for Disease Control and Prevention and the American Heart Association provided the first guidelines for use of inflammatory biomarkers in clinical practice in January 2003.[38] This report confirmed the importance of hsCRP in clinical risk detection and recommended use of hsCRP as part of global risk prediction, particularly among those deemed at "intermediate risk" by standard risk factors. One of the most important groups likely to benefit from hsCRP evaluation is

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Dr Ridker is listed as a coinventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease.

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*Justification for the Use of statins in Primary prevention: an Intervention Trial Evaluating Rosuvastatin.

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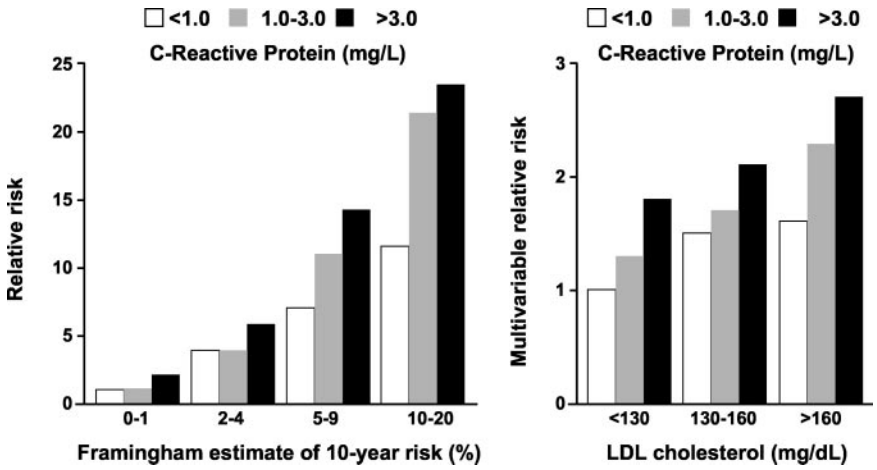


**Figure 1.** hsCRP adds prognostic information on vascular risk at all levels of LDL-C (right) and at all levels of the Framingham Risk Score (left). Data are derived from Ridker et al.[11,15,21]

composed of those with normal or low levels of LDL-C. As shown in Figure 3 in data from the large-scale Women's Health Study, apparently healthy individuals with low levels of LDL-C but high levels of hsCRP are at higher absolute risk of future vascular events than are those with high levels of LDL-C but low levels of hsCRP.[15] Such patients, however, are not currently considered for statin therapy, as they have LDL-C levels <130 mg/dL, the current treatment target in primary prevention. Nonetheless, both experimental and clinical studies indicate that statins may have direct anti-inflammatory effects, and it is now established that statins lower hsCRP levels on a population basis.[39–41] Thus, it has been hypothesized that hsCRP screening might provide a method to improve the targeting of statin therapy, particularly among those with low to normal levels of LDL-C.[42]

## hsCRP, Statin Therapy, and the Prevention of Cardiovascular Events

To address this issue, a hypothesis-generating study was recently completed in which hsCRP levels were measured at baseline among 5742 participants enrolled in AFCAPS/TexCAPS, a randomized, double-blind, placebo-controlled trial of lovastatin in the primary prevention of cardiovascular events conducted among American men and women with average cholesterol levels and below-average HDL cholesterol levels.[43] In that trial, lovastatin allocation was associated with a 37% reduction in the primary clinical end point of fatal or nonfatal myocardial infarction, hospitalization for unstable angina, or sudden cardiac death. However, after measuring baseline hsCRP as well as lipid levels in the AFCAPS/TexCAPS population, several critical observations regarding the efficacy of statin therapy in primary prevention were observed.[43]

First, coronary event rates increased with entry hsCRP levels such that the relative risks from lowest to highest quartiles of baseline hsCRP among those allocated to placebo were 1.0, 1.2, 1.3, and 1.7 ($P=0.01$), an effect that was independent of traditional risk factors included in the Framingham Risk Score.

Second, compared with placebo, allocation to lovastatin in AFCAPS/TexCAPS resulted in a statistically significant reduction in median hsCRP levels at the end of the first year of treatment (95% CI of the median, −17.4 to −12.5%, $P<0.001$); data were consistent with those of other statins.[39–41] As also demonstrated in the Pravastatin INflammation CRP Evaluation (PRINCE),[41] this reduction in hsCRP was not related to the effect of statin therapy on lipid levels.

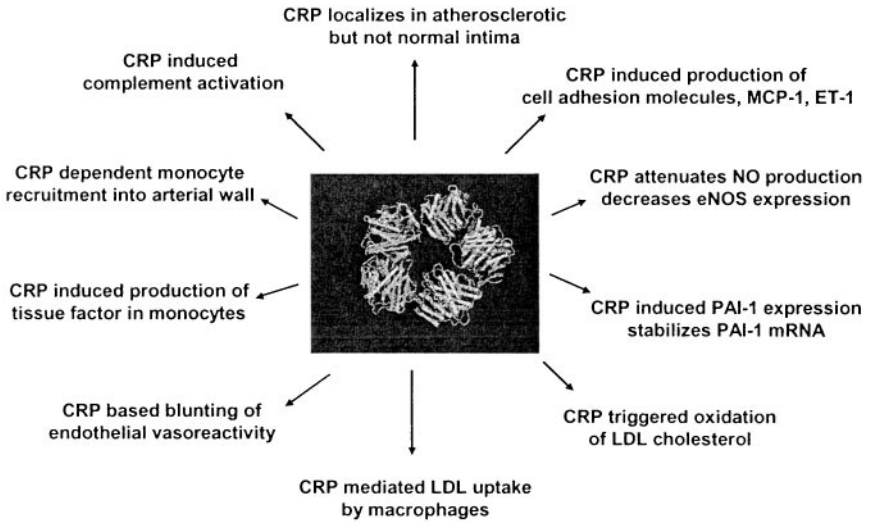


**Figure 2.** Mechanisms relating C-reactive protein (CRP) to development and progression of the atherothrombotic process. eNOS indicates endothelial NO synthase; ET-1, endothelin-1; MCP-1, monocyte chemoattractant protein-1; and PAI-1, plasminogen activator inhibitor-1.

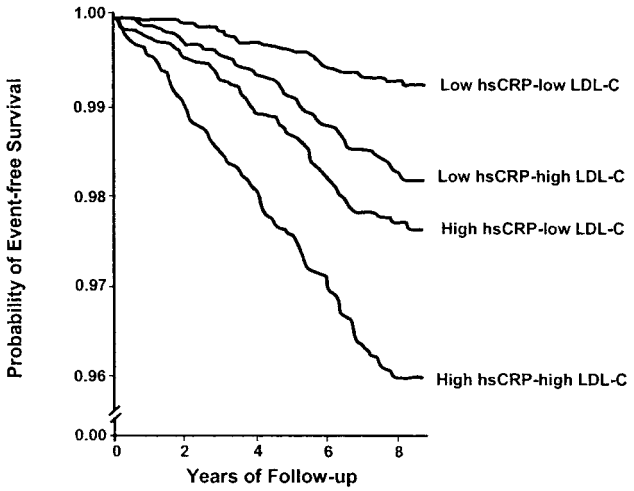


**Figure 3.** Cardiovascular event–free survival according to baseline levels of LDL-C and hsCRP. Note that those with elevated levels of hsCRP but low LDL-C (the target population for the JUPITER trial) appear at higher vascular risk than those with high LDL-C but low hsCRP. Data are derived from Ridker et al.[15]

Third, and most importantly, there were major differences in the observed efficacy of lovastatin when AFCAPS/TexCAPS participants were stratified into 4 groups on the basis of median LDL-C and median hsCRP levels[43] (Table). As expected, lovastatin was highly effective in preventing first vascular events among participants with elevated levels of LDL-C. However, lovastatin was also highly effective in reducing coronary events among those with low LDL-C levels but who had elevated levels of hsCRP, data that suggest that statin therapy may well have efficacy in the presence of systemic inflammation even in the absence of hyperlipidemia. In fact, the low LDL-C/high hsCRP subgroup in AFCAPS/TexCAPS had a risk of future vascular events just as high as that observed in the subgroups with overt hyperlipidemia. In marked contrast, event rates were low among AFCAPS/TexCAPS participants with low LDL-C and low hsCRP, a subgroup in which there was no evidence that lovastatin reduced the risk of future cardiovascular events. These hypothesis-generating data in primary prevention parallel the data in secondary prevention from the Cholesterol and Recurrent Events (CARE) trial that previously suggested that the benefit of statin therapy was greater among those with elevated hsCRP levels.[24]

Since publication of the AFCAPS/TexCAPS[43] and CARE[24] trial data for hsCRP, several clinical registries have corroborated the observation that individuals with elevated hsCRP levels benefit preferentially from the use of statins both among those with angiographically severe coronary disease[44,45] and in the setting of percutaneous coronary interventions and stent placement.[46,47] Moreover, a number of studies have suggested direct anti-inflammatory mechanisms for statin therapy that appear largely independent of LDL reduction.[48–51] One recent study has shown a dose-response relationship between statin therapy and hsCRP reduction that was augmented by the addition of ezetimibe.[52]

For some physicians, these data have been interpreted as evidence that hsCRP screening should be broadly applied and that those with elevated levels of hsCRP should be placed on statin therapy for the primary prevention of cardiovascular events. It is critical to recognize, however, that observations regarding hsCRP in both the AFCAPS/TexCAPS and CARE trials were made on a post hoc basis and that the total number of events within the low LDL-C/high hsCRP strata in each of those studies was small. Thus, a large-scale, prospective, placebo-controlled trial of statin therapy among individuals without overt hyperlipidemia but with evidence of systemic inflammation is needed to directly test this hypothesis.

## The JUPITER Trial

### Study Objectives

The primary objective of the JUPITER trial is to determine whether long-term treatment with rosuvastatin (20 mg orally per day) will reduce the rate of first major cardiovascular events, defined as the combined end point of cardiovascular death, stroke, myocardial infarction, hospitalization for unstable angina, or arterial revascularization among individuals with LDL-C levels <130 mg/dL (3.36 mmol/L) who are at high vascular risk because of an enhanced inflammatory response as indicated by hsCRP levels ≥2 mg/L. Secondary objectives of JUPITER are to evaluate the safety of long-term treatment with rosuvastatin in terms of total mortality, noncardiovascular mortality, and adverse events and to determine whether rosuvastatin reduces the incidence of type 2 diabetes. This latter objective reflects the fact that hsCRP levels also predict the onset of diabetes[53] and that inflammation appears to be a critical link between diabetes and atherothrombosis.[54] Finally, on the basis of observational evidence regarding statins, osteoporosis, and hypercoagulability, the JUPITER trial will also determine whether rosuvastatin reduces the incidence of bone fractures and venous thromboembolic events.[55,56]

**Crude Event Rates, Relative Risks (RR), and the No. Needed to Treat (NNT) Associated With Lovastatin Allocation Among AFCAPS/TexCAPS Participants, According to Baseline Levels of LDL Cholesterol and hsCRP**

| | Lovastatin | | Placebo | | | | |
|---|---|---|---|---|---|---|---|
| Study Group | N | Rate* | N | Rate* | RR | 95% CI | NNT* |
| Low LDLC/low hsCRP | 19/726 | 0.025 | 17/722 | 0.022 | 1.08 | 0.56–2.08 | ... |
| Low LDLC/high hsCRP | 22/718 | 0.029 | 37/710 | 0.051 | 0.58 | 0.34–0.98 | 48 |
| High LDLC/low hsCRP | 15/709 | 0.020 | 37/711 | 0.050 | 0.38 | 0.21–0.70 | 33 |
| High LDLC/high hsCRP | 29/741 | 0.038 | 40/705 | 0.055 | 0.68 | 0.42–1.10 | 58 |

*Event rates and NNT calculated on the basis of 5 patient-years at risk. Data are derived from Ridker et al.[43]

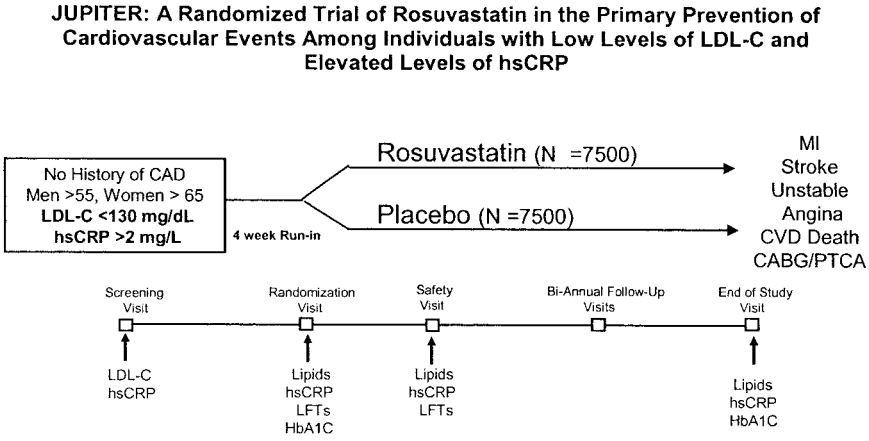


**Figure 4.** Overall design of the JUPITER trial. CABG indicates coronary artery bypass graft; CAD, coronary artery disease; CVD, cardiovascular disease; $HbA_{1c}$, hemoglobin $A_{1c}$; LFTs, liver function tests; MI, myocardial infarction; and PTCA, percutaneous transluminal coronary angioplasty.

## Study Population

The JUPITER trial will enroll up to 15 000 men age 55 years and older and women age 65 years and older, who, on initial screening, are found to have hsCRP ≥2 mg/L, LDL-C <130 mg/dL, and triglycerides <500 mg/dL (5.65 mmol/L), and who have no history of myocardial infarction, stroke, arterial revascularization, or coronary risk equivalent as defined by current NCEP guidelines. Additional exclusion criteria are as follows: current use of statins or other lipid-lowering therapies, including fibrates, niacin, and bile-acid sequestrants; known hypersensitivity to statin therapy; current use of postmenopausal oral hormone therapy; current use of immunosuppressants; active liver disease or elevated liver enzymes (alanine aminotransferase [ALT] >2 times upper limit of normal [ULN]); creatine kinase [CK] >3 times ULN; diabetes mellitus (fasting serum glucose >126 mg/dL [7.0 mmol/L], or use of insulin or oral hypoglycemic agent); uncontrolled hypertension (systolic or diastolic blood pressure >190 or 100 mm Hg, respectively); history of cancer, except nonmalignant skin cancer, within the past 5 years; uncontrolled hypothyroidism (thyroid-stimulating hormone >1.5 above ULN); chronic inflammatory conditions such as severe arthritis, lupus, or inflammatory bowel disease; history of alcohol or drug abuse within the past year; and serious medical or psychological conditions that may compromise successful study participation.

## Study Design

The overall design of the JUPITER trial is shown in Figure 4. At the initial screening visit, informed consent will be sought, a preliminary assessment of subject eligibility will occur, and a fasting blood sample will be obtained for analysis of hsCRP and lipid levels. At a second screening visit, a physical examination and medical history focusing on cardiovascular risk factors will be conducted, and fasting blood and urine samples will be collected for further lipid analysis, hematologic indices, creatinine, thyroid-stimulating hormone, ALT, CK, glucose, and hemoglobin $A_{1c}$. For participants who provide additional consent, plasma and buffy coat samples will be stored for future genomic and proteomic analyses relating to lipid metabolism, inflammatory function, and statin therapy. Eligible subjects will then be enrolled in a 4-week prerandomization run-in period designed to ensure a group of study participants capable of long-term protocol compliance.

Following the run-in period, participants will be randomly assigned to either oral rosuvastatin (20 mg/d; supplied as CRESTOR by AstraZeneca [Wilmington, Del]) or placebo for a period of 3 to 4 years, the estimated time needed to accrue the 520 cardiovascular end points on which the study is powered. The dose of rosuvastatin selected should result in ≈50% reductions in LDL cholesterol[57] as well as a substantial reduction in hsCRP.

All study participants will visit the clinic sites for evaluation at 3 and 6 months after randomization and thereafter at 6-month intervals for the duration of follow-up. At these visits, staff will dispense study medication; assess compliance with pill taking, the use of concomitant medications, and the development of major illnesses, study end points, or adverse effects; and collect fasting blood and urine samples to evaluate changes in lipid and inflammatory parameters and to monitor safety. Study medication will be discontinued among subjects who develop myopathy (CK >10 times ULN and muscle aches or weakness) or a persistent elevation in ALT (>3 times ULN on 2 consecutive tests). Subjects whose blinded LDL-C levels rise to ≥130 mg/dL during the study will be counseled to adopt lifestyle changes recommended by the NCEP. If, after 3 months, LDL-C levels remain elevated and the calculated Framingham Risk Score exceeds 10% despite lifestyle changes, investigators will be encouraged to consider lipid-lowering therapy with bile-acid sequestrants or cholesterol-absorption inhibitors for those subjects. However, if the responsible study physician believes statin therapy is indicated, the study medication will be discontinued and open-label statin therapy will be initiated. All subjects in whom study medication is discontinued will be followed for the duration of the trial and included in data analyses.

## Data Analysis, Power, and Trial Organization

The primary end point under study is the first occurrence of a major cardiovascular event defined as cardiovascular death, stroke, myocardial infarction, hospitalization for unstable angina, or arterial revascularization. Secondary end points are total mortality, noncardiovascular mortality, diabetes mellitus, venous thromboembolic events, bone fractures, and discontinuation of the study medication because of adverse

effects. All primary analyses will be on an intention-to-treat basis. Event rates for the rosuvastatin and placebo groups will be compared using the proportional-hazards regression model to adjust for variable length of follow-up.

Power estimates are based on the assumption of a mean follow-up of 3.5 years, a placebo event rate of 1.5 per 100 patient-years at risk, and a net attrition rate of 5% per year. Given a sample size of 15 000, the power of the trial to detect a 25% reduction in risk of major vascular events associated with rosuvastatin exceeds 90%.

The JUPITER trial was designed as an investigator-initiated protocol from the Center for Cardiovascular Disease Prevention at the Brigham and Women's Hospital, Harvard Medical School, Boston, Mass.[42] Members of the JUPITER Steering Committee are listed in Appendix A.

A fully independent 5-member Data and Safety Monitoring Board has been established and will review unblinded safety data at least twice yearly. Frequency of interim efficacy analyses and rules for early trial termination have been prespecified and approved by all members of this board (listed in Appendix B).

## What Will the JUPITER Trial Teach Us?

The JUPITER trial has been carefully designed to address a critical unanswered question regarding inflammation, statins, and atherothrombosis, as follows: Will statin therapy prevent first-ever cardiovascular events among those with LDL-C <130 mg/dL, but who are nonetheless at increased vascular risk because of elevated levels of hsCRP? This issue is of exceptional clinical importance, as half of all vascular events occur among those with normal or even low levels of LDL-C. Within the United States alone, as many as 25 to 30 million adults fall into this potentially high-risk category. Thus, a strong positive finding from JUPITER will dramatically affect public health and prevention and would provide a clear rationale for much broader use of statin therapy for the primary prevention of cardiovascular events than currently endorsed. On the other hand, a negative finding would also be of great importance, as it would direct the use of scarce prevention resources to other nonstatin methods for coronary risk reduction.

By using rosuvastatin, JUPITER will also be addressing whether aggressive LDL-C reduction[57] has efficacy in primary prevention among those with relatively low LDL-C levels. However, because JUPITER is evaluating an agent that dramatically lowers LDL-C as well as hsCRP, the JUPITER trial will not directly answer whether CRP reduction alone leads to reduced vascular risk. This hypothesis will require testing of agents with targeted vascular anti-inflammatory effects that lack proven beneficial effects such as LDL-C reduction.

Initial site recruitment for the JUPITER trial within the United States and Canada began in mid-2003. Further information on the JUPITER trial can be obtained at www.JUPITERstudy.com or by calling (888) 660-8254.

## Appendix A: JUPITER Steering Committee (United States and Canada)

Paul M Ridker, Harvard Medical School (Study Chairman)
Antonio Gotto, Weill Medical College of Cornell University
Jacques Genest, McGill University
Peter Libby, Harvard Medical School
James Willerson, University of Texas
James Blasetto, Astra-Zeneca (nonvoting)

## Appendix B: JUPITER Independent Data and Safety Monitoring Board

Rory Collins, Oxford University (Chair)
Gervasio Lamas, Miami Heart Institute
Douglas Vaughan, Vanderbilt University
Sidney Smith, University of North Carolina
Kent Bailey, Mayo Clinic
Robert J Glynn, Harvard Medical School (nonvoting)

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# C-Reactive Protein in Healthy Subjects: Associations With Obesity, Insulin Resistance, and Endothelial Dysfunction

## A Potential Role for Cytokines Originating From Adipose Tissue?

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***Abstract***—C-reactive protein, a hepatic acute phase protein largely regulated by circulating levels of interleukin-6, predicts coronary heart disease incidence in healthy subjects. We have shown that subcutaneous adipose tissue secretes interleukin-6 in vivo. In this study we have sought associations of levels of C-reactive protein and interleukin-6 with measures of obesity and of chronic infection as their putative determinants. We have also related levels of C-reactive protein and interleukin-6 to markers of the insulin resistance syndrome and of endothelial dysfunction. We performed a cross-sectional study in 107 nondiabetic subjects: (1) Levels of C-reactive protein, and concentrations of the proinflammatory cytokines interleukin-6 and tumor necrosis factor-$\alpha$, were related to all measures of obesity, but titers of antibodies to *Helicobacter pylori* were only weakly and those of *Chlamydia pneumoniae* and cytomegalovirus were not significantly correlated with levels of these molecules. Levels of C-reactive protein were significantly related to those of interleukin-6 ($r=0.37$, $P<0.0005$) and tumor necrosis factor-$\alpha$ ($r=0.46$, $P<0.0001$). (2) Concentrations of C-reactive protein were related to insulin resistance as calculated from the homoeostasis model assessment model, blood pressure, HDL, and triglyceride, and to markers of endothelial dysfunction (plasma levels of von Willebrand factor, tissue plasminogen activator, and cellular fibronectin). A mean standard deviation score of levels of acute phase markers correlated closely with a similar score of insulin resistance syndrome variables ($r=0.59$, $P<0.00005$), this relationship being weakened only marginally by removing measures of obesity from the insulin resistance score ($r=0.53$, $P<0.00005$). These data suggest that adipose tissue is an important determinant of a low level, chronic inflammatory state as reflected by levels of interleukin-6, tumor necrosis factor-$\alpha$, and C-reactive protein, and that infection with *H pylori*, *C pneumoniae*, and cytomegalovirus is not. Moreover, our data support the concept that such a low-level, chronic inflammatory state may induce insulin resistance and endothelial dysfunction and thus link the latter phenomena with obesity and cardiovascular disease. **(*Arterioscler Thromb Vasc Biol*. 1999;19:972-978.)**

**Key Words:** C-reactive protein ■ insulin resistance ■ obesity ■ endothelial dysfunction ■ interleukin-6

Inflammatory processes have important roles in the etiology of coronary heart disease (CHD),[1,2] but the mechanisms underlying this relationship are poorly understood. Several studies have shown that elevated plasma levels of fibrinogen, C-reactive protein (CRP), and interleukin-6 (IL-6) are associated with the risk of CHD and the severity of atherosclerosis.[3–6] Whether these molecules play a causative role, or simply act as markers of the acute phase reaction, is debatable. Elevated IL-6 levels have been reported in patients with unstable angina where inflammatory processes may facilitate the transition from the clinically stable to unstable atherosclerotic plaques.[6] However, it has also been shown that CRP levels are associated with CHD in healthy subjects, both in a cross-sectional study in general practice,[7] and longitudinally in the US Physicians Health Study,[8] the MONICA-Augsburg Cohort Study,[9] and the MRFIT Study,[10] where CRP levels predicted cardiovascular events or CHD mortality during a follow-up of between 2 and 17 years. These observations imply that atheroma progression, as well as plaque rupture, may be predicted by raised CRP levels. It has nevertheless remained an issue of debate as to whether the relationship between CRP and cardiovascular disease reflects inflammation in the vascular wall, perhaps because of chronic infections such as *Chlamydia pneumonia*,[11] or inflammation originating in a more remote site, with secondary effects on the vascular wall through cytokines and other mediators.

The synthesis of CRP by the liver is largely regulated by IL-6.[12] Although the activated leukocyte is widely assumed to be the major source of circulating IL-6, with additional contributions from fibroblasts and endothelial cells,[12] novel

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observations from our laboratory have proposed a previously unsuspected source for this cytokine. Using the technique of arteriovenous difference measures across a subcutaneous adipose tissue bed and radio-xenon measures of adipose tissue blood flow, we have demonstrated IL-6 production by human subcutaneous adipose tissue in vivo.[13] The production of IL-6, as well as systemic concentrations, increase with adiposity, and we have estimated that ≈30% of total circulating concentrations of IL-6 originate from adipose tissue in healthy subjects.[13] Both IL-6 and tumor necrosis factor-$\alpha$ (TNF-$\alpha$) are expressed in adipose tissue[14,15] and in vitro release of TNF-$\alpha$ by adipocytes has been reported.[16] Among the known effects of these cytokines are inhibition of insulin signaling[17] and induction of both hypertriglyceridemia[18] and endothelial activation.[19]

These observations have led us to explore the links of levels of acute phase markers and concentrations of proinflammatory cytokines with two of their proposed *determinants*, ie, obesity and chronic infection with 3 organisms proposed to be related to risk of CHD.[11,20–22] We have also explored relationships of acute phase markers with features of the insulin resistance syndrome[23] and of markers of endothelial dysfunction, ie, with the proposed *consequences* of a chronic low-level inflammatory state.[17,19] We hypothesized that: (1) If adipose tissue were responsible for production of proinflammatory cytokines, then circulating concentrations of C-reactive protein and of proinflammatory cytokines would be related to measures of obesity; (2) If IL-6 were responsible for the metabolic and vascular consequences of obesity, then measures of IL-6 and of CRP would relate to insulin resistance syndrome and endothelial markers, independently of measures of adiposity.

We have explored these relationships in a population of 107 healthy subjects in whom a large number of measures had been assessed, recognizing that this size of study, and its cross-sectional design, must, by its nature be hypothesis generating. We have explored associations both between individual measures of obesity, insulin resistance syndrome, endothelial and acute phase activation, as well as between predefined groups of these variables.

## Methods

### Subjects

We studied 107 white nondiabetic subjects as a follow-up investigation of cardiovascular risk factors.[24–28] In summary, we originally investigated subjects aged 40 to 75 randomly selected from the age-sex register of a north London general practice, and 36 (SD5) months later restudied 125 of those with normal glucose tolerance. In 107 of the recall subjects, sufficient serum and plasma was available to study a range of other variables, these being similar to the total population in age, gender ratio, and levels of the risk factors under investigation. The details of the study methods for anthropometry, blood pressure, daytime and overnight albumin excretion rate, cardiovascular disease history, and Minnesota code classification of electrocardiograms have been described previously.[26–28]

### Methods

Fasting blood from these 107 subjects was collected, spun at 2000*g* for 15 minutes, and used for assay of total and HDL cholesterol, triglycerides, insulin, proinsulin, des 32,32 proinsulin, plasminogen activator inhibitor-1 (PAI-1) activity, and fibrinogen as previously described,[24–27] and for additional measures of endothelial and acute phase markers and other related variables. Tumor necrosis factor-$\alpha$ (TNF-$\alpha$) and interleukin-6 (IL-6) were measured by ELISA (R&D Systems). Thrombomodulin, von Willebrand factor, cellular fibronectin,[29] tissue plasminogen activator (tPA) antigen, PAI-1 antigen, and C-reactive protein (CRP) were measured at the Gaubius Laboratory, TNO-PG, Leiden, Netherlands. Thrombomodulin was assayed using an ELISA kit (Stago)[30]; von Willebrand factor antigen was measured by an ELISA essentially as described[31] using polyclonal antibodies from DAKO; and cellular fibronectin was measured with a sandwich ELISA using a monoclonal antibody IST-9 (Harlan Sera Labs) against the ED-A domain for capture, and a peroxidase-conjugated polyclonal fibronectin antibody (DAKO) for detection. Tissue plasminogen activator and PAI-1 antigens were measured by ELISA (Organon Teknika), which recognizes both free forms of the factors and complexes of tPA with PAI-1. C-reactive protein was measured using a highly sensitive ELISA procedure,[32] with a range of 0.25 to 10.25 $\mu$g/mL and an interassay coefficient of variation (CV) of 8%. Antibody titers to *Helicobacter pylori* were measured using an enzyme immunoassay (Helico-G, Porton Cambridge). *C pneumoniae* IgG antibody titers were determined by ELISA according to a published method.[33] Cytomegalovirus (CMV) IgG titers were determined using a standard microtiter complement fixation assay, using in-house CMV antigen prepared from the AD169 strain of CMV. These assays were performed in the Departments of Microbiology and Virology, UCL Hospitals, London. Insulin sensitivity was calculated using the homeostasis model assessment (HOMA) model,[34] a mathematical estimate of insulin sensitivity based on fasting glucose and insulin concentrations.

### Statistical Methods

Linear correlation was used to look at relationships between variables, with logarithmic transformation of skewed variables. Comparison of groups was performed using unpaired Student's *t* test. Multiple regression analysis was used to explore the independence of observed relationships between clusters of variables, with forced entry of age, gender, smoking, and prevalent CHD, followed by the standard deviation scores (see below) for the putative independent variables. Data are presented as mean±SD or as median (interquartile range) for skewed variables. Significance levels are shown for all comparisons and relationships where $P<0.05$ although, because of the number of tests being performed, a more rigorous criterion of significance should be applied. Nevertheless, because the purpose is to explore relationships of acute phase markers with obesity, insulin resistance syndrome, and endothelial activation, another approach has also been used.

To explore the association between predefined clusters of variables, we created *mean standard deviation scores* for insulin resistance variables, endothelial markers, and acute phase markers for each subject. This approach was taken to reduce the influences of biological variability of each measure,[35] which would make the usual multivariate approach less suitable, as well as to reduce the number of associations explored. We also preferred this approach to a formal factor analysis, as we were interested in possible etiological relationships between three predefined, and ostensibly distinct, groups of variables. For each subject, each variable was expressed as standard deviations of difference from the population mean, if necessary after logarithmic transformation, a value that ranged from ≈−2.5 to 2.5. The mean scores were calculated as the mean of these standard deviation scores as follows: (1) *Insulin resistance score*={systolic blood pressure+diastolic blood pressure+triglyceride+[HDL cholesterol×(−1)]+[insulin sensitivity×(−1)]+body mass index +waist-to-hip ratio+subscapular-to-triceps ratio}/8. (2) *Endothelial marker score*=(thrombomodulin+cellular fibronectin+von Willebrand factor+mean albumin excretion rate)/4. (3) *Acute phase marker score*=(fibrinogen+C-reactive protein+IL-6+TNF-$\alpha$)/4.

For some of the analyses, including those shown in Table 2, the *obesity variables were omitted from the insulin resistance score* as follows: {systolic blood pressure+diastolic blood pressure+triglyceride+[HDL cholesterol×(−1)]+[insulin sensitivity×(−1)]}/5.

For some analyses we also derived an *obesity score* as a mean standard deviation score: (body mass index+waist-to-hip ratio+subscapular-to-triceps ratio)/3.

Where results were missing, for insulin (n=2), albumin excretion rate (n=1), thrombomodulin (n=10), or fibrinogen (n=4), the mean standard deviation scores were calculated for the smaller denomina-

**TABLE 1. Characteristics of Study Subjects**

| | |
|---|---|
| Number (M/F) | 107 (59/48) |
| Age (y) | 59.0±10.9 |
| Body mass index (kg/m$^2$) | 25.9±4.5 |
| Waist-to-hip ratio | 0.86±0.08 |
| Subscapular-to-triceps ratio | 1.31±0.59 |
| Systolic blood pressure (mm Hg) | 124.8±18.4 |
| Diastolic blood pressure (mm Hg) | 80.4±11.0 |
| Triglyceride (mmol/L) | 1.3 (1.0, 1.7) |
| HDL cholesterol (mmol/L) | 1.38±0.37 |
| LDL cholesterol (mmol/L) | 3.62±1.05 |
| Fasting plasma glucose (mmol/L) | 4.8±0.5 |
| 2-h plasma glucose (mmol/L) | 4.9±1.1 |
| PAI-1 activity (AU/mL) | 8.1 (4.2, 15.9) |
| PAI-1 antigen (ng/mL) | 95.6±58.9 |
| tPA (ng/mL) | 21.0±9.4 |
| von Willebrand factor (%) | 109.7±40.9 |
| Thrombomodulin (ng/mL) | 33.7 (10.9, 121.3) |
| Cellular fibronectin (%) | 108 (71, 159) |
| Mean albumin excretion rate ($\mu$g/min) | 10.2 (7.0, 20.6) |
| Fibrinogen (mg/mL) | 289.2±75.9 |
| CRP ($\mu$g/mL) | 1.35 (0.57, 2.18) |
| TNF-$\alpha$ (pg/mL) | 3.65 (2.98, 4.53) |
| IL-6 (pg/mL) | 2.19 (1.18, 4.40) |

Variables are presented as mean±SD, or as median (interquartile range) for skewed variables.

tor, but the relationships observed were almost identical if all data from these subjects were omitted. Data for PAI-1 antigen were not used as a component of the insulin resistance score because this molecule is also an acute phase protein. Furthermore, tPA antigen was excluded from the endothelial score because tPA circulates partly bound to PAI-1, the complex being measured by the assay we used.

## Results

The characteristics of these middle-aged white subjects with normal glucose tolerance are shown in Table 1. The low levels of CRP are similar to those found in other healthy populations.[7,8]

To explore the possible determinants of the acute phase markers and of the levels of proinflammatory cytokines, we explored their relationships with titers of IgG antibodies to 3 organisms which have been proposed as playing a potential role in atherogenesis.[11,20–22] Concentrations of C-reactive protein correlated weakly with titers of *H pylori, C pneumoniae*, and cytomegalovirus antibodies (Table 2). However, the only significant correlation seen between titers of such antibodies and concentrations of cytokines was that of IL-6 with *H pylori*.

Both IL-6 and TNF-$\alpha$ are expressed in adipose tissue,[14,15] and we have recently described the release of the former, but not the latter, from a subcutaneous adipose tissue bed in vivo.[13] Concentrations of IL-6, TNF-$\alpha$, and C-reactive protein were strongly related to measures of total, and particularly central, obesity (Table 2).

**TABLE 2. Relationships of Concentrations of Proinflammatory Cytokines and C-reactive Protein With Antibody Titers and Obesity**

| | TNF-$\alpha$§ | IL-6§ | CRP§ |
|---|---|---|---|
| *H pylori* titer (n=80) | 0.18 | 0.28* | 0.24* |
| *C pneumoniae* titer (n=70) | 0.21 | 0.15 | 0.25* |
| CMV titer (n=80) | 0.21 | 0.17 | 0.23* |
| Body mass index | 0.33‡ | 0.19* | 0.41‡ |
| Waist-to-hip ratio | 0.51‡ | 0.41‡ | 0.32† |
| Subscapular-to-triceps ratio | 0.37‡ | 0.26† | 0.21* |
| CRP | 0.46‡ | 0.37‡ | – |

Values are shown as Pearson correlation coefficients.
*$P<0.05$.
†$P<0.01$.
‡$P<0.001$.
§Data logarithmically transformed.

Concentrations of CRP correlated both with those of IL-6 ($r=0.37$, $P<0.0005$) and of TNF-$\alpha$ ($r=0.46$, $P<0.0001$). In Table 3 the relationships of concentrations of IL-6, TNF-$\alpha$, and C-reactive protein with the components of the insulin resistance syndrome and with endothelial markers are shown. Univariate correlations are given as these were little affected by adjustment for age and gender. Concentrations of TNF-$\alpha$ were related to all insulin resistance variables, including proinsulin-like molecules, tPA, and PAI-1. Concentrations of IL-6 were also related to several of the insulin resistance syndrome and endothelial markers, including albumin excretion rate, although the relationships for C-reactive protein were generally stronger. Although there is a weak relationship between concentrations of low-density LDL cholesterol and those of CRP, no such relationships are seen with TNF-$\alpha$ or IL-6.

The population was dichotomized into those with high and those with low concentrations of CRP, based on the median

**TABLE 3. Relationship of Concentrations of Proinflammatory Cytokines and of C-Reactive Protein With Components of Insulin Resistance Cluster and Endothelial Markers**

| | TNF-$\alpha$§ | IL-6§ | CRP§ |
|---|---|---|---|
| Insulin sensitivity§ | −0.35‡ | −0.09 | −0.22* |
| Triglyceride§ | 0.37‡ | 0.03 | 0.27† |
| HDL cholesterol | 0.27† | −0.26† | −0.21* |
| LDL cholesterol | 0.08 | 0.04 | 0.25* |
| Systolic blood pressure | 0.33‡ | 0.31† | 0.34‡ |
| Intact proinsulin§ | 0.33‡ | 0.16 | 0.16 |
| Des 31,32 proinsulin§ | 0.28† | 0.11 | 0.08 |
| PAI-1 antigen | 0.35‡ | 0.18 | 0.19* |
| tPA antigen | 0.40‡ | 0.32† | 0.40‡ |
| von Willebrand factor | 0.38‡ | 0.11 | 0.31‡ |
| Thrombomodulin§ | 0.32† | −0.05 | 0.13 |
| Cellular fibronectin§ | 0.36‡ | 0.13 | 0.28† |
| Mean albumin excretion rate§ | 0.25* | 0.20* | 0.07 |

Values are shown as Pearson correlation coefficients.
*$P<0.05$.
†$P<0.01$.
‡$P<0.001$.
§Data logarithmically transformed.

**TABLE 4. Characteristics of Subjects With Low (<1.35 μg/mL) and High (≥1.35 μg/mL) of C-Reactive Protein**

| | Low CRP | High CRP | *P* |
|---|---|---|---|
| Number (M/F) | 53 (27/26) | 54 (32/22) | 0.38 |
| Age (y) | 55.9±11.0 | 62.1±9.9 | 0.003 |
| Smokers (non/ex/current) | 37/3/13 | 26/6/22 | 0.17 |
| Body mass index (kg/m$^2$) | 24.3±4.0 | 27.5±4.5 | <0.001 |
| Waist-to-hip ratio | 0.84±0.08 | 0.89±0.07 | 0.001 |
| Subscapular-to-triceps ratio | 1.15±0.51 | 1.47±0.62 | 0.004 |
| *H pylori* titer | 1/<10 (1/<10, 1/48) | 1/24 (1<10, 1/70) | 0.57 |
| *C pneumoniae* titer | 1/100 (1<100, 1/400) | 1/200 (1/<100, 1/200) | 0.55 |
| Cytomegalovirus titre | 1/20 (1/<5, 1/80) | 1/40 (1/10, 1/80) | 0.11 |
| Insulin (pmol/L) | 21.4 (14.4, 42.9) | 32.8 (19.1, 46.0) | 0.032 |
| Insulin sensitivity (HOMA) (%) | 107.6 (55.9, 165.6) | 73.8 (51.0, 127.6) | 0.027 |
| Systolic blood pressure (mm Hg) | 118.7±15.7 | 130.8±18.9 | 0.001 |
| Diastolic blood pressure (mm Hg) | 77.7±11.0 | 83.0±10.3 | 0.011 |
| Triglyceride (mmol/L) | 1.2 (0.9, 1.6) | 1.35 (1.18, 1.93) | 0.036 |
| HDL cholesterol (mmol/L) | 1.44±0.39 | 1.33±0.35 | 0.14 |
| Fasting plasma glucose (mmol/L) | 4.8±0.4 | 4.8±0.5 | 0.40 |
| PAI-1 activity (AU/mL) | 6.5 (3.6, 12.2) | 10.7 (4.8, 17.2) | 0.051 |
| PAI-1 antigen (ng/mL) | 83.4±55.1 | 107.6±60.5 | 0.033 |
| tPA (ng/mL) | 17.1±8.6 | 24.9±8.6 | <0.001 |
| von Willebrand factor (%) | 101.5±37.8 | 117.8±42.6 | 0.038 |
| Thrombomodulin (ng/mL) | 33.2 (27.7, 39.6) | 34.8 (28.1, 41.1) | 0.35 |
| Cellular fibronectin (%) | 84.0 (62.0, 138.5) | 130.0 (83.3, 178.5) | 0.001 |
| Mean albumin excretion rate (μg/min) | 10.8 (7.3, 19.4) | 9.6 (6.8, 21.6) | 0.34 |
| Fibrinogen (mg/mL) | 275.2±68.7 | 301.8±80.4 | 0.22 |
| TNF-α (pg/mL) | 3.18 (2.63, 3.69) | 4.12 (3.58, 5.06) | <0.001 |
| IL-6 (pg/mL) | 1.35 (0.89, 3.11) | 3.22 (1.79, 5.39) | <0.001 |

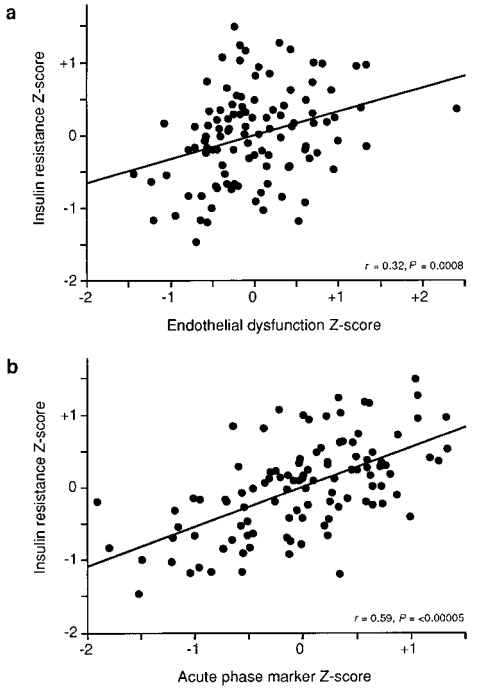
Variables are presented as mean±SD, or as median (interquartile range) for skewed variables.

value of 1.35 mg/mL (Table 4). Subjects with high concentrations of CRP were more obese than those with lower levels, and had higher levels of blood pressure, triglyceride, von Willebrand factor, cellular fibronectin, PAI-1, tPA, and of the proinflammatory cytokines TNF-α and IL-6, but did not differ in titers of antibodies to *Helicobacter*, *Chlamydia*, or cytomegalovirus.

To overcome the problems of biological variability of the different measures, and to explore these inter-relationships further while controlling for potential confounds, we used summary scores for the insulin resistance syndrome variables, for endothelial dysfunction, and for acute phase markers by calculating a mean of a standard deviation score for each group of variables (see Methods). The relationships of these are shown in Figure 1. Whereas the insulin resistance syndrome and endothelial scores correlate with a coefficient of 0.32 ($P=0.0008$), there is a strong relationship between the insulin resistance syndrome and acute phase scores ($r=0.59$, $P<0.00005$). The third of these correlations, between endothelial and acute phase scores, is also significant ($r=0.43$, $P<0.00005$). A sum obesity score correlated with measures of both endothelial ($r=0.33$, $P=0.001$) and acute phase ($r=0.54$, $P<0.0005$) scores. Nevertheless, if the 3 measures of obesity are removed from the insulin resistance syndrome score, the relationship with the acute phase score was only slightly weakened ($r=0.53$, $P<0.00005$). Moreover, the strength of the relationship was not substantially affected by omitting any particular variable from either score. In multiple regression models, controlling for age, gender, smoking, and prevalent CHD, if the acute phase and endothelial scores were included in the same model, the former remained significantly associated with insulin resistance syndrome score (partial $r=0.61$, $P<0.00005$), but not the latter (partial $r=-0.02$, $P=0.82$). We have also approached the analysis of clustering of the variables using factor analysis, with generally similar results. The insulin resistance variables associate as two clusters, one comprising altered lipid concentrations with central obesity, and the other blood pressure with body mass index. Although both clusters correlate with acute phase markers, it is the second that relates more closely to endothelial dysfunction (data not shown).

## Discussion

There has been much interest in the prognostic significance of raised levels of C-reactive protein in patients with angina,[36] with the proposal that it points to release of IL-6 by activated macrophages in an unstable plaque.[37] More recently, however, the observations that raised concentrations of CRP in




(a) The relationship between a derived score of insulin resistance variables and a score for measures of endothelial dysfunction. (b) The relationship between a derived score of insulin resistance variables and a score for acute phase markers and proinflammatory cytokines. For the derivation of these scores, see Methods.

healthy subjects predicted the incidence of CHD over a period of years[8–10] have suggested a role for inflammation in the initiation of atherosclerosis as well as in the precipitation of an acute event. The synthesis of CRP is predominantly under the control of IL-6,[12] which in turn has been assumed to originate largely from activated leukocytes, either in the vessel wall itself or at a remote site of infection.[2,11] We found that concentrations of C-reactive protein were related to titers of IgG antibodies, evidence of previous infection, for each of the 3 organisms we investigated. However, the correlations between these antibody titers and concentrations of fibrinogen, TNF-$\alpha$, and IL-6 were weaker and generally insignificant. In this study, *Chlamydia* antibodies were measured using an ELISA method, and it is possible that antibody titers by an immunofluorescence assay would have been more closely related to markers of inflammation.[11,38]

We have found close relationships between circulating CRP and cytokine concentrations and each of the anthropometric measures of obesity, compatible with an adipose tissue origin for TNF-$\alpha$ and IL-6. Mendall et al have previously shown associations of circulating concentrations of CRP,[7] and of TNF-$\alpha$,[39] but not of IL-6,[39] with BMI, and the relationship of CRP levels with obesity were also noted in the MRFIT cohort,[10] and, among nonsmokers, in the Cardiovascular Health Study.[40] By contrast, we found no significant influence of smoking status on the relationships between acute phase markers and obesity. Both cytokines are expressed in, and released by, adipose tissue.[13–16] We have recently reported significant in vivo release of IL-6, but not TNF-$\alpha$, by a subcutaneous adipose tissue depot.[13] However, the relationships of circulating concentrations of TNF-$\alpha$ with obesity suggests that adipose tissue, perhaps in other sites, may contribute to circulating levels. Alternatively, the expression of one of the TNF-$\alpha$ soluble receptors by adipose tissue[41] raises the possibility that the circulating cytokine is in the form of a complex, the relationships with measures of obesity representing secretion of the soluble receptor. Even if free, it is likely that the presence of this cytokine in the circulation represents spillover from the interstitial compartment in adipose tissue, and perhaps from adipocytes within muscle.

We report a relationship between circulating concentrations both of CRP and of two proinflammatory cytokines with a number of features of the insulin resistance syndrome,[23] reflecting our previous report of a relationship between fibrinogen concentrations and measures of insulin resistance.[25] Although relationships of CRP levels with triglycerides, HDL, glucose, and diabetes have been noted previously,[7,40] no such relationship appears to have been reported with insulin concentrations or measures of insulin resistance. It is clearly not possible, in a cross-sectional study, to attribute causality to one of a set of correlated variables, but we have explored some hypotheses in this setting. The relationship between elevated concentrations of CRP and of the proinflammatory cytokines with the insulin resistance syndrome could represent associations produced by a confounding variable, such as adiposity. However, the relationships between a derived insulin resistance syndrome standard deviation score and one for the acute phase variables was only slightly weakened by removing all obesity measures from the former score. We also excluded PAI-1 from the calculation of an insulin resistance score, both because the measure of antigen may represent inactive PAI-1 (complexed to tPA or released from platelets), and also because PAI-1 is recognized to respond to acute phase stimuli.

Our observations could suggest that the cytokines, arising in part from adipose tissue, might themselves be partly responsible for the metabolic, hemodynamic, and hemostatic abnormalities that cluster with insulin resistance. Although not itself an inducer of acute phase proteins, TNF-$\alpha$ induces production of IL-6,[42] which is itself the major determinant of the acute phase response.[12] Among the known metabolic effects of TNF-$\alpha$ are inhibition of the action of lipoprotein lipase[43] and stimulation of lipolysis,[18] these actions being shared with IL-6.[44,45] Furthermore, TNF-$\alpha$ impairs the function of the insulin signaling pathway by effects on phosphorylation of both the insulin receptor and its substrate, IRS-1.[17,46]

In addition to their associations with insulin resistance syndrome variables, elevated levels of CRP and of cytokines were associated with a series of indicators of endothelial dysfunction. Tracy et al have previously reported associations of levels of CRP with a variety of measures of procoagulant activity and fibrinolysis,[40] and have suggested that these represent consequences either of inflammation in underlying atherothrombotic disease or of inflammatory cells activated by products of ongoing coagulation processes. TNF-$\alpha$ is known to influence endothelial cell function,[19,47] and a recent study suggests that IL-6 may also induce endothelial expression of chemokines and adhesion molecules in the presence of IL-6 soluble receptor, which is released in inflammatory states.[48] If endothelial dysfunction, perhaps as a consequence of elevated concentrations of cytokines, resulted in impair-

ment of vasodilatation of resistance vessels,[49] it could be postulated that the cluster of variables that have been attributed to insulin resistance (dyslipidemia, hypertension, and impaired fibrinolysis), as well as insulin resistance itself, might all result as consequences of a common antecedent. The strong relationship between concentrations of C-reactive protein and insulin resistance variables (Table 3), compared with those seen for IL-6, may simply reflect the longer half-life of C-reactive protein providing a more stable marker of acute phase mediators. Levels of C-reactive protein are predominantly modulated by hepatic effects of IL-6,[12] which suggests a more important role for this cytokine in the cluster than suggested by the correlations shown in Table 3. If circulating TNF-$\alpha$ represents spillover from adipose tissue and muscle, where the local concentrations would be more likely to approximate to those required to exert metabolic effects in vitro,[50,51] this might imply autocrine or paracrine, and not endocrine, metabolic effects of TNF-$\alpha$. Adipose tissue release of IL-6, also induced by TNF-$\alpha$,[42] may then be responsible for systemic effects on endothelium[48] and lipids.[44,45]

In conclusion, we have shown, in healthy subjects, relationships between levels of CRP and measures of obesity, consistent with our finding of adipose tissue release of IL-6 in vivo[13] and implicating adipose tissue as a major source for circulating IL-6. We have also found associations between levels of acute phase proteins and of proinflammatory cytokines not only with blood pressure and dyslipidemia, but both with a measure of insulin resistance and with markers of endothelial dysfunction. Furthermore, the association of acute phase markers with insulin resistance variables is independent of anthropometric measures of obesity. We are suggesting a more general role for both IL-6 and TNF-$\alpha$ in atherogenesis and thrombosis, influencing as they do, the risk factors which have been termed the insulin resistance syndrome, endothelial function and expression of prothrombotic factors and adhesion molecules, and acute phase proteins, which in turn may increase cardiovascular risk. Our paradigm provides a novel explanation for the association of insulin resistance and cardiovascular risk, as well as a putative mechanism for the deleterious effects of obesity, and in particular central adiposity,[52] in heart disease risk. Of necessity, however, this study has merely developed a hypothesis about the common antecedence of adipose tissue-generated proinflammatory cytokines in insulin resistance and endothelial dysfunction, which will require further testing in both epidemiological and clinical investigative studies.

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# Chronic Subclinical Inflammation as Part of the Insulin Resistance Syndrome

## The Insulin Resistance Atherosclerosis Study (IRAS)

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***Background***—Inflammation has been suggested as a risk factor for the development of atherosclerosis. Recently, some components of the insulin resistance syndrome (IRS) have been related to inflammatory markers. We hypothesized that insulin insensitivity, as directly measured, may be associated with inflammation in nondiabetic subjects.

***Methods and Results***—We studied the relation of C-reactive protein (CRP), fibrinogen, and white cell count to components of IRS in the nondiabetic population of the Insulin Resistance Atherosclerosis Study (IRAS) (n=1008; age, 40 to 69 years; 33% with impaired glucose tolerance), a multicenter, population-based study. None of the subjects had clinical coronary artery disease. Insulin sensitivity ($S_I$) was measured by a frequently sampled intravenous glucose tolerance test, and CRP was measured by a highly sensitive competitive immunoassay. All 3 inflammatory markers were correlated with several components of the IRS. Strong associations were found between CRP and measures of body fat (body mass index, waist circumference), $S_I$, and fasting insulin and proinsulin (all correlation coefficients $>0.3$, $P<0.0001$). The associations were consistent among the 3 ethnic groups of the IRAS. There was a linear increase in CRP levels with an increase in the number of metabolic disorders. Body mass index, systolic blood pressure, and $S_I$ were related to CRP levels in a multivariate linear regression model.

***Conclusions***—We suggest that chronic subclinical inflammation is part of IRS. CRP, a predictor of cardiovascular events in previous reports, was independently related to $S_I$. These findings suggest potential benefits of anti-inflammatory or insulin-sensitizing treatment strategies in healthy individuals with features of IRS. **(*Circulation*. 2000;102:42-47.)**

**Key Words:** inflammation ■ proteins ■ insulin resistance syndrome ■ insulin ■ atherosclerosis

A relationship between C-reactive protein (CRP), a sensitive marker of inflammation, and the development of atherosclerotic disease has been observed in experimental[1–4] and epidemiological studies.[5–8] It is still unknown, however, whether elevated CRP levels merely reflect an epiphenomenon accompanying established atherosclerotic disease or whether the protein itself is involved in the initiation and/or progression of atherosclerosis.

Previous reports suggest a positive association between components of the insulin resistance syndrome (IRS) and markers of the acute-phase response, including CRP[6,8–12] and fibrinogen.[13] CRP levels were associated with body mass index (BMI),[6,8–10,12] serum lipids,[6,8–10,12] and fasting glucose.[9] Elevated levels of inflammatory markers (including CRP) were also found in type 2 diabetic patients with features of IRS.[11]

The nature of the association of CRP with IRS, however, is poorly understood. We hypothesized that insulin insensitivity and/or hyperinsulinemia may be associated with circulating CRP levels. Such an association would potentially provide insights into the role of CRP in atherosclerotic disease and further clarify the association of hyperinsulinemia with cardiovascular disease.[14]

We studied the relation of inflammatory markers (CRP, fibrinogen, white cell count) and components of IRS, including insulin sensitivity, as directly measured by a frequently sampled intravenous glucose tolerance test. Furthermore, we sought to investigate whether CRP levels were independently related to insulin (or its precursors), insulin sensitivity, or both. The analyses were restricted to nondiabetic subjects without clinical coronary artery disease to avoid possible confounding by preexisting cardiovascular disease. It has been shown previously that patients with type 2 diabetes present with higher levels of inflammatory markers[8,11] and a high prevalence of atherosclerosis, including clinically undetected disease.[15]

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## Methods

### Study Subjects

The Insulin Resistance Atherosclerosis Study (IRAS) is a multicenter, population-based epidemiological study exploring relationships between insulin resistance, cardiovascular risk factors, and cardiovascular disease across different ethnic groups and various states of glucose tolerance. A full description of the design and methods of the IRAS has been published.[16] The IRAS protocol was approved by local institutional review committees, and all subjects gave informed consent.

A total of 1088 nondiabetic individuals participated in the IRAS. Subjects with a current acute illness (including clinically significant infectious disease) were excluded from IRAS examination. Subjects with clinically overt coronary artery disease, defined as past myocardial infarction, PTCA or CABG, or ECG evidence of ischemic heart disease, were excluded from the present analyses. This report includes data on 1008 nondiabetic subjects in whom CRP and fibrinogen levels were measured. Cigarette smoking was dichotomized into "never" and "ever" (including past and current) by use of a standard questionnaire. BMI (weight/height$^2$ [kg/m$^2$]) was used as an estimate of overall adiposity. Waist circumference (estimate of visceral fat) was measured at the natural indentation between the 10th rib and the iliac crest (minimum waist).

A standard 75-g oral glucose tolerance test was performed. Glucose tolerance status was based on World Health Organization criteria.[17]

### Laboratory Measurements

Plasma glucose was measured with the glucose oxidase technique on an automated autoanalyzer (Yellow Springs Equipment Co). Insulin was measured with the dextran-charcoal radioimmunoassay.[18] This insulin assay cross-reacts with proinsulin. Fasting serum intact proinsulin and 32–33 split proinsulin were determined at the Department of Clinical Biochemistry at Addenbrook's Hospital, Cambridge, UK (Professor C.N. Hales), by means of highly specific 2-site monoclonal antibody–based immunoradiometric assays.[19]

Insulin sensitivity was assessed by a frequently sampled intravenous glucose tolerance test[20] with minimal model analysis.[21] Two modifications of the original protocol were used: (1) an injection of regular insulin, rather than tolbutamide, to ensure adequate plasma insulin levels for the accurate computation of insulin sensitivity across a broad range of glucose tolerance[22] and (2) the reduced sampling protocol[23] because of the large number of subjects. Insulin sensitivity, expressed as the insulin sensitivity index ($S_I$), was calculated by mathematical modeling methods (MINMOD, version 3.0, 1994).

Plasma lipoprotein measurements were obtained from fasting single fresh plasma samples through Lipid Research Clinic methods at the central IRAS laboratory at Medlantic Research Institute, Washington, DC (Professor B.V. Howard).

CRP was measured by in-house ultrasensitive competitive immunoassay (antibodies and antigens from Calbiochem) with an interassay coefficient of variation of 8.9%.[24] Fibrinogen was measured in citrated plasma with a modified clot-rate assay by use of the Diagnostica STAGO ST4 instrument, as described previously.[25] Complete blood cell counts were performed with standard techniques.

### Statistical Analysis

Statistical analyses were performed with the SAS statistical software system. Descriptive statistics (mean±SE) and number/percent are shown on Table 1. CRP levels differed by sex and ethnicity in the present population, and age and smoking were determinants of CRP levels in previous reports; therefore, multivariate models (partial Spearman correlations, multiple linear regression analysis) were tailored to account for these possible confounders. Partial Spearman correlations (adjusting for age, sex, ethnicity, clinic, and smoking status) for inflammatory markers with components of the IRS were estimated for the overall population (Table 2) and stratified by ethnicity and glucose tolerance status (normal [NGT] versus impaired [IGT] glucose tolerance). In these models, we also tested for interactions between the independent variables of interest (BMI, fasting glucose, insulin, proinsulin, split proinsulin, and $S_I$) and ethnicity and glucose tolerance status, respectively. The distribution of CRP levels was highly skewed. Logarithmically transformed values of CRP (log CRP) were used because the distribution of the residuals from the fitted models became normally distributed after log transformation. Thus, mean values of log CRP (adjusted for age, sex, ethnicity, clinic, and smoking status) in relation to the number of metabolic disorders were calculated by ANCOVA (Figure 1). Fur-

**TABLE 1. Descriptive Data in Nondiabetic Subjects Without Clinical Coronary Artery Disease: The IRAS**

| | |
|---|---|
| n | 1008 |
| Male sex, % | 43 |
| Impaired glucose tolerance, % | 33 |
| Age, y | 54.7±0.3 |
| BMI, kg/m$^2$ | 28.4±0.2 |
| Waist circumference, cm | 90.4±0.4 |
| Systolic BP, mm Hg | 119.6±0.5 |
| Diastolic BP, mm Hg | 77.1±0.3 |
| Triglyceride, mmol/L | 1.51±0.03 |
| Total cholesterol, mmol/L | 5.48±0.03 |
| LDL cholesterol, mmol/L | 3.66±0.03 |
| HDL cholesterol, mmol/L | 1.23±0.01 |
| Fasting glucose, mmol/L | 5.47±0.02 |
| Fasting insulin, pmol/L | 94.1±2.7 |
| Proinsulin, pmol/L | 6.0±0.2 |
| Split proinsulin, pmol/L | 8.3±0.3 |
| $S_I \times 10^{-4}$, $min^{-1} \cdot \mu U^{-1} \cdot mL^{-1}$ | 2.19±0.07 |
| CRP, mg/L | 3.53±0.18 |
| CRP >10 mg/L, n (%) | 70 (6.9) |
| White cell count, $\times 10^3$/mm$^3$ | 5.81±0.07 |
| Fibrinogen, mg/dL | 276.4±1.8 |

BP indicates blood pressure. Data are mean±SE.

**TABLE 2. Partial Spearman Correlation Analysis of Inflammation Markers With Variables of IRS Adjusted for Age, Sex, Clinic, Ethnicity, and Smoking Status**

| | CRP | WBC | Fibrinogen |
|---|---|---|---|
| BMI | 0.40‡ | 0.17‡ | 0.22‡ |
| Waist | 0.43‡ | 0.18‡ | 0.27‡ |
| Diastolic BP | 0.17‡ | 0.01 | 0.11† |
| Systolic BP | 0.20‡ | 0.08* | 0.11† |
| Triglyceride | 0.23‡ | 0.15‡ | 0.03 |
| Cholesterol (total) | 0.10† | −0.01 | 0.01 |
| HDL cholesterol | −0.11† | −0.12† | −0.15‡ |
| LDL cholesterol | 0.09* | −0.01 | 0.05 |
| Fasting glucose | 0.18‡ | 0.13‡ | 0.07* |
| Fasting insulin | 0.33‡ | 0.24‡ | 0.18‡ |
| Proinsulin (intact) | 0.30‡ | 0.17‡ | 0.20‡ |
| Proinsulin (split) | 0.32‡ | 0.21‡ | 0.20‡ |
| $S_I$ | −0.37‡ | −0.24‡ | −0.18‡ |

WBC indicates white cell count; BP, blood pressure.
*$P<0.05$, †$P<0.005$, ‡$P<0.0001$.

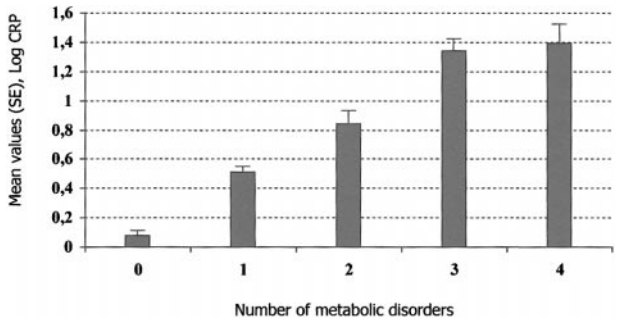


**Figure 1.** Mean levels of log CRP (SE represented by bars) adjusted for age, sex, ethnicity, clinic, and smoking status according to number of metabolic disorders (0 to 4), including (1) dyslipidemia (high triglyceride >2.27 mmol/L [200 mg/dL] and/or low HDL: men ≤0.91 mmol/L [35 mg/dL] and women ≤1.16 mmol/L [45 mg/dL]), (2) upper body adiposity (≥75th percentile for waist circumferences: men=103.0 cm and women=99.3 cm), (3) insulin resistance (<25th percentile for $S_I$: $<0.88\times10^{-4}$ $min^{-1} \cdot \mu U^{-1} \cdot mL^{-1}$ or in 47 subjects without frequently sampled intravenous glucose tolerance test, ≥75th percentile for fasting insulin: 114 pmol/L), and (4) hypertension (systolic blood pressure of ≥140 mm Hg and/or diastolic blood pressure of ≥90 mm Hg or current use of antihypertensive medication). All comparisons, $P=0.0001$, except for 2 versus 4 ($P<0.005$) and 3 versus 4 ($P$=NS).

thermore, we calculated (unadjusted) mean values of log CRP by tertile for $S_I$, BMI, and triglycerides and for hypertension (Figure 2).

Stepwise linear regression models were fit for log CRP as a dependent variable, including all variables of interest at the same time as independent variables to demonstrate the relative contribution of each of these variables to the outcome variable. After age, sex, ethnicity, clinic, and smoking status were forced into the model, the following independent variables were considered for the model: BMI, diastolic and systolic blood pressures, fasting glucose, $S_I$, fasting insulin, and proinsulin (intact). Only variables that had a $P\leq0.05$ were considered in the final fitted model (Table 3). A value of $P<0.05$ (2-sided) was considered statistically significant.

**TABLE 3. Stepwise Multiple Linear Regression Analysis With Log of CRP as the Dependent Variable**

| Independent Variable | B | SE(B) | $P$ | Partial $R^2$, % |
|---|---|---|---|---|
| BMI | 0.06 | 0.006 | 0.0001 | 14.2 |
| $S_I$ | −0.11 | 0.02 | 0.0001 | 3.1 |
| SBP | 0.008 | 0.002 | 0.0001 | 1.2 |

After forcing age, sex, clinic, ethnicity, and smoking status into the model, BMI, diastolic and systolic blood pressures (SBP), fasting glucose, fasting insulin, $S_I$, and proinsulin (intact) were analyzed as independent variables. Only variables that had a $P\leq0.05$ were considered in the final fitted model. $R^2$ for the model=26.0%.

## Results

Table 1 shows descriptive data. All 3 inflammatory markers were correlated with several components of IRS (Table 2). The associations were generally stronger for CRP than for white cell count and fibrinogen. Strong associations (correlation coefficients >0.3) were found between CRP and measures of body fat (BMI, waist circumference), $S_I$, fasting insulin, and proinsulin. There was a linear increase in CRP levels with an increase in the number of metabolic disorders (dyslipidemia, upper body adiposity, insulin resistance, hypertension; Figure 1). The respective mean log of CRP levels

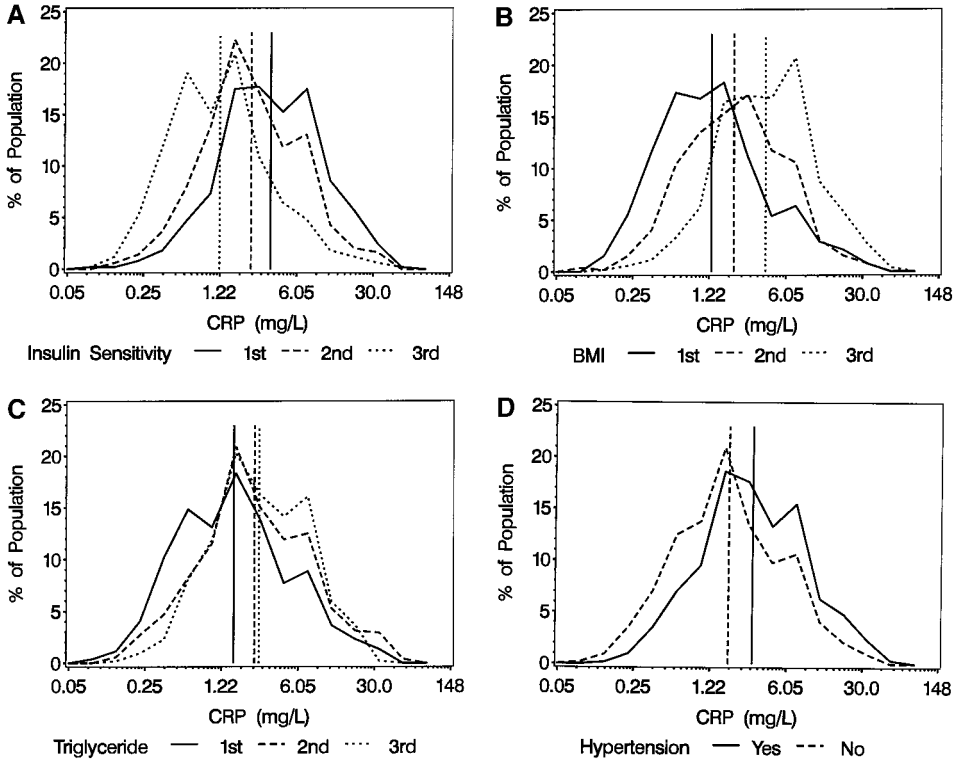


**Figure 2.** A through D, Distributions and mean values of log CRP stratified by tertiles for $S_I$ (A), BMI (B), and triglycerides (C). Mean values by tertiles are depicted by vertical solid lines (first tertile), dashed lines (second tertile), and dotted lines (third tertile). A, First tertile represents subjects with high insulin resistance (low $S_I$). D, Solid vertical line depicts mean value of log CRP in hypertensive subjects; dashed line, mean value in nonhypertensive subjects.

(±SE) were 0.075±0.06, 0.511±0.06, 0.845±0.07, 1.34±0.10, and 1.39±0.17 in the presence of 0, 1, 2, 3, or 4 metabolic disorders. The percentage of subjects presenting with 0, 1, 2, 3, and 4 disorders was 30.3%, 32.7%, 22.7%, 10.4%, and 4.0%, respectively. When mean values of log CRP were analyzed by tertiles of $S_I$, BMI, and triglycerides and by hypertension (Figure 2), higher levels of log CRP were found in the lower tertiles of $S_I$ (indicating a relation of higher CRP levels with higher insulin resistance), in the higher tertiles of BMI, in the highest tertile of triglycerides, and in hypertensive subjects. Furthermore, the distribution curves of log CRP values showed a shift to the right for hypertensive subjects and for increasing tertiles of BMI and triglycerides or a shift to the left for increasing tertiles of $S_I$.

The associations as shown in the overall population (Table 2) were also consistent among the 3 ethnic groups. Correlation coefficients for CRP in non-Hispanic whites (n=399), blacks (n=267), and Hispanics (n=342) were 0.43, 0.43, and 0.38 (BMI); 0.43, 0.46, and 0.39 (waist); 0.35, 0.29, and 0.36 (fasting insulin); 0.30, 0.31, and 0.29 (intact proinsulin); 0.38, 0.28, and 0.29 (split proinsulin); and −0.41, −0.33, and −0.38 ($S_I$), respectively (all $P<0.0001$). The correlation coefficient of CRP with fasting glucose was 0.16 ($P<0.005$) in non-Hispanic whites, 0.12 ($P$=NS) in blacks, and 0.25 ($P<0.0001$) in Hispanics. The association of fasting insulin with CRP and fibrinogen was less pronounced in blacks compared with non-Hispanic whites and Hispanics ($P<0.005$ and $P<0.05$ for interaction terms), respectively, although the associations were clearly in the same direction and, for CRP, highly significant in all 3 ethnic groups. All other interaction terms were not statistically significant.

The associations were also consistently seen in subjects with NGT and IGT. Correlation coefficients for CRP in subjects with NGT and IGT were 0.34 and 0.41 (BMI), 0.37 and 0.40 (waist), 0.32 and 0.23 (fasting insulin), 0.23 and 0.29 (intact proinsulin), 0.25 and 0.31 (split proinsulin), and −0.35 and −0.26 ($S_I$), respectively (all $P<0.0001$). The correlation of CRP with fasting glucose was weak in NGT ($r=0.11$, $P<0.05$) and not significant in IGT ($r=0.09$). The association of fasting insulin with CRP was stronger in subjects with NGT than with IGT ($P<0.0001$ for interaction term). All other interaction terms were not statistically significant.

Multivariate linear regression analyses showed that BMI, systolic blood pressure, and $S_I$ (inversely) were independently associated with log CRP levels in the overall population (Table 3).

## Discussion

In the present study, we have shown that in healthy, nondiabetic subjects, CRP, a sensitive marker of inflammation that has previously been associated with cardiovascular disease,[5–8] was independently related to insulin sensitivity. Chronic subclinical inflammation emerged as part of IRS. The findings were consistent across the 3 ethnic groups of the IRAS (non-Hispanic whites, blacks, and Hispanics) and in subjects with NGT and IGT.

Previously, in various populations, CRP levels were associated with BMI,[6,8–10,12] triglyceride level,[6,8–10,12] HDL cholesterol level (inversely),[9,10,12] total cholesterol level,[9] and blood pressure.[8,12] Mendall et al[9] found an association between CRP levels and fasting glucose, confirmed by Tracy et al[10] in the elderly but only in a nonsmoking subset. However, information about the association of serum levels of inflammatory markers with insulinemia and insulin sensitivity, hallmarks of the IRS, is scarce. Recently, we have reported from the IRAS associations of fibrinogen and plasminogen activator-1 with several components of IRS, including insulin, proinsulin, and $S_I$.[26] In 3 other studies, fibrinogen levels were independently associated with fasting insulin levels in nondiabetic subjects.[13,27,28] In 107 nondiabetic subjects, CRP levels were related to insulin resistance, as calculated with the homeostasis model assessment model.[12] The present study clearly corroborates and extends these results, indicating that chronic, subclinical inflammation is part of IRS. We have shown that various components of IRS were correlated to inflammatory markers (Table 2 and Figure 2) and that an increasing number of components of IRS (dyslipidemia, abdominal obesity, low $S_I$, and hypertension) paralleled increasing levels of CRP (Figure 1). The results were consistent across a variety of ethnic groups that differ in insulin sensitivity,[29] indicating that the relations found in our study apply to populations with high and low $S_I$.

There are several possible explanations for these findings, which are not necessarily exclusive. First, it is possible that chronic inflammation may represent a triggering factor in the origin of IRS, and eventually type 2 diabetes, as previously discussed by Pickup and Crook.[30] According to this hypothesis, stimuli such as overnutrition would result in cytokine hypersecretion and eventually lead to insulin resistance and diabetes in genetically or metabolically predisposed individuals. Cytokines, mainly interleukin (IL)-1, IL-6, and tumor necrosis factor (TNF)-$\alpha$, exert major stimulatory effects on hepatic synthesis of acute-phase proteins.[31]

Second, clustering of cardiovascular risk factors as typically encountered in subjects with the IRS may yield cardiovascular disease (yet undetected), and elevated CRP levels thus would be the result of preexisting atherosclerosis.[32] Previous cross-sectional analyses show an association between CRP levels and atherosclerotic disease.[6,9] CRP was also elevated in elderly women with subclinical atherosclerosis in the Cardiovascular Health Study[7]; however, in a larger cohort from the same population, no significant association of CRP with carotid intimal-medial thickness was found.[10] Atherosclerosis starts very early in life, and insulin resistance potentially accelerates this process.[33] Therefore, it is highly likely that in our "healthy" middle-aged population, atherosclerosis prevails, particularly in those with features of IRS. However, the degree and extent of atherosclerosis operative in increasing CRP levels is unknown; therefore, even relatively sensitive methods of assessing preclinical atherosclerosis (such as carotid ultrasound) may lack accuracy in this respect.

Third, decreased insulin sensitivity may lead to enhanced CRP expression by counteracting the physiological effect of insulin on hepatic acute-phase protein synthesis.[34] Clamp studies in normal subjects showed that insulin exerts selective effects on hepatic protein synthesis, with an increase in

albumin synthesis and a decrease in fibrinogen synthesis,[35] the inverse of the picture typically seen during the acute-phase response.[36] Resistance to this effect would then lead to increased synthesis of acute-phase proteins, such as fibrinogen and CRP.

Finally, the effect could be indirect via body fat; we observed a strong and independent association of CRP levels with measures of body fat and triglycerides. This is in accordance with results of previous cross-sectional analyses.[6,8–10,12] In a previous interventional study, the synthesis of proinflammatory cytokines by peripheral monocytes (TNF-$\alpha$, IL-1) was suppressed by dietary fish oil supplementation,[37] suggesting an effect of dietary fat on cytokine production.[38] Another (speculative at this time) mechanism would be a generally enhanced adipose tissue–derived cytokine expression (TNF-$\alpha$, IL-6). Accordingly, weight loss was associated with a decrease in CRP in the Women's Healthy Lifestyle Project (E. Meilahn, personal communication, 1996), also supporting an association of body fat and chronic inflammation.

We found a strong and independent association of elevations in inflammatory markers, namely CRP, with decreased $S_I$. The association of low $S_I$ (indicating high insulin resistance) with elevated CRP levels found in the present study could potentially explain the association of hyperinsulinemia (another indicator of insulin resistance) with cardiovascular disease.[14] Several experimental studies suggest a direct role of CRP in the initiation and/or progression of atherosclerotic lesions. CRP has been shown to (1) be a potent stimulator of tissue factor production by macrophages[4]; (2) activate the complement system in vivo[39]; (3) accumulate in early atherosclerotic lesions in human aorta[2] and coronary arteries[3]; (4) bind to lipoproteins, such as LDL and VLDL, thus inducing their aggregation[1]; and (5) be expressed by monocytes.[40] In epidemiological studies, CRP levels in the upper normal range have consistently been predictive of cardiovascular disease in various populations.[5–8] Moreover, sensitive assays and the biological properties of CRP (such as its stable half-life[41]) make this protein a clinically useful marker of chronic subclinical inflammation.

The results of the present study are potentially clinically important. As previously shown, treatment of several components of IRS (adiposity, dyslipidemia, hypertension) may have beneficial effects in terms of preventing type 2 diabetes[42] and cardiovascular disease.[43,44] Therefore, if subclinical inflammation is indeed another facet of the IRS, anti-inflammatory treatment may also be beneficial. Accordingly, it has been suggested that the effects of aspirin may, at least partly, be mediated through its anti-inflammatory rather than its antiplatelet properties.[5] Furthermore, treatment aiming at improving insulin resistance, whether nonpharmacological, such as exercise and weight reduction, or pharmacological, such as metformin and thiazolidinediones, may lower CRP levels and thus provide additional therapeutic benefits beyond mere glucose lowering. Alternatively, if elevated CRP levels were merely a marker of prevalent or developing atherosclerosis, these treatment strategies would then be clinically fruitless. Prospective studies are clearly needed to address these issues.

## Acknowledgments

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# C-Reactive Protein Modulates Risk Prediction Based on the Framingham Score

## Implications for Future Risk Assessment: Results From a Large Cohort Study in Southern Germany

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***Background***—The Framingham Coronary Heart Disease (CHD) prediction score is recommended for global risk assessment in subjects prone to CHD. Recently, C-reactive protein (CRP) has emerged as an independent predictor of CHD. We sought to assess the potential of CRP measurements to enhance risk prediction based on the Framingham Risk Score (FRS) in a large cohort of middle-aged men from the general population.

***Methods and Results***—We measured CRP and traditional cardiovascular risk factors at baseline in 3435 white men of German nationality, 45 to 74 years of age. All men were drawn from 3 random samples of the general population in the Augsburg area located in Southern Germany in 1984 to 1985, 1989 to 1990, and 1994 to 1995 (response rate, 80%), and the FRS was calculated in all of them. Outcome was defined as nonfatal and fatal coronary events, including sudden cardiac death. During an average follow-up of 6.6 years, a total of 191 coronary events occurred. Cox regression showed a significant contribution of CRP to coronary event risk prediction independent of the FRS ($P=0.0002$). In stratified analysis for 5 categories of FRS, CRP significantly added prognostic information to the FRS in subjects in 2 intermediate risk categories ($P=0.03$ and $P=0.02$).

***Conclusions***—Our results suggest that CRP enhances global coronary risk as assessed by the FRS, especially in intermediate risk groups. This might have implications for future risk assessment. **(*Circulation*. 2004;109:1349-1353.)**

**Key Words:** inflammation ■ risk factors ■ coronary disease ■ epidemiology ■ prevention

The Framingham Risk Score (FRS) is widely recommended to assess global risk for coronary heart disease (CHD) events.[1] However, classic risk factors do not account for all incident coronary events, and there may be a substantial number of subjects with normal lipoprotein concentrations who have the disease.[2] This has led to the search for additional diagnostic tools,[3] and in more than 15 large prospective studies, C-reactive protein (CRP), through the use of new high-sensitivity (hs) assays, has emerged as a strong and consistent predictor of an incident cardiovascular event in initially healthy subjects.[4] A recent prospective study in women has suggested that CRP may even better predict future cardiovascular events than LDL cholesterol[2] and that it may add to the prediction of the estimated 10-year risk according to the FRS.

We sought to investigate the potential of CRP measurements to modify risk prediction based on the FRS in a large CHD-event free cohort of middle-aged white men of German nationality sampled from the general population.

## Methods

### Study Design, Population, and Follow-Up

The population-based MONICA (MONItoring of trends and determinants in CArdiovascular disease) Augsburg studies (Southern Germany) conducted between 1984 and 1998 were used as the database.[5]

Three independent cross-sectional surveys covering the city of Augsburg and two adjacent counties were conducted in 1984 to 1985 (S1), 1989 to 1990 (S2), and 1994 to 1995 (S3) to estimate the prevalence of cardiovascular risk factors among men and women. Altogether, 13,428 white subjects of German nationality (6725 men, 6703 women; response rate, 77%), 25 to 74 years of age, randomly drawn from the general population, participated in at least one of the three studies. In a follow-up study, vital status was assessed for all persons of the three surveys in 1998. During the observation period, 772 participants (531 men, 241 women) had died, and vital status could not be assessed for 56 persons (31 men, 25 women) who had moved to an unknown location.

The outcome variable was a combination of incident fatal or nonfatal acute myocardial infarction (MI) and sudden cardiac death. They were identified through the MONICA/KORA (KOoperative Gesundheitsforschung in der Region Augsburg) coronary event

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**TABLE 1. Age- and Survey-Adjusted Means and Prevalences (%) for Men With and Without Incident Coronary Event: MONICA/KORA Augsburg Cohort Study, 1984 to 1998**

| | Men With Event (n=191) | Men Without Event (n=3244) | *P* |
|---|---|---|---|
| Age,* y | 59.2 | 56.2 | <0.0001 |
| TC/HDL-C ratio (antilog) | 5.44 | 4.85 | <0.0001 |
| CRP, mg/L (antilog) | 2.56 | 1.64 | <0.0001 |
| Cholesterol | | | |
| In mg/dL | 257.4 | 244.4 | 0.0001 |
| In mmol/L | 6.65 | 6.32 | 0.0001 |
| HDL-C | | | |
| In mg/dL | 48.4 | 51.8 | 0.0034 |
| In mmol/L | 1.25 | 1.33 | 0.0034 |
| Systolic blood pressure, mm Hg | 142.6 | 138.5 | 0.0029 |
| Diastolic blood pressure, mm Hg | 83.8 | 83.3 | 0.5768 |
| BMI, kg/m$^2$ | 28.0 | 27.7 | 0.2083 |
| Normal weight (BMI <25.0 kg/m$^2$), % | 18.3 | 20.8 | 0.4136 |
| Overweight (BMI 25 to 29.9 kg/m$^2$), % | 53.3 | 57.4 | 0.2783 |
| Obesity (BMI ≥30.0 kg/m$^2$), % | 28.2 | 21.7 | 0.0431 |
| Alcohol intake, % | | | |
| 0 g/d | 18.1 | 15.4 | 0.3288 |
| 1 to 39.9 g/d | 41.8 | 48.0 | 0.1016 |
| ≥40 g/d | 39.6 | 35.9 | 0.3264 |
| History of diabetes (yes), % | 12.0 | 5.4 | 0.0004 |
| Education (<12 y), % | 76.6 | 73.5 | 0.36 |
| Physical activity (≥1 h/wk), % | 14.0 | 18.0 | 0.1580 |
| Current smoker, % | 44.2 | 26.4 | 0.0001 |

*Only survey-adjusted.

register of the 25- to 74-year-old study population and censored at the 75th year of age.[6] According to the MONICA manual,[5] the diagnosis of a major nonfatal MI event was based on symptoms, cardiac enzymes, and typical ECG changes. Deaths from cardiovascular causes were validated by autopsy reports, death certificates, chart review, and information from the last treating physician.

The present analysis was restricted to men 45 to 74 years of age (response rate, 80%) at the baseline examination (n=3667). Of those, 990 (27%) subjects were from survey S1, 1324 (36%) from S2, and 1353 (37%) from S3. A total of 48 subjects had missing values on CRP or other variables. Participants with prevalent MI (n=184) were excluded. Thus, 3435 subjects were available for the present analysis.

## Survey Methods

All participants completed a standardized questionnaire, including medical history, lifestyle, and drug history. Blood pressure, body height (meters) and body weight (kilograms), body mass index (BMI, kg/m$^2$), smoking behavior, and alcohol consumption (g/d) were determined as described elsewhere.[7] The number of education years was calculated on the basis of the highest level of formal education completed.

## Laboratory Procedures

A nonfasting venous blood sample was collected from all participants in a supine resting position. Samples for measurement of CRP were stored at −70°C until analysis. Serum CRP concentrations were measured with the use of an hs-immunoradiometric assay (range, 0.05 to 10 mg/L), as previously described.[8] The coefficient of variation (CV) for repeated measurements was 12% over all ranges. Total serum cholesterol (TC) and HDL cholesterol (HDL-C) were measured in multiple batches by routine enzymatic methods. Corresponding CVs were between 1% and 3% for TC and between 3% and 4% for HDL-C.

## Statistical Analysis

Means or proportions (adjusted for age and survey) for baseline demographic and clinical characteristics were computed by ANCOVA for men with and without incident coronary events. Cox proportional hazards analysis was used to assess the independent risk for the incidence of a first fatal or nonfatal acute coronary event separately in quartiles of TC/HDL-C and in quartiles of CRP. Relative risks for both variables were adjusted for age and survey (S1, S2, or S3) and were further adjusted for BMI (according to Bray, 7), current smoking (yes/no), hypertension (blood pressure less than versus ≥140/90 mm Hg), education years (less than versus ≥12 years), alcohol consumption (0, 0.1 to 39.9, versus ≥40 g/d), physical activity (inactive versus active, that is, ≥1 hour in at least 1 season), and history of diabetes (yes versus no) in all other models. Results are presented as hazard ratios (HR), together with their 95% confidence intervals. Probability values are based on the Wald statistic.

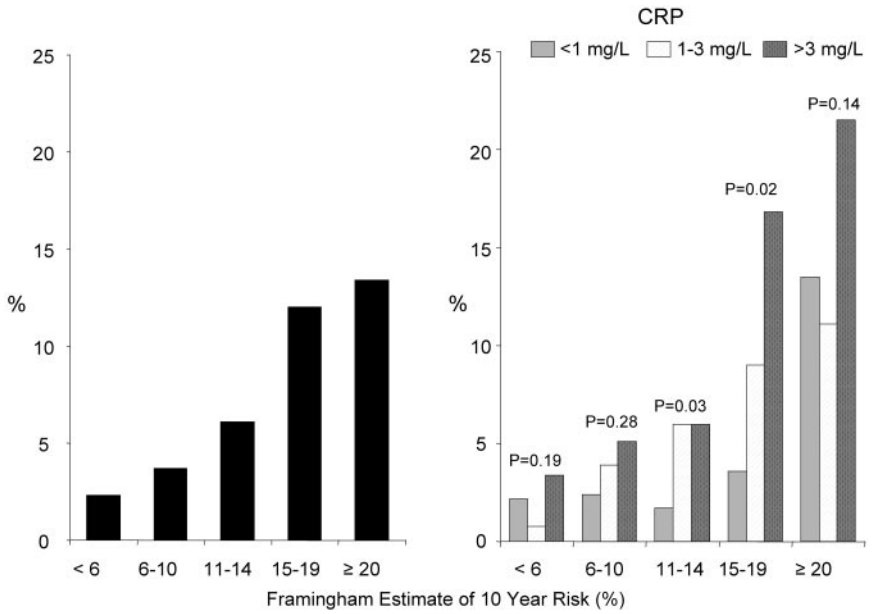
Finally, using Cox proportional hazards analysis, we assessed whether CRP contributed information on risk beyond that conveyed by the 10-year risk calculated with the FRS according to the formula of Wilson et al[9] and beyond the risk associated with the ratio of TC/HDL-C. In these analyses, CRP concentrations were categorized according to recently proposed cut-points (<1.0 mg/L; 1.0 to 3.0 mg/L; >3.0 mg/L).[10] Moreover, to estimate the effects of the additional value of CRP on the prediction of coronary events in

**TABLE 2. Risk of a First Coronary Event Estimated by Cox Proportional Hazards Model Without and With CRP for the Ratio of TC/HDL-C (A) and the Framingham Risk Score With Three Categories (B) and Five Categories (C)**

| | | Model Without CRP | | Model With CRP | |
|---|---|---|---|---|---|
| Factor | Events/n | HR (95% CI) | *P* | HR (95% CI) | *P* |
| A | | | | | |
| TC/HDL-C ratio | | | <0.0001 | | 0.0023 |
| <4.3 | 46/1100 | Reference | | Reference | |
| 4.3 to 5.6 | 55/1152 | 1.25 (0.85–1.86) | | 1.18 (0.80–1.74) | |
| ≥5.6 | 90/1183 | 2.06 (1.45–2.95) | | 1.80 (1.26–2.57) | |
| CRP, mg/L | | | ... | | <0.0001 |
| <1 | 37/1178 | ... | | Reference | |
| 1 to 3 | 64/1262 | ... | | 1.73 (1.15–2.60) | |
| >3 | 90/995 | ... | | 2.91 (1.98–4.29) | |
| AIC | | 2853 | | 2824 | ΔAIC: 29 |
| AUC | | 0.704 | | 0.725 | 0.1029 |
| B | | | | | |
| FRS 1, % | | | <0.0001 | | <0.0001 |
| <6 | 18/809 | Reference | | Reference | |
| 6 to 19 | 117/2090 | 2.81 (1.71–4.62) | | 2.39 (1.45–3.94) | |
| ≥20 | 56/536 | 6.19 (3.64–10.54) | | 4.85 (2.82–8.33) | |
| CRP, mg/L | | | ... | | <0.0001 |
| <1 | 37/1178 | ... | | Reference | |
| 1 to 3 | 64/1262 | ... | | 1.54 (1.02–2.32) | |
| >3 | 90/995 | ... | | 2.47 (1.67–3.65) | |
| AIC | | 2816 | | 2797 | ΔAIC: 19 |
| AUC | | 0.713 | | 0.740 | 0.0077 |
| C | | | | | |
| FRS 2, % | | | <0.0001 | | <0.0001 |
| <6 | 18/809 | Reference | | Reference | |
| 6 to 10 | 32/914 | 1.63 (0.91–2.90) | | 1.46 (0.82–2.61) | |
| 11 to 14 | 35/650 | 2.70 (1.53–4.77) | | 2.35 (1.32–4.16) | |
| 15 to 19 | 50/526 | 5.61 (3.27–9.62) | | 4.50 (2.59–7.80) | |
| ≥20 | 56/536 | 6.21 (3.65–10.57) | | 5.01 (2.91–8.62) | |
| CRP, mg/L | | | ... | | 0.0002 |
| <1 | 37/1178 | ... | | Reference | |
| 1 to 3 | 64/1262 | ... | | 1.44 (0.95–2.17) | |
| >3 | 90/995 | ... | | 2.21 (1.49–3.27) | |
| AIC | | 2789 | | 2776 | ΔAIC: 13 |
| AUC | | 0.735 | | 0.750 | 0.0163 |

different FRS categories, separate Cox proportional hazards analyses stratified for FRS categories (3 or 5, respectively) were performed. For each of the 3, respective 5 FRS categories, a Cox proportional hazards model was calculated with the inclusion of CRP (3 categoreis) as exposition variable and FRS (continuous) and survey as confounding covariates. The percentages of a first incident coronary event within 10 years estimated by these Cox models were compared according to percentages estimated by Cox models with FRS categories (3 or 5, respectively) adjusted only for survey.

To assess the goodness of fit of the different prediction models, we calculated Akaike's Information Criterion (AIC) regarding an AIC difference between two models of >10 as essentially different.[11,12] Although ROC analysis can be only a rough estimate for the predictive value of a Cox proportional hazards model in our study design with censored data, we performed such analysis for reasons of comparison with other studies. We estimated the area under the curve (AUC) as a measure for the predictive ability of a Cox proportional hazards model. Different AUCs were tested by a nonparametric approach of DeLong et al.[13] The "change-in-estimate" method (CIE) was used to evaluate the impact of the addition of CRP on the HRs of the FRS with a 10% criterion.[14] Significance tests are 2 tailed, and probability values <0.05 were considered statistically significant. All analyses were performed with the use of the Statistical Analysis System (Version 8.2 for Unix, SAS Institute Inc).




Occurrence of a first coronary event within 10 years, estimated by Cox proportional hazards models in percentages. Left, Percentage estimated by a model with FRS (5 categories) adjusted for survey. Right, Percentage estimated for each of 5 FRS categories by a model with CRP (3 categories) adjusted for FRS (continuous) and survey. Probability values indicate significance status of CRP in the Cox model. MONICA/KORA-Augsburg Cohort Study, 1984 to 1998.

## Results

### Baseline Characteristics

During an average follow-up of 6.6 years, a total of 191 coronary events occurred. Men with an incident event were significantly older; had a higher TC/HDL-C ratio, higher systolic blood pressures, and elevated CRP concentrations; were more frequently smokers; and had a higher prevalence of obesity and diabetes (Table 1).

### Relative Risks Associated With CRP and the Ratio of TC/HDL-C

In multivariable analyses, the relative risks associated with increasing quartiles as compared with the bottom quartiles were 1.21, 1.43, and 2.03 for CRP ($P=0.0079$) and 1.72, 1.76, and 2.62 for the ratio of TC/HDL-C ($P=0.0006$).

### Additive Effect of CRP to the Risk Associated With the Ratio of TC/HDL-C and the FRS

We performed Cox proportional hazards analyses to assess the influence of CRP measurements on the risk of a first coronary event, independent of the TC/HDL-C ratio and the calculated FRS. For this purpose, TC/HDL-C was categorized into tertiles and the FRS was divided into low (<6%), intermediate (6% to 19%), and high (≥20%) risk over 10 years (FRS 1), according to Greenland et al,[15] and into 5 risk categories <6%, 6% to 10%, 11% to 14%, 15% to 19%, and ≥20% (FRS 2) (Table 2). For each of these factors, two Cox regression models were calculated: The first model included only the factor under concern as covariate (model without CRP) and the second model additionally included CRP as covariate (model with CRP). As shown in Table 2, CRP significantly contributed to the prediction of incident coronary events through the use of the TC/HDL-C ratio, FRS 1, and FRS 2 (probability values <0.001). As suggested by AIC, with differences >10 between models without CRP and those including CRP, the fit of each model containing CRP was superior to that of the TC/HDL-C ratio and FRS alone.

The AUC as a rough estimate (in our study design) for the predictive value of the different Cox models revealed a statistically significant increase from 0.735 (model with FRS 2) to 0.750 (model with FRS 2 and CRP) ($P=0.0163$). The inclusion of CRP was associated with a remarkable decrease in the HR of the FRS. The HR of the highest FRS 2 category (risk >20%/10 years) compared with the lowest FRS 2 category (risk <6%/10 years) decreased from 6.2 to 5.0, indicating a CIE of 19.4%. All CIEs are greater than the 10% criterion, indicating a strong impact of CRP on the HRs of FRS 2. Similar results were observed for the FRS 1 model.

Moreover, we compared the proportions of incident coronary events within 10 years estimated by the Cox model for the five categories of FRS 2 alone (Figure, left panel) and for different CRP categories in each category of FRS 2, adjusted for survey and FRS (Figure, right panel). Probability values of the stratified analyses are given in the Figure (right panel, above each FRS category). Cox regression revealed a considerable modification in coronary event incidence based on CRP concentrations and, more importantly, in categories of FRS 2 associated with a 10% to 20% risk per 10 years, elevated concentrations of CRP were consistently and statistically significantly associated with a further increased risk ($P=0.03$ and $P=0.02$). In contrast, in men with a risk <6% and 6% to 10% per 10 years, CRP had no statistically significant additional effect on the prediction of a first coronary event. Regarding the different AUCs, a remarkable increase was found for the intermediate FRS categories of 11% to 14% and 15% to 19% (increase in the AUC from 0.725 to 0.776 and from 0.695 to 0.751).

## Discussion

In this prospective population-based study, increased CRP concentrations and an elevated TC/HDL-C ratio were both independently related to incident coronary events. However, even if the strongest lipid/lipoprotein variable was chosen for risk assessment, our data clearly show that the measurement

of CRP contributes significantly to the prediction of a first coronary event and adds clinically relevant information to the TC/HDL-C ratio. Finally, and most importantly, these prospective data from a large European cohort of middle-aged men clearly suggest that CRP modulates the risk conveyed by the FRS as the HRs from the top to the bottom category decreased remarkably after inclusion of CRP in the various models. This was observed in particular in those with an FRS between 10% and 20% over a period of 10 years. These men may benefit from additional noninvasive tests such as determination of CRP by a hs assay.

Such conclusions are in agreement with recent findings by Albert et al.[16] They found significant correlations between CRP concentrations and the FRS but only minimal correlations with its individual components. However, our data are in contrast to a report from the Rotterdam Study,[17] which found no additional value of CRP in elderly people. Such discrepancy may be explained by differences in age, the additional inclusion of left ventricular hypertrophy, another important risk marker, and the fact that CRP concentrations in incident cases were considerably lower than in our study despite the older average age in the Rotterdam population.

In summary, our data confirm and extend results from the Women's Health Study[2] in a large sample of men from the general population. If these findings can be replicated in other populations, this may represent a strong argument for the inclusion of CRP as an additional variable to further improve risk prediction in asymptomatic subjects at intermediate risk of CHD. This would be in line with recent American Heart Association/Centers for Disease Control guidelines.[10]

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ORIGINAL ARTICLE

# Statin Therapy, LDL Cholesterol, C-Reactive Protein, and Coronary Artery Disease

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## ABSTRACT

### BACKGROUND

Recent trials have demonstrated better outcomes with intensive than with moderate statin treatment. Intensive treatment produced greater reductions in both low-density lipoprotein (LDL) cholesterol and C-reactive protein (CRP), suggesting a relationship between these two biomarkers and disease progression.

### METHODS

We performed intravascular ultrasonography in 502 patients with angiographically documented coronary disease. Patients were randomly assigned to receive moderate treatment (40 mg of pravastatin orally per day) or intensive treatment (80 mg of atorvastatin orally per day). Ultrasonography was repeated after 18 months to measure the progression of atherosclerosis. Lipoprotein and CRP levels were measured at baseline and follow-up.

### RESULTS

In the group as a whole, the mean LDL cholesterol level was reduced from 150.2 mg per deciliter (3.88 mmol per liter) at baseline to 94.5 mg per deciliter (2.44 mmol per liter) at 18 months ($P<0.001$), and the geometric mean CRP level decreased from 2.9 to 2.3 mg per liter ($P<0.001$). The correlation between the reduction in LDL cholesterol levels and that in CRP levels was weak but significant in the group as a whole ($r=0.13$, $P=0.005$), but not in either treatment group alone. In univariate analyses, the percent change in the levels of LDL cholesterol, CRP, apolipoprotein B-100, and non–high-density lipoprotein cholesterol were related to the rate of progression of atherosclerosis. After adjustment for the reduction in these lipid levels, the decrease in CRP levels was independently and significantly correlated with the rate of progression. Patients with reductions in both LDL cholesterol and CRP that were greater than the median had significantly slower rates of progression than patients with reductions in both biomarkers that were less than the median ($P=0.001$).

### CONCLUSIONS

For patients with coronary artery disease, the reduced rate of progression of atherosclerosis associated with intensive statin treatment, as compared with moderate statin treatment, is significantly related to greater reductions in the levels of both atherogenic lipoproteins and CRP.

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*The REVERSAL Investigators are listed in the Appendix.

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Two recent trials demonstrated that intensive lipid-lowering therapy with statins improved clinical outcomes[1] and reduced the progression of atherosclerosis.[2] Many authorities attributed the greater benefits of intensive statin therapy, as compared with moderate statin therapy, to greater reductions in the levels of atherogenic lipoproteins, particularly low-density lipoprotein (LDL) cholesterol.[3] However, statins have a wide range of biologic effects in addition to lipid lowering, including reductions in the levels of C-reactive protein (CRP), a phenomenon commonly termed a "pleiotropic effect."[4-6] In both recent comparisons, at the conclusion of the trials, CRP levels were 30 to 40 percent lower after intensive statin therapy than after moderate treatment.[4] This finding raises a provocative scientific question: Do reductions in CRP represent an independent factor influencing the benefits of more intensive statin therapy?

Large observational studies have established a strong relationship between CRP levels and the morbidity and mortality associated with coronary disease.[7-9] However, the precise mechanism underlying the association between CRP levels and adverse outcomes remains incompletely described. Theoretically, by decreasing the levels of atherogenic lipoproteins, statins could decrease systemic inflammation, thereby reducing CRP levels. An alternative hypothesis proposes that statins have direct antiinflammatory effects, independent of their lipid-lowering capabilities. In this model, CRP plays a more direct role in the pathogenesis of atherosclerosis, and a statin-mediated reduction in inflammation contributes directly to reduced disease activity. Because statins decrease the levels of both LDL cholesterol and CRP, it is difficult to determine whether CRP is an indirect biomarker reflecting the benefits of statins or a direct participant in atherogenesis.

Intravascular ultrasonography is a useful technique for assessing the effect of therapies on the vascular wall, providing a precise and continuous measure of the progression of atherosclerosis. In the Reversal of Atherosclerosis with Aggressive Lipid Lowering (REVERSAL) trial, intensive therapy with 80 mg of atorvastatin per day slowed the pro-

**Table 1. Laboratory Values at Baseline and Follow-up and Change in Values from Baseline.***

| Characteristic | Both Groups (N=502) | Pravastatin Group (N=249) | Atorvastatin Group (N=253) | P Value† |
|---|---|---|---|---|
| **Baseline** | | | | |
| Total cholesterol (mg/dl) | 232.2±34.2 | 232.6±34.1 | 231.8±34.2 | 0.80 |
| LDL cholesterol (mg/dl) | 150.2±26.9 | 150.2±25.9 | 150.2±27.9 | 0.99 |
| HDL cholesterol (mg/dl) | 42.6±10.7 | 42.9±11.4 | 42.3±9.9 | 0.51 |
| Non-HDL cholesterol (mg/dl) | 189.6±32.5 | 189.7±32.3 | 189.5±32.7 | 0.96 |
| Triglycerides (mg/dl) | 197.4±100.6 | 197.7±105.6 | 197.2±95.7 | 0.96 |
| Apo B-100 (mg/dl) | 152.7±23.4 | 153.0±22.4 | 152.4±24.3 | 0.79 |
| CRP (mg/liter)‡ | | | | 0.46 |
| Geometric mean | 2.9 | 3.0 | 2.8 | |
| Interquartile range | 1.4 to 6.1 | 1.4 to 6.1 | 1.3 to 6.3 | |
| **18-Mo follow-up** | | | | |
| Total cholesterol (mg/dl) | 169.2±40.0 | 187.5±32.2 | 151.3±38.9 | <0.001 |
| LDL cholesterol (mg/dl) | 94.5±32.2 | 110.4±25.8 | 78.9±30.2 | <0.001 |
| HDL cholesterol (mg/dl) | 43.8±11.3 | 44.6±11.3 | 43.1±11.3 | 0.15 |
| Non-HDL cholesterol (mg/dl) | 125.4±39.6 | 142.9±32.2 | 108.1±38.6 | <0.001 |
| Triglycerides (mg/dl) | 157.0±93.8 | 165.8±92.1 | 148.4±94.9 | 0.04 |
| Apo B-100 (mg/dl) | 104.8±29.1 | 118.1±24.0 | 91.8±27.9 | <0.001 |
| CRP (mg/liter)‡ | | | | <0.001 |
| Geometric mean | 2.3 | 2.9 | 1.8 | |
| Interquartile range | 0.9 to 5.4 | 1.3 to 6.2 | 0.8 to 4.3 | |

gression of atherosclerosis more than did moderate treatment with 40 mg of pravastatin per day.[2] We applied statistical methods to examine the relationship between the reductions in LDL cholesterol and CRP levels and the rate of disease progression measured by intravascular ultrasonography.

## METHODS

### STUDY DESIGN

The institutional review board of each participating center approved the protocol, and all patients provided written informed consent. Intravascular ultrasonography was performed in a single vessel in patients who had a clinical indication for coronary angiography and had stenosis of at least 20 percent on angiography. Eligible patients had to have an LDL cholesterol level of 125 to 210 mg per deciliter (3.23 to 5.43 mmol per liter) after a statin-free washout period of 4 to 10 weeks. Patients were randomly assigned to receive either 40 mg of pravastatin or 80 mg of atorvastatin orally daily. The patients and all study personnel were unaware of the treatment assignments or the results of laboratory measurements.

### INTRAVASCULAR ULTRASONOGRAPHY

Investigators performed intravascular ultrasonography in the longest and least angulated target vessel that met the inclusion criteria. After the adminis-

**Table 1. (Continued.)**

| Characteristic | Both Groups (N=502) | Pravastatin Group (N=249) | Atorvastatin Group (N=253) | P Value† |
|---|---|---|---|---|
| **Change from baseline** | | | | |
| Total cholesterol | | | | <0.001 |
| Mean (mg/dl) | −63±44 | −45±37 | −81±43 | |
| Percent | −26.3 | −18.4 | −34.1 | |
| LDL cholesterol | | | | <0.001 |
| Mean (mg/dl) | −56±37 | −40±29 | −71±37 | |
| Percent | −35.8 | −25.2 | −46.3 | |
| HDL cholesterol | | | | 0.11 |
| Mean (mg/dl) | 1.2±7.9 | 1.6±7.7 | 0.8±8.0 | |
| Percent | 4.2 | 5.6 | 2.9 | |
| Non-HDL cholesterol | | | | <0.001 |
| Mean (mg/dl) | −64±43 | −47±35 | −81±43 | |
| Percent | −33.0 | −23.6 | −42.2 | |
| Triglycerides | | | | 0.002 |
| Mean (mg/dl) | −40±96 | −32±94 | −49±98 | |
| Percent | −13.5 | −6.8 | −20.0 | |
| Apo B-100 | | | | <0.001 |
| Mean (mg/dl) | −48±30 | −35±25 | −61±30 | |
| Percent | −30.6 | −22.0 | −39.1 | |
| CRP‡ | | | | <0.001 |
| Geometric mean (mg/liter) | −0.2 | 0.2 | −0.7 | |
| Interquartile range (mg/liter) | −1.9 to 0.8 | −1.5 to 1.6 | −2.8 to 0.1 | |
| Percent | −22.4 | −5.2 | −36.4 | |

* Plus–minus values are means ±SD. To convert values for cholesterol to millimoles per liter, multiply by 0.02586. To convert values for triglycerides to millimoles per liter, multiply by 0.01129.
† P values were calculated by means of the two-sample t-test.
‡ CRP levels were not available for six patients at baseline or follow-up (one in the pravastatin group and five in the atorvastatin group).

tration of intracoronary nitroglycerin, the transducer was positioned in the distal vessel and withdrawn at a rate of 0.5 mm per second (the "pullback") with the use of a motor drive. A core laboratory evaluated the image quality of each ultrasonogram, and only patients whose ultrasonograms met prespecified image-quality requirements were eligible for randomization. After an 18-month treatment period, patients again underwent intravascular ultrasonography under identical conditions. This method of intravascular ultrasonography has been described previously in detail.[2,10,11]

### CORE LABORATORY MEASUREMENTS

Personnel who were unaware of the patients' clinical characteristics and treatment assignments used manual planimetry to measure, on a computer screen, a series of cross-sections of ultrasonographic images selected exactly 1.0 mm apart along the long axis of the vessel. Measurements were performed in accordance with the standards of the American College of Cardiology and the European Society of Cardiology.[12] For each cross-section analyzed, the operator measured the area of the external elastic membrane and the lumen. The accuracy and reproducibility of this method have been reported previously.[2,13]

### CALCULATION OF END POINTS

The average area of atheroma per cross-section was calculated as follows:

$$\frac{\Sigma(\mathrm{EEM_{CSA}} - \mathrm{LUMEN_{CSA}})}{n},$$

where $\mathrm{EEM_{CSA}}$ is the cross-sectional area of the external elastic membrane, $\mathrm{LUMEN_{CSA}}$ is the cross-sectional area of the lumen, and n is the number of cross-sections in the pullback. To compensate for pullbacks of differing lengths, the total atheroma volume for each patient was calculated as the average area of atheroma multiplied by the median number of cross-sections in the pullbacks for all patients in the study. The efficacy variable "change in normalized total atheroma volume" (TAV) was calculated as $\mathrm{TAV_{18\ months}} - \mathrm{TAV_{baseline}}$. The percent atheroma volume (PAV) was calculated with the use of the following formula:

$$\left[\frac{\Sigma(\mathrm{EEM_{CSA}} - \mathrm{LUMEN_{CSA}})}{\Sigma \mathrm{EEM_{CSA}}}\right] \times 100.$$

The efficacy variable "change in PAV" was calculated as $\mathrm{PAV_{18\ months}} - \mathrm{PAV_{baseline}}$.

### LABORATORY TESTS

A central laboratory performed all biochemical determinations (Medical Research Laboratory, Highland Heights, Ky.).

### STATISTICAL ANALYSIS

For continuous variables with a normal distribution, means ±SD are reported. For CRP levels, the geometric means and interquartile ranges are reported. Because the ultrasonographic end points were not normally distributed, we applied an analysis-of-covariance model to rank-transformed data to determine P values. Correlations between variables are described with the use of Spearman rank-correlation coefficients, and multivariate regression analyses based on rank-transformed data were used to obtain partial correlation coefficients adjusted for the effects of covariates.[14] The ultrasonographic variable served as the dependent variable; the independent variables consisted of the change in CRP coupled with the change in non–high-density lipoprotein (non-HDL) cholesterol, LDL cholesterol, or apolipoprotein B-100 (apo B-100). For a further description of bivariate relationships with ultrasonographic end points, we used the locally weighted scatterplot smoothing (LOWESS) technique.[15] This technique is designed to produce a smooth fit to the data and reduces the influence of extreme outliers. Analyses were performed with the use of SAS software, version 6.12.

## RESULTS

### PATIENT POPULATION

Between June 1999 and September 2001, 502 patients were enrolled at 34 U.S. centers and underwent intravascular ultrasonography at both baseline and 18 months of follow-up that could be evaluated (249 in the pravastatin group and 253 in the atorvastatin group). The average age was 56 years, 72 percent were men, 89 percent were white (race was recorded by the study coordinators on the case-report form), 26 percent were current smokers, 69 percent had a history of hypertension, and 19 percent had a history of diabetes.[2]

### LABORATORY FINDINGS AND RESULTS OF INTRAVASCULAR ULTRASONOGRAPHY

Table 1 summarizes laboratory values at baseline and at the completion of the study (18 months) for the entire population and each treatment group. For

all 502 patients, the mean baseline LDL cholesterol level was 150.2 mg per deciliter (3.88 mmol per liter), the non-HDL cholesterol level was 189.6 mg per deciliter (4.90 mmol per liter), and the geometric mean CRP level was 2.9 mg per liter. After 18 months of treatment, the mean LDL cholesterol level was 94.5 mg per deciliter (2.44 mmol per liter), the non-HDL cholesterol level was 125.4 mg per deciliter (3.24 mmol per liter), and the geometric mean CRP level was 2.3 mg per liter. There were greater reductions in LDL cholesterol, non-HDL cholesterol, and CRP levels in the atorvastatin group than in the pravastatin group (P<0.001 for each comparison).[2]

Table 2 summarizes measures of disease burden as determined by intravascular ultrasonography at baseline and the completion of the study for the entire population and the two treatment groups. Both measures of the progression of atherosclerosis — total atheroma volume and percent atheroma volume — reflected a slower rate of progression in the group that received intensive treatment with atorvastatin than in the group that received moderate treatment with pravastatin.

### CORRELATION BETWEEN REDUCTIONS IN LIPOPROTEIN AND CRP

There was a weak but significant correlation between the percent reductions in LDL cholesterol and in CRP levels only for the study group as a whole (r=0.13, P=0.005) — not for the pravastatin group alone (r=–0.008, P=0.90) or the atorvastatin group alone (r=0.09, P=0.17). Changes in other atherogenic lipoproteins, such as apo B-100 and non-HDL cholesterol, had similarly weak correlations with the reduction in CRP levels in the regression analysis.

### EFFECT OF CHANGES IN CRP AND LIPIDS ON PROGRESSION

Table 3 summarizes the correlations between the changes in the levels of atherogenic lipoproteins, CRP, and HDL cholesterol and the rate of progres-

**Table 2. Baseline and Follow-up Values for Intravascular Ultrasonographic End Points and Change in Values from Baseline.***

| Atheroma Volume | Both Groups (N=502) | | Pravastatin Group (N=249) | | Atorvastatin Group (N=253) | | P Value† |
|---|---|---|---|---|---|---|---|
| | Mean ±SD | Median | Mean ±SD | Median | Mean ±SD | Median | |
| **Baseline** | | | | | | | |
| Total (mm³) | 189.4±115.3 | 165.9 (113.8 to 238.9) | 194.5±114.8 | 168.6 (117.4 to 246.2) | 184.4±115.7 | 161.9 (111.0 to 228.2) | 0.20 |
| Normalized total (mm³)‡ | 184.1±83.1 | 174.5 (122.1 to 232.3) | 189.1±86.5 | 187.2 (122.1 to 239.1) | 179.1±79.4 | 166.6 (122.4 to 226.6) | 0.26 |
| Percent | 38.9±11.0 | 38.9 (32.2 to 46.2) | 39.5±10.8 | 40.0 (32.5 to 46.3) | 38.4±11.3 | 38.2 (31.7 to 45.8) | 0.18 |
| **18-Mo follow-up** | | | | | | | |
| Total (mm³) | 191.7±110.7 | 169.9 (113.3 to 244.0) | 199.6±112.3 | 180.0 (125.5 to 255.3) | 183.9±108.8 | 160.9 (107.4 to 240.3) | 0.04 |
| Normalized total (mm³)‡ | 186.5±81.5 | 175.7 (124.5 to 239.2) | 194.2±86.0 | 179.7 (128.9 to 248.2) | 178.9±76.2 | 170.5 (119.8 to 222.2) | 0.08 |
| Percent | 40.2±10.5 | 39.9 (33.8 to 47.1) | 41.4±10.0 | 41.8 (35.0 to 47.7) | 39.0±10.8 | 38.7 (31.6 to 45.7) | <0.001 |
| **Change from baseline** | | | | | | | |
| Total (mm³) | 2.3±31.7 | 1.4 (–14.4 to 19.5) | 5.1±31.4 | 4.4 (–13.3 to 21.9) | –0.4±31.8 | –0.9 (–14.5 to 13.8) | 0.04 |
| Normalized total (mm³)‡ | 2.4±29.4 | 1.5 (–15.3 to 20.1) | 5.1±27.6 | 4.1 (–13.2 to 23.5) | –0.2±31.0 | –0.9 (–17.9 to 15.3) | 0.03 |
| Percent | 1.3±5.1 | 0.9 (–1.9 to 4.4) | 1.9±4.9 | 1.6 (–1.6 to 4.7) | 0.6±5.1 | 0.2 (–2.5 to 3.9) | 0.002 |

* Values in parentheses are interquartile ranges.
† P values were calculated with the use of the Wilcoxon rank-sum test.
‡ Values were adjusted for pullbacks of different lengths by multiplying the average area of atheroma volume for each patient by the median number of cross-sections in the pullbacks for all patients in the study.

**Table 3. Relationships between Changes in Laboratory Measures and Intravascular Ultrasonographic End Points.**

| Laboratory Measure | Percent Atheroma Volume | | Total Atheroma Volume | |
|---|---|---|---|---|
| | Correlation Coefficient* | P Value | Correlation Coefficient* | P Value |
| **Univariate analysis** | | | | |
| LDL cholesterol | | | | |
| Change | 0.10 | 0.03 | 0.09 | 0.04 |
| Percent change | 0.14 | 0.002 | 0.12 | 0.005 |
| HDL cholesterol | | | | |
| Change | –0.04 | 0.40 | –0.01 | 0.84 |
| Percent change | –0.04 | 0.42 | –0.01 | 0.78 |
| Triglycerides | | | | |
| Change | 0.05 | 0.23 | 0.06 | 0.19 |
| Percent change | 0.08 | 0.08 | 0.08 | 0.09 |
| Non-HDL cholesterol | | | | |
| Change | 0.09 | 0.05 | 0.07 | 0.10 |
| Percent change | 0.13 | 0.004 | 0.10 | 0.02 |
| apo B-100 | | | | |
| Change | 0.09 | 0.05 | 0.08 | 0.06 |
| Percent change | 0.13 | 0.004 | 0.12 | 0.008 |
| CRP | | | | |
| Change | 0.11 | 0.01 | 0.11 | 0.02 |
| Percent change | 0.11 | 0.01 | 0.11 | 0.02 |
| **Multivariate analysis (adjusted for changes in CRP and non-HDL cholesterol)** | | | | |
| Percent change in non-HDL cholesterol | 0.11 | 0.01 | 0.08 | 0.06 |
| Percent change in CRP | 0.09 | 0.04 | 0.09 | 0.05 |
| **Multivariate analysis (adjusted for changes in CRP and LDL cholesterol)** | | | | |
| Percent change in LDL cholesterol | 0.12 | 0.008 | 0.11 | 0.02 |
| Percent change in CRP | 0.09 | 0.04 | 0.08 | 0.06 |
| **Multivariate analysis (adjusted for changes in CRP and apo B-100)** | | | | |
| Percent change in apo B-100 | 0.11 | 0.01 | 0.10 | 0.03 |
| Percent change in CRP | 0.09 | 0.05 | 0.08 | 0.07 |

* Values are Spearman rank-correlation coefficients.

sion of atherosclerosis for both end points assessed by means of intravascular ultrasonography. Univariate analysis revealed significant correlations between ultrasonographic measures of disease progression and laboratory measures of atherogenic lipoproteins, including LDL cholesterol, apo B-100, and non-HDL cholesterol. The percent change in the LDL cholesterol level had the closest correlation with progression, with a correlation coefficient of 0.12 for total atheroma volume (P=0.005) and of 0.14 for percent atheroma volume (P=0.002).

The correlations between the reduction in CRP levels and the rates of progression on intravascular ultrasonography were also significant and similar in strength to the relationships observed for the atherogenic lipoproteins. Univariate analysis yielded a correlation coefficient of 0.11 for both total and percent atheroma volume (P=0.02 and P=0.01, respectively). Most correlations between the rates of progression on ultrasonography and the percent change in non-HDL cholesterol, LDL cholesterol, and CRP levels remained significant on multivariate analysis but were weaker than those obtained by univariate analyses (Table 3).

As shown in Figure 1, greater reductions in LDL cholesterol levels were associated with slower rates of progression on intravascular ultrasonography. Figure 2 shows this same relationship for the reduction in CRP levels. Patients with the largest reductions in CRP levels had regression of atheroma, as evidenced by progression rates of less than zero.

Table 4 shows the rates of progression of atherosclerosis on ultrasonography for subgroups defined according to whether the reductions in LDL cholesterol or CRP levels were greater than or less than the median decreases. For both efficacy measures, the highest rates of progression were in the subgroup in which decreases in both LDL cholesterol and CRP levels were less than the median. Significantly lower progression rates were observed in the subgroup with decreases in both LDL cholesterol and CRP levels that were greater than the median (P=0.001 for both efficacy measures).

## DISCUSSION

Epidemiologic evidence has established a strong relationship between elevated levels of atherogenic lipoproteins, particularly LDL cholesterol, and the risk of death and complications from cardiovascular causes. Placebo-controlled trials of statins have demonstrated that pharmacologic therapies that reduce LDL cholesterol levels also proportionally decrease cardiovascular risk.[16-19] Accordingly, the clinical benefits of statin therapy have largely been attributed to reductions in the levels of atherogenic lipoproteins. However, observational studies have also established a strong relationship between the levels of CRP, the most stable and reliable laboratory measure of systemic inflammation, and adverse

cardiovascular outcomes. Statins have a variety of pleiotropic properties, including their ability to induce dose-dependent decreases in the levels of CRP and other inflammatory biomarkers.[5,6] Since statins reduce the levels of both LDL cholesterol and CRP, it is difficult to determine the relative contribution of the reduction in each of these biomarkers to the observed clinical benefits.

We sought to close this gap in knowledge by analyzing the correlation among lipid levels, CRP levels, and the rate of progression of atherosclerosis, using intravascular ultrasonography to measure disease progression in patients who were being treated with statins.[2] Intravascular ultrasonography is a useful technique for assessing the effect of therapies on the vascular wall, providing a precise and continuous measure of disease progression.[20] In the REVERSAL trial, intensive therapy with 80 mg of atorvastatin per day slowed the rate of progression of atherosclerosis more than did moderate treatment with 40 mg of pravastatin per day. Because we studied two different intensities of statin therapy, we evaluated a broad range of reductions in LDL cholesterol and CRP, permitting a post hoc analysis of the relationship between these two biomarkers and the rate of progression of atherosclerosis across a clinically important range of values.

Correlation analysis revealed that reductions in the levels of atherogenic lipoproteins were not closely correlated with reductions in CRP levels. There was a weak but significant correlation between the reduction in LDL cholesterol levels and the reduction in CRP levels for the overall group of 502 patients (r=0.13, P=0.005), but not in either treatment group alone. These data demonstrate that statin-mediated reductions in CRP are largely unrelated to the decrease in LDL cholesterol levels. These findings confirm the work of other investigators and strongly suggest that the statin-mediated reduction in CRP is unlikely to be a secondary consequence of a reduction in LDL cholesterol but, rather, is potentially mediated by independent pathways.[21]

Analysis of the relationship among lipoprotein levels, CRP levels, and the rate of progression of atherosclerosis yielded particularly informative results. Reductions in both LDL cholesterol and CRP levels were significantly correlated to the rate of progression. In univariate analyses, both ultrasonographic measures of progression — the change in the normalized total atheroma volume and the change in percent atheroma volume — correlated significantly with the reduction in the levels of atherogenic lipoproteins, including LDL cholesterol, non-HDL cholesterol, and apo B-100. The closest correlation was between the LDL cholesterol level and the percent atheroma volume (r=0.14, P=0.002). However, similar correlations were observed for the relationship between the reduction in CRP levels and the rate of progression on intravascular ultrasonography (r=0.11, P=0.01). Substituting non-HDL cholesterol for LDL cholesterol, to account for the broad range of atherogenic lipoproteins, did not increase the correlation. Since the levels of both CRP and LDL cholesterol showed relatively weak correlations with the ultrasonographic end points (r values of 0.11 to 0.14), this analysis

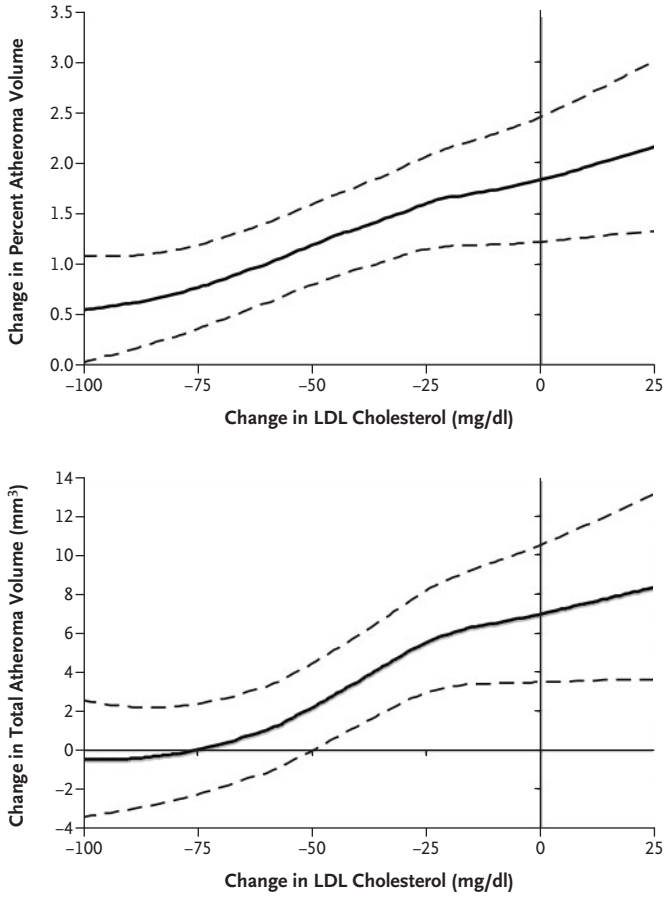


**Figure 1. Locally Weighted Smoothed Scatterplots Showing the Relationship between the Change in LDL Cholesterol Levels and the Rate of Progression of Atherosclerosis in the Entire Group of 502 Patients.**

In each plot, the solid line represents the point estimates and the upper and lower lines the 95 percent confidence intervals. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

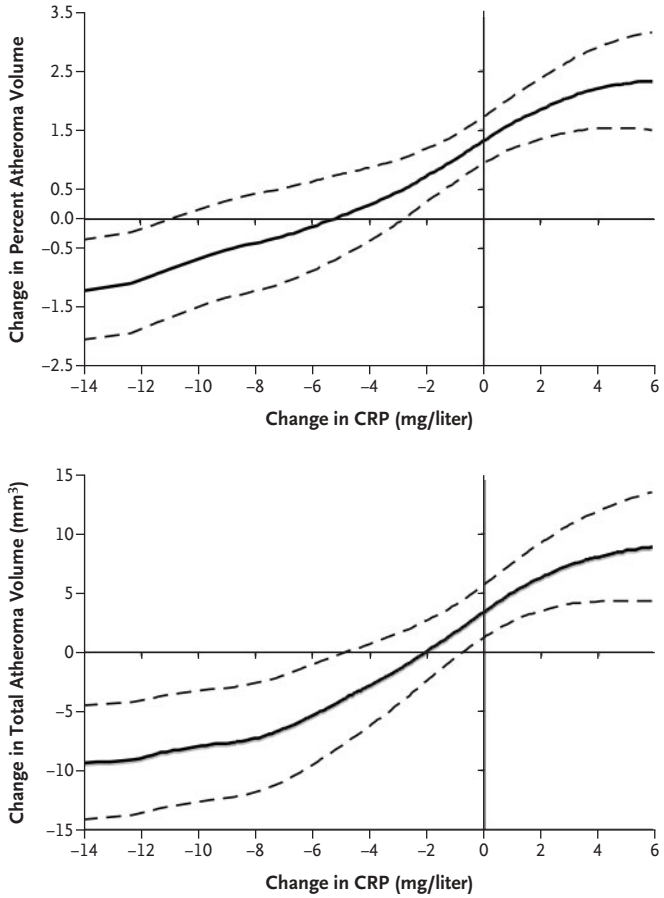


**Figure 2. Locally Weighted Smoothed Scatterplots Showing the Relationship between the Changes in CRP Levels and the Rate of Progression of Atherosclerosis in the Entire Group of 502 Patients.**

In each plot, the solid line represents the point estimates and the upper and lower lines the 95 percent confidence intervals.

demonstrates that biomarkers can account for only a small fraction of the observed progression rate.

To determine whether the reduction in CRP levels represented an independent factor influencing the progression of atherosclerosis, we adjusted the CRP correlations for the effects of atherogenic lipoproteins. In this multivariate analysis, CRP remained significant in most analyses, regardless of which measure of atherogenic lipoproteins was used — LDL cholesterol, apo B-100, or non-HDL cholesterol. Patients with reductions in the levels of both LDL cholesterol and CRP that were greater than the median reduction had significantly lower progression rates than patients in whom the reductions were less than the median decrease (P=0.001). These data provide evidence that the reduction in CRP levels plays an independent role in the beneficial effects of statins on the progression of coronary atherosclerosis.

Since measures of progression reflected by intravascular ultrasonography are not normally distributed, we used LOWESS methods to illustrate the relationships between the reductions in LDL cholesterol and CRP levels and the rates of progression determined by ultrasonography (Fig. 1 and 2). These plots demonstrated a continuous relationship between the magnitude of reduction in either LDL cholesterol or CRP levels and the rates of progression of atherosclerosis for both measures of efficacy. Atherosclerosis regressed in patients with the greatest reduction in CRP levels, but not in those with the greatest reduction in LDL cholesterol levels. Although the data are not provided in this article, LOWESS plots showed slower rates of progression in the intensively treated atorvastatin subgroup across a broad range of reductions in lipids and CRP. The slower rate of progression in the atorvastatin group for any magnitude of reduction in LDL cholesterol levels can be partially explained by the additional effects of treatment on the reduction in CRP levels, just as the differences in the CRP plots can be partially explained by the additional reduction in LDL cholesterol levels effected by atorvastatin therapy. Thus, the effects of the reductions in both LDL cholesterol and CRP levels must be considered to explain the observed differences in progression between atorvastatin and pravastatin treatment.

Our findings have important implications for understanding the pathogenesis of the progression of atherosclerosis and the mechanism of benefit of statin therapy. The Pravastatin or Atorvastatin and Infection Therapy (PROVE IT) trial demonstrated improved outcomes[1] and the REVERSAL trial demonstrated reduced rates of progression of atherosclerosis[2] after intensive, as compared with moderate, statin therapy. Although a single trial had previously shown that the effects of statins are evident within 16 weeks,[22] the rapidity of the divergence in results between the treatment groups in both trials was unexpected.[4] In most earlier placebo-controlled trials, differences between statins and placebo were not evident for the first two years after randomization.[16-18] However, in both the REVERSAL and PROVE IT trials, CRP levels were 30 to 40 percent lower at the conclusion of the trial in the intensively treated patients than in the group that received moderate treatment, which may ex-

**Table 4. Rates of Progression According to the Change in LDL Cholesterol and CRP Levels.***

| Subgroup | No. of Patients | Percent Atheroma Volume† | | | Total Atheroma Volume (mm³)† | | |
|---|---|---|---|---|---|---|---|
| | | Median | 95% CI | Mean ±SD | Median | 95% CI | Mean ±SD |
| Reduction in LDL cholesterol and CRP both greater than median | 141 | 0.24 (–2.8 to 3.5)‡ | –0.77 to 0.54 | 0.33±5.3 | –1.98 (–23.0 to 10.8)‡ | –6.26 to 3.67 | –2.41±31.6 |
| Reduction in LDL cholesterol greater than median, reduction in CRP less than median | 106 | 0.81 (–2.0 to 4.8) | –0.32 to 1.81 | 1.62±4.7 | 2.06 (–12.8 to 21.5) | –3.26 to 6.41 | 4.04±28.7 |
| Reduction in LDL cholesterol less than median, reduction in CRP greater than median | 108 | 1.21 (–2.0 to 4.0) | –0.31 to 2.08 | 0.91±4.9 | –1.04 (–18.6 to 22.5) | –6.78 to 8.74 | 1.42±29.2 |
| Reduction in LDL cholesterol and CRP both less than median | 141 | 1.82 (–1.5 to 5.1) | 1.0 to 2.84 | 2.25±5.0 | 8.21 (–11.8 to 27.5) | 0.40 to 13.05 | 7.49±27.5 |

* CRP levels were not available for six patients at baseline or follow-up. The subgroups were formed on the basis of the median percent change in LDL cholesterol of –37.1 percent and the median percent change in CRP of –21.4 percent.
† Values in parentheses are interquartile ranges. Confidence intervals (CIs) are for the medians.
‡ P=0.001 for the comparison with the subgroup in which the reduction in the levels of both LDL cholesterol and CRP was less than the median reduction (by Wilcoxon's rank-sum test).

plain the magnitude and unexpectedly rapid divergence of outcomes reported by Ridker et al. elsewhere in this issue of the *Journal*.[23]

Our findings are consistent with a variety of experimental observations that suggest a direct role for CRP in the pathogenesis of atherosclerosis. CRP renders oxidized LDL more susceptible to uptake by macrophages, induces the expression of vascular-cell adhesion molecules, stimulates the production of tissue factor, and impairs the production of nitric oxide.[24-27] Children with elevated CRP levels have increased carotid intimal medial thickness and reduced vasodilatation mediated by brachial-artery flow.[28] A recent study suggested that the presence of above-average levels of CRP attenuates the benefits of intensive statin therapy with respect to the carotid intimal media thickness.[29]

Evidence of a dual mechanism of benefit for statins — lipid lowering and a reduction in inflammation — has important implications for current and future treatment of atherosclerosis. Current guidelines emphasize the use of lipid-lowering therapies to reach target levels of LDL cholesterol, non-HDL cholesterol, or both. However, individual agents differ in their ability to reduce the levels of inflammatory biomarkers. Accordingly, our study raises the provocative question of whether the effects of statins on CRP, as well as LDL cholesterol, should be considered in decisions regarding therapy.

Our study has important limitations. It is a hypothesis-generating post hoc analysis examining the effect of a single inflammatory marker on disease progression, not morbidity or mortality. Nonetheless, our findings suggest that the level of CRP may ultimately represent an important therapeutic target. We do not believe that these data are sufficient to recommend routine serial measurement of CRP in order to modulate statin therapy, but further study is warranted. An ongoing clinical trial is assessing the use of CRP levels to guide therapy in patients who do not have elevated LDL cholesterol levels.[30] Since approaches to the reduction of LDL cholesterol levels that do not involve statins have uncertain antiinflammatory effects, the ability of such therapies to improve the outcome requires testing in clinical trials.[31]

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## APPENDIX

In addition to the authors, the following investigators participated in this study: Wake Forest University, Winston-Salem, N.C., M. Kutcher; University of Colorado Health Sciences Center, Denver, J. Burchenal; University of Texas–San Antonio, San Antonio, S. Bailey; Heart Institute at Borgess, Kalamazoo, Mich., T. Fischell; University of Florida, Gainesville, R. Kerensky; Heart Care Center, Blue Island, Ill., R. Iaffaldano; University of Chicago, Chicago, J. Lopez; William Beaumont Hospital, Royal Oak, Mich., C. Grines; University of California, San Diego, San Diego, A. DeMaria; UCLA Medical Center for Health Sciences, Los Angeles, J. Tobis; LeBauer Cardiovascular Research Foundation, Greensboro, N.C., B. Brodie; University of Washington Medical Center, Seattle, D. Linker; Cedars-Sinai Medical Center, Los Angeles, J. Forrester; University of North Carolina, Chapel Hill, S. Smith; Androscoggin Cardiology Research, Auburn, Me., R. Weiss; Medical College of Ohio, Toledo, C. Cooper; Rhode Island Hospital, Providence, B. Sharaf; East Carolina University, Greenville, N.C., M. Miller; Buffalo Cardiology and Pulmonary Associates, Buffalo, N.Y., J. Corbelli; Heart Care Group, Allentown, Pa., J. Kleaveland; University of Arkansas for Medical Sciences, Little Rock, L. Garza; University of Louisville, Louisville, Ky., M. Leesar; Capital Cardiology Associates, Albany, N.Y., A. DeLago; Cardiology of Georgia–Piedmont Hospital, Atlanta, C. Wickliffe; New England Medical Center, Boston, J. Kuvin; Kramer & Crouse Cardiology, Kansas City, Mo., P. Kramer; Miriam Hospital, Providence, R.I., P. Gordon; Mount Sinai Hospital, New York, S. Sharma; Oklahoma Heart Institute, Tulsa, W. Leimbach; Eastlake Cardiovascular Associates, St. Clair Shores, Mich., R. Cleary, Jr.; University Hospitals of Cleveland, R. Nair.

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# C-REACTIVE PROTEIN AND OTHER MARKERS OF INFLAMMATION IN THE PREDICTION OF CARDIOVASCULAR DISEASE IN WOMEN

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## ABSTRACT

***Background*** Since inflammation is believed to have a role in the pathogenesis of cardiovascular events, measurement of markers of inflammation has been proposed as a method to improve the prediction of the risk of these events.

***Methods*** We conducted a prospective, nested case–control study among 28,263 apparently healthy postmenopausal women over a mean follow-up period of three years to assess the risk of cardiovascular events associated with base-line levels of markers of inflammation. The markers included high-sensitivity C-reactive protein (hs-CRP), serum amyloid A, interleukin-6, and soluble intercellular adhesion molecule type 1 (sICAM-1). We also studied homocysteine and several lipid and lipoprotein measurements. Cardiovascular events were defined as death from coronary heart disease, nonfatal myocardial infarction or stroke, or the need for coronary-revascularization procedures.

***Results*** Of the 12 markers measured, hs-CRP was the strongest univariate predictor of the risk of cardiovascular events; the relative risk of events for women in the highest as compared with the lowest quartile for this marker was 4.4 (95 percent confidence interval, 2.2 to 8.9). Other markers significantly associated with the risk of cardiovascular events were serum amyloid A (relative risk for the highest as compared with the lowest quartile, 3.0), sICAM-1 (2.6), interleukin-6 (2.2), homocysteine (2.0), total cholesterol (2.4), low-density lipoprotein (LDL) cholesterol (2.4), apolipoprotein B-100 (3.4), high-density lipoprotein (HDL) cholesterol (0.3), and the ratio of total cholesterol to HDL cholesterol (3.4). Prediction models that incorporated markers of inflammation in addition to lipids were significantly better at predicting risk than models based on lipid levels alone ($P<0.001$). The levels of hs-CRP and serum amyloid A were significant predictors of risk even in the subgroup of women with LDL cholesterol levels below 130 mg per deciliter (3.4 mmol per liter), the target for primary prevention established by the National Cholesterol Education Program. In multivariate analyses, the only plasma markers that independently predicted risk were hs-CRP (relative risk for the highest as compared with the lowest quartile, 1.5; 95 percent confidence interval, 1.1 to 2.1) and the ratio of total cholesterol to HDL cholesterol (relative risk, 1.4; 95 percent confidence interval, 1.1 to 1.9).

***Conclusions*** The addition of the measurement of C-reactive protein to screening based on lipid levels may provide an improved method of identifying women at risk for cardiovascular events. (N Engl J Med 2000;342:836-43.)



HALF of all myocardial infarctions occur in persons in whom plasma lipid levels are normal.[1] In an effort to better identify patients at high risk for cardiovascular events, several markers of risk have been proposed for use in screening, including homocysteine and fibrinogen levels, fibrinolytic capacity, and levels of apolipoprotein A-I, apolipoprotein B-100, and Lp(a) lipoprotein. However, the clinical value of many of these markers has been limited because of inadequate standardization of assay conditions, inconsistency of prospective data, or lack of evidence of significant improvement in the prediction of risk over that afforded by standard lipid screening alone.[2]

With the recognition that atherosclerosis is an inflammatory process,[3] several plasma markers of inflammation have also been evaluated as potential tools for prediction of the risk of coronary events. Among them are markers of systemic inflammation produced in the liver, such as high-sensitivity C-reactive protein (hs-CRP) and serum amyloid A; cytokines such as interleukin-6; and adhesion molecules such as soluble intercellular adhesion molecule type 1 (sICAM-1).[4-11] However, as with other proposed predictors of the risk of cardiovascular events, the prognostic value of these markers of inflammation remains uncertain. For example, a widely held clinical view is that levels of markers of inflammation vary too greatly over time to allow accurate prediction of risk. Furthermore, few prospective studies have measured all these markers of inflammation in a single group of patients, so the relative usefulness of each marker cannot be easily evaluated. In addition, data supporting the hypothesis that markers of inflammation significantly increase the predictive value of lipid screening are scant and are limited almost exclusively to data from studies of hs-CRP in middle-aged men.[7,12] Finally, clinical application of these findings has been limited, since standardized, commercial assays for most markers of inflammation are only now being developed.

In a previous study, in which we used an experimental assay for hs-CRP, we found higher levels of this marker among healthy postmenopausal women participating in the Women's Health Study who subse-

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quently had cardiovascular events than among those who did not have such events.[13] On the basis of that finding and in the effort to address the clinical issues outlined above, we used a commercial assay to measure hs-CRP in the same cohort and simultaneously measured plasma levels of serum amyloid A, interleukin-6, sICAM-1, total cholesterol, low-density lipoprotein (LDL) cholesterol, high-density lipoprotein (HDL) cholesterol, the ratio of total cholesterol to HDL cholesterol, apolipoprotein A-I, and apolipoprotein B-100. In addition, to allow comparison with other proposed markers, we measured plasma levels of Lp(a) lipoprotein and homocysteine. We thus were able to evaluate directly the relative value of each of these 12 measurements as an independent predictor of future cardiovascular events in a large cohort of apparently healthy women. We also sought to determine whether the measurement of markers of inflammation in addition to standard screening of lipid levels might provide a clinically useful method for improving overall prediction of the risk of cardiovascular events.

## METHODS

### Study Participants

We designed a prospective, nested case–control study involving participants in the Women's Health Study, an ongoing trial of aspirin and vitamin E for primary prevention among postmenopausal women with no history of cardiovascular disease or cancer.[14] Blood samples were collected in tubes containing EDTA at base line from 28,263 women (71 percent of the Women's Health Study participants) and stored in liquid nitrogen until the time of analysis.

For this analysis, case subjects were study participants from whom a base-line blood sample was obtained who subsequently had a cardiovascular event (defined as death from coronary heart disease, nonfatal myocardial infarction or stroke, or a coronary-revascularization procedure) during a mean follow-up period of three years. Myocardial infarction was classified as confirmed if symptoms met the criteria of the World Health Organization[15] and if the event was associated with abnormal levels of cardiac enzymes or diagnostic electrocardiographic changes. Stroke was classified as confirmed if the patient had a new neurologic deficit that lasted more than 24 hours. Computed tomographic scans or magnetic resonance images were available for the majority of women in whom stroke occurred. Performance of revascularization procedures was confirmed by review of hospital records. Death from coronary heart disease was confirmed by review of the autopsy report, the death certificate, medical records, or information from family members regarding the circumstances of death.

For each woman who had a confirmed cardiovascular event during follow-up, two control subjects of the same age (within one year) and smoking status (former smoker, current smoker, or nonsmoker) were selected from among the remaining study participants from whom a base-line blood sample had been obtained and who remained free of reported cardiovascular disease during follow-up. With use of these criteria, 122 case subjects and 244 control subjects were selected.

### Procedures

Base-line plasma samples from each woman with an event and each control subject were thawed and assayed for hs-CRP, serum amyloid A, and Lp(a) lipoprotein with use of latex-enhanced immunonephelometric assays on a BN II analyzer (Dade Behring, Newark, Del.). Apolipoprotein A-I and apolipoprotein B-100 were simultaneously measured with this device by immunoassay. Total cholesterol, HDL cholesterol, and directly obtained LDL cholesterol levels were measured on a Hitachi 911 analyzer (Roche Diagnostics, Indianapolis) with reagents from Roche Diagnostics and Genzyme (Cambridge, Mass.). Plasma levels of sICAM-1 and interleukin-6 were measured by enzyme-linked immunosorbent assay (R & D Systems, Minneapolis), and the total plasma homocysteine level was measured with an IMx homocysteine assay (Abbott Laboratories, Abbott Park, Ill.) as previously reported.[16] Samples were handled in identical and in blinded fashion throughout the study. Samples were analyzed in triplicate and in random order so as to reduce systematic bias and interassay variation.

### Statistical Analysis

Means and proportions for risk factors for cardiovascular events at base line were calculated for women who had cardiovascular events during follow-up and those who did not. The significance of differences in means between the two groups was assessed with Student's t-test, and the significance of differences in proportions was tested with use of the chi-square statistic. Analysis of trends was used to test for associations between increasing levels of each plasma variable and the risk of future cardiovascular events, after the sample was divided into quartiles according to the distribution of control values for that marker. Adjusted risk estimates were obtained with use of logistic-regression models that, in addition to accounting for the variables used for matching (age and smoking status), adjusted for random assignment to aspirin or vitamin E in the Women's Health Study; several risk factors for cardiovascular events, including a history of hypertension, body-mass index, a history of diabetes, and a parental history of myocardial infarction before the age of 60 years; and other measured plasma markers.

We evaluated the combined role of lipid levels and markers of inflammation as predictors of the risk of future cardiovascular events in a series of analyses in which we explored the sensitivity and robustness of our findings from a clinical perspective. First, we used the likelihood-ratio test to determine whether logistic-regression models that included measurements of lipid variables and markers of inflammation provided a significantly better fit than did logistic-regression models limited to lipid measurements alone. Second, to estimate the clinical relevance of these effects, we computed the area under receiver-operating-characteristic curves for prediction models based on lipid measurements alone and for models based on measurements of both lipid levels and markers of inflammation. Third, we divided the study participants into nine groups according to low, medium, and high levels of total cholesterol and low, medium, and high levels of each marker of inflammation. In these analyses, logistic regression was used to evaluate simultaneously the risk of future cardiovascular events in each of the nine groups; the group of women in the lowest third for total cholesterol and in the lowest third for the respective marker of inflammation was considered the reference group. Finally, to address the clinical need for improved assessment of risk among persons with cholesterol levels currently considered safe, we performed a subgroup analysis of study participants with LDL cholesterol levels of less than 130 mg per deciliter (3.4 mmol per liter), the target level for the primary prevention of coronary heart disease according to the current guidelines of the National Cholesterol Education Program.[17]

All P values were two-tailed, and values of less than 0.05 were considered to indicate statistical significance. All confidence intervals were calculated at the 95 percent level.

## RESULTS

The base-line characteristics of the women who subsequently had cardiovascular events (case subjects) and those who remained free of reported cardiovascular disease (controls) are shown in Table 1. As expected, women who had cardiovascular events were heavier at base line than those who remained free of cardiovascular disease and were more likely to have hypertension, diabetes, or a parental history of prema-

**TABLE 1.** BASE-LINE CLINICAL CHARACTERISTICS OF THE STUDY PARTICIPANTS.*

| CHARACTERISTIC | WOMEN WITH CARDIOVASCULAR EVENTS (N=122) | WOMEN FREE OF CARDIOVASCULAR EVENTS (N=244) | P VALUE† |
|---|---|---|---|
| Mean age (yr) | 59.3 | 59.3 | — |
| Mean body-mass index‡ | 27.1 | 26.0 | 0.04 |
| History of hypertension (%) | 55.5 | 31.3 | 0.001 |
| History of diabetes (%) | 9.8 | 2.1 | 0.001 |
| Parental history of myocardial infarction before 60 yr (%) | 21.3 | 12.7 | 0.04 |
| Smoking status (%) | | | — |
| Former smoker | 29.5 | 29.5 | |
| Current smoker | 27.9 | 27.9 | |
| Nonsmoker | 42.6 | 42.6 | |
| Frequency of exercise (%) | | | 0.9 |
| >3 times/wk | 6.6 | 8.2 | |
| 1–3 times/wk | 27.9 | 27.1 | |
| <1 time/wk | 21.3 | 20.1 | |
| Rarely or never | 44.3 | 44.5 | |
| Frequency of alcohol consumption (%) | | | 0.6 |
| Daily | 12.3 | 8.2 | |
| Weekly | 27.9 | 31.2 | |
| Monthly | 14.8 | 13.9 | |
| Rarely or never | 45.1 | 46.7 | |
| Current use of hormone-replacement therapy (%) | 44.3 | 41.0 | 0.1 |

*Because of rounding, not all percentages total 100.

†P values were not calculated for variables used in matching of case and control subjects, since the distribution of these variables was identical in the two groups.

‡The body-mass index is the weight in kilograms divided by the square of the height in meters.

**TABLE 2.** BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

| VARIABLE | WOMEN WITH CARDIOVASCULAR EVENTS | WOMEN FREE OF CARDIOVASCULAR EVENTS | P VALUE |
|---|---|---|---|
| High-sensitivity C-reactive protein (mg/dl) | | | <0.001 |
| Median | 0.42 | 0.28 | |
| Interquartile range | 0.21–0.83 | 0.11–0.55 | |
| Serum amyloid A (mg/dl) | | | 0.003 |
| Median | 0.63 | 0.52 | |
| Interquartile range | 0.45–1.01 | 0.35–0.78 | |
| Soluble intercellular adhesion molecule type 1 (ng/ml) | 349.7±121.3 | 321.3±107.4 | 0.03 |
| Interleukin-6 (pg/ml) | | | 0.003 |
| Median | 1.65 | 1.30 | |
| Interquartile range | 1.14–2.62 | 1.00–2.03 | |
| Total cholesterol (mg/dl) | 230.5±41.2 | 219.2±37.5 | 0.01 |
| LDL cholesterol (mg/dl) | 132.2±34.6 | 121.5±30.2 | 0.003 |
| HDL cholesterol (mg/dl) | 45.4±14.6 | 51.1±15.4 | <0.001 |
| Apolipoprotein A-I (mg/dl) | 163.8±40.3 | 168.5±36.1 | 0.3 |
| Apolipoprotein B-100 (mg/dl) | 128.5±31.0 | 115.0±26.7 | <0.001 |
| Lp(a) lipoprotein (mg/liter) | | | 0.3 |
| Median | 79 | 74 | |
| Interquartile range | 34–247 | 29–203 | |
| Ratio of total cholesterol to HDL cholesterol | 5.5±1.9 | 4.6±1.4 | <0.001 |
| Homocysteine (μmol/liter) | 14.1±8.0 | 12.4±5.8 | 0.02 |

*Plus–minus values are means ±SD. For normally distributed variables, P values were computed with t-tests; for non-normally distributed variables, P values were computed with the Wilcoxon rank-sum test for the difference in medians. LDL denotes low-density lipoprotein, and HDL high-density lipoprotein. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

ture myocardial infarction (before the age of 60 years). The frequency of exercise, the frequency of alcohol consumption, and rate of use of hormone-replacement therapy were similar in the two groups. Because of matching, the women who had cardiovascular events and the control subjects were virtually identical with respect to mean age and smoking status.

Base-line plasma levels of the inflammation markers hs-CRP ($P<0.001$), serum amyloid A ($P=0.003$), sICAM-1 ($P=0.03$), and interleukin-6 ($P=0.003$) were higher among the women who subsequently had cardiovascular events than among those who did not (Table 2). Similarly, base-line plasma levels of total cholesterol ($P=0.01$), LDL cholesterol ($P=0.003$), apolipoprotein B-100 ($P<0.001$), and homocysteine ($P=0.02$) and the ratio of total cholesterol to HDL cholesterol ($P<0.001$) were significantly higher among women with subsequent events than those without such events, whereas levels of HDL cholesterol were significantly lower among women with subsequent events ($P<0.001$). Base-line levels of Lp(a) lipoprotein were somewhat higher and levels of apolipoprotein A-I somewhat lower among the women with events than among control subjects, but these differences were not significant.

Table 3 shows the relative risks of cardiovascular events according to the quartile of each marker of inflammation or lipid measured in plasma. Measurements of hs-CRP, serum amyloid A, sICAM-1, and interleukin-6 were predictive of the risk of future cardiovascular events. Of the 12 measures, the level of hs-CRP was the most powerful predictor of risk in the univariate analysis (relative risk for women in the highest quartile as compared with the lowest quartile, 4.4; 95 percent confidence interval, 2.2 to 8.9; $P<0.001$). Of the lipid variables, the ratio of total cholesterol to HDL cholesterol (relative risk, 3.4; $P=0.001$) and the apolipoprotein B-100 level (relative risk, 3.4; $P=0.001$) were the most powerful predictors of risk. Nonsignificant trends were observed for apolipoprotein A-I and Lp(a) lipoprotein. As reported previously,[16] increasing levels of homocysteine were also associated with increased risk.

Levels of several markers of inflammation were highly correlated. For example, the correlation coefficient for the relation between hs-CRP and serum

**TABLE 3.** RELATIVE RISK OF CARDIOVASCULAR EVENTS ACCORDING TO BASE-LINE PLASMA LEVELS OF MARKERS OF INFLAMMATION AND LIPIDS.*

| VARIABLE | QUARTILE OF PLASMA LEVEL | | | | P VALUE FOR TREND |
|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | |
| High-sensitivity C-reactive protein | | | | | |
| Median — mg/dl | 0.06 | 0.19 | 0.38 | 0.85 | |
| Relative risk (95% CI) | 1.0 | 2.1 (1.0–4.5) | 2.1 (1.0–4.4) | 4.4 (2.2–8.9) | <0.001 |
| Serum amyloid A | | | | | |
| Median — mg/dl | 0.25 | 0.43 | 0.62 | 1.17 | |
| Relative risk (95% CI) | 1.0 | 1.8 (0.9–3.6) | 1.9 (0.9–3.8) | 3.0 (1.5–6.0) | 0.002 |
| Soluble intercellular adhesion molecule type 1 | | | | | |
| Median — ng/ml | 228.7 | 273.9 | 319.1 | 439.3 | |
| Relative risk (95% CI) | 1.0 | 1.5 (0.7–3.1) | 2.0 (1.0–4.1) | 2.6 (1.3–5.1) | 0.004 |
| Interleukin-6 | | | | | |
| Median — pg/ml | 0.82 | 1.15 | 1.58 | 2.70 | |
| Relative risk (95% CI) | 1.0 | 1.3 (0.6–2.7) | 1.4 (0.7–2.8) | 2.2 (1.1–4.3) | 0.02 |
| Total cholesterol | | | | | |
| Median — mg/dl | 176 | 206 | 224 | 267 | |
| Relative risk (95% CI) | 1.0 | 1.2 (0.6–2.3) | 1.7 (0.9–3.3) | 2.4 (1.3–4.7) | 0.003 |
| LDL cholesterol | | | | | |
| Median — mg/dl | 88.4 | 108.9 | 127.4 | 156.6 | |
| Relative risk (95% CI) | 1.0 | 0.9 (0.4–1.9) | 1.7 (0.9–3.3) | 2.4 (1.3–4.6) | 0.001 |
| HDL cholesterol | | | | | |
| Median — mg/dl | 34.5 | 44.5 | 54.9 | 68.5 | |
| Relative risk (95% CI) | 1.0 | 0.5 (0.3–0.8) | 0.5 (0.2–0.8) | 0.3 (0.2–0.6) | 0.001 |
| Apolipoprotein A-I | | | | | |
| Median — mg/dl | 127 | 152 | 176 | 212 | |
| Relative risk (95% CI) | 1.0 | 0.8 (0.4–1.4) | 0.4 (0.2–0.8) | 0.8 (0.4–1.4) | 0.1 |
| Apolipoprotein B-100 | | | | | |
| Median — mg/dl | 86 | 104 | 121 | 149 | |
| Relative risk (95% CI) | 1.0 | 1.1 (0.5–2.3) | 1.6 (0.8–3.3) | 3.4 (1.8–6.8) | <0.001 |
| Lp(a) lipoprotein | | | | | |
| Median — mg/liter | 16 | 55 | 107 | 329 | |
| Relative risk (95% CI) | 1.0 | 1.0 (0.5–1.9) | 1.1 (0.6–2.1) | 1.3 (0.7–2.4) | 0.4 |
| Ratio of total cholesterol to HDL cholesterol | | | | | |
| Median | 3.06 | 4.00 | 4.80 | 6.34 | |
| Relative risk (95% CI) | 1.0 | 0.8 (0.3–1.5) | 1.7 (0.9–3.4) | 3.4 (1.8–5.9) | <0.001 |
| Homocysteine | | | | | |
| Median — μmol/liter | 8.2 | 10.3 | 12.1 | 15.7 | |
| Relative risk (95% CI) | 1.0 | 1.1 (0.6–2.2) | 1.1 (0.5–2.1) | 2.0 (1.1–3.8) | 0.02 |

*P values were calculated by logistic-regression analyses. In all models, subjects were matched according to age and smoking status, and all models were adjusted for random assignment to aspirin or vitamin E. CI denotes confidence interval, LDL low-density lipoprotein, and HDL high-density lipoprotein. To convert values for cholesterol to millimoles per liter, multiply by 0.02586.

amyloid A was 0.81 ($P<0.001$). In contrast, correlations between markers of inflammation and lipid measures were low; less than 10 percent of the variance in any marker of inflammation was explained by any of the lipid measures.

To determine the independent predictive value of each of the 12 measures, we performed a series of logistic-regression analyses that simultaneously controlled for increasing quartiles of hs-CRP, serum amyloid A, sICAM-1, interleukin-6, homocysteine, and Lp(a) lipoprotein and the ratio of total cholesterol to HDL cholesterol (because of colinearity with this ratio, levels of apolipoprotein A-I, apolipoprotein B-100, and LDL cholesterol were not included in these analyses). As shown in Table 4, only the level of hs-CRP and the ratio of total cholesterol to HDL cholesterol were found to be independent predictors of risk in models in which women were matched for smoking status and age or in models that included further adjustments for body-mass index, hypertension, diabetes, and parental history of premature coronary artery disease. In similar models that were limited to markers of inflammation, hs-CRP remained an independent predictor of the risk of future cardiovascular events. In contrast, the beta coefficients associated with serum amyloid A, sICAM-1, and interleukin-6 decreased substantially and were no longer statistically significant in analyses that included control for the quartile of hs-CRP.

To explore whether any of the markers of inflam-

**TABLE 4.** ADJUSTED RELATIVE RISK OF CARDIOVASCULAR EVENTS ASSOCIATED WITH AN INCREASE OF ONE QUARTILE IN THE CONCENTRATION OF EACH PLASMA MARKER.*

| VARIABLE | ADJUSTED FOR OTHER PLASMA MARKERS | | ADJUSTED FOR OTHER PLASMA MARKERS AND RISK FACTORS† | |
|---|---|---|---|---|
| | RELATIVE RISK (95% CI) | P VALUE | RELATIVE RISK (95% CI) | P VALUE |
| High-sensitivity C-reactive protein | 1.4 (1.1–1.9) | 0.02 | 1.5 (1.1–2.1) | 0.02 |
| Serum amyloid A | 1.1 (0.8–1.4) | 0.5 | 1.1 (0.8–1.6) | 0.4 |
| Soluble intercellular adhesion molecule type 1 | 1.1 (0.9–1.4) | 0.4 | 1.1 (0.8–1.4) | 0.6 |
| Interleukin-6 | 0.9 (0.7–1.2) | 0.6 | 0.8 (0.6–1.1) | 0.2 |
| Homocysteine | 1.1 (0.9–1.4) | 0.2 | 1.1 (0.8–1.4) | 0.6 |
| Lp(a) lipoprotein | 1.1 (0.9–1.3) | 0.6 | 1.0 (0.8–1.2) | 0.8 |
| Ratio of total cholesterol to HDL cholesterol | 1.4 (1.1–1.7) | 0.01 | 1.4 (1.1–1.9) | 0.02 |

*In all models, subjects were matched according to age and smoking status, and all models were adjusted for random assignment to aspirin or vitamin E. CI denotes confidence interval, and HDL high-density lipoprotein.

†These models were adjusted for the following additional risk factors: body-mass index (the weight in kilograms divided by the square of the height in meters), a history of hypertension, a history of diabetes, and a parental history of myocardial infarction.

mation added to the predictive value of lipid-based screening, several additional analyses were performed. First, we computed the relative risk of cardiovascular events in analyses in which study participants were stratified into nine groups according to total cholesterol level as well as each marker of inflammation. As shown in Figure 1, for each marker of inflammation included in this analysis, the risk of cardiovascular events was lowest among women with low total cholesterol levels and low levels of the marker in question. In contrast, the risk tended to be highest among women with high total cholesterol levels and high levels of a marker of inflammation. However, even among the women with low total cholesterol levels, the risk of cardiovascular events was significantly higher among those with high levels of hs-CRP and serum amyloid A than among those with low levels of these markers (Fig. 1). These associations were also evident, but to a lesser extent, for interleukin-6 and sICAM-1. In all of the analyses, these additive effects were robust with respect to the choice of cutoff point and the choice of the lipid variable analyzed. For example, the addition of hs-CRP to lipid screening produced a significant and additive predictive effect when regression analyses were based on cutoff points for quartiles (rather than cutoff points for the division of the study group into thirds) and on analysis of the ratio of total cholesterol to HDL cholesterol (rather than on total cholesterol alone).

Second, likelihood-ratio tests were used to compare the fit of predictive models that were based on measurement of a marker of inflammation in combination with lipids to the fit of models based on lipid measurements alone. In these analyses, each of the markers of inflammation significantly improved the usefulness of lipid screening in predicting risk. For example, models including both hs-CRP and total cholesterol were significantly better in the prediction of the risk of cardiovascular events than were models including only total cholesterol ($P<0.001$). Likewise, models involving both hs-CRP and the ratio of total cholesterol to HDL cholesterol allowed significantly better prediction of risk than did models based solely on this lipid ratio alone ($P<0.001$). Similar additive effects were seen for serum amyloid A, sICAM-1, and interleukin-6 when these markers were added to models based on total cholesterol or the ratio of total cholesterol to HDL cholesterol alone ($P<0.01$ for all comparisons).

Third, as a measure of clinical usefulness, we computed the area under the receiver-operating-characteristic curve associated with risk-prediction models based on lipid screening alone and compared it with those based on a combination of lipids and markers of inflammation. In these analyses, the use of hs-CRP levels in addition to total cholesterol increased the area under the receiver-operating-characteristic curve from 0.59 to 0.66 ($P<0.001$) and in addition to the ratio of total cholesterol to HDL cholesterol increased the area under the curve from 0.64 to 0.68 ($P<0.001$). Similar effects were observed for analyses that included serum amyloid A, sICAM-1, and interleukin-6: the addition of these markers to screening based on total cholesterol increased the area under the curve from 0.59 to 0.63, 0.63, and 0.64, respectively ($P<0.003$ for all three comparisons). Use of the serum amyloid A level in addition to the ratio of total cholesterol to HDL cholesterol increased the area under the curve from 0.64 to 0.67 ($P=0.007$); the use of sICAM-1 in addition to this ratio led to a smaller change (area under the curve, 0.65; $P=0.01$), as did the use of interleukin-6 (area under the curve, 0.65; $P=0.01$).

Finally, to address the clinical observation that many persons with "safe" lipid levels nonetheless have cardiovascular events, we performed a subgroup analysis limited to women whose levels of LDL cholesterol were less than 130 mg per deciliter, the target level currently recommended for primary prevention of coronary heart disease by the National Cholesterol Education Program.[17] In this analysis, women with increased base-line levels of hs-CRP, serum amyloid A, interleukin-6, or sICAM-1 were found to be at increased risk for future cardiovascular events. This effect was strongest for hs-CRP and serum amyloid A. In this subgroup, the relative risks of cardiovascular events for women in the lowest to the highest quartiles of hs-CRP were 1.0, 2.4, 2.9, and 4.1 (95 percent confidence interval for women in the highest as compared with the lowest quartile, 1.7 to 11.3; $P=0.002$; P for trend across quartiles, 0.005). After adjustment

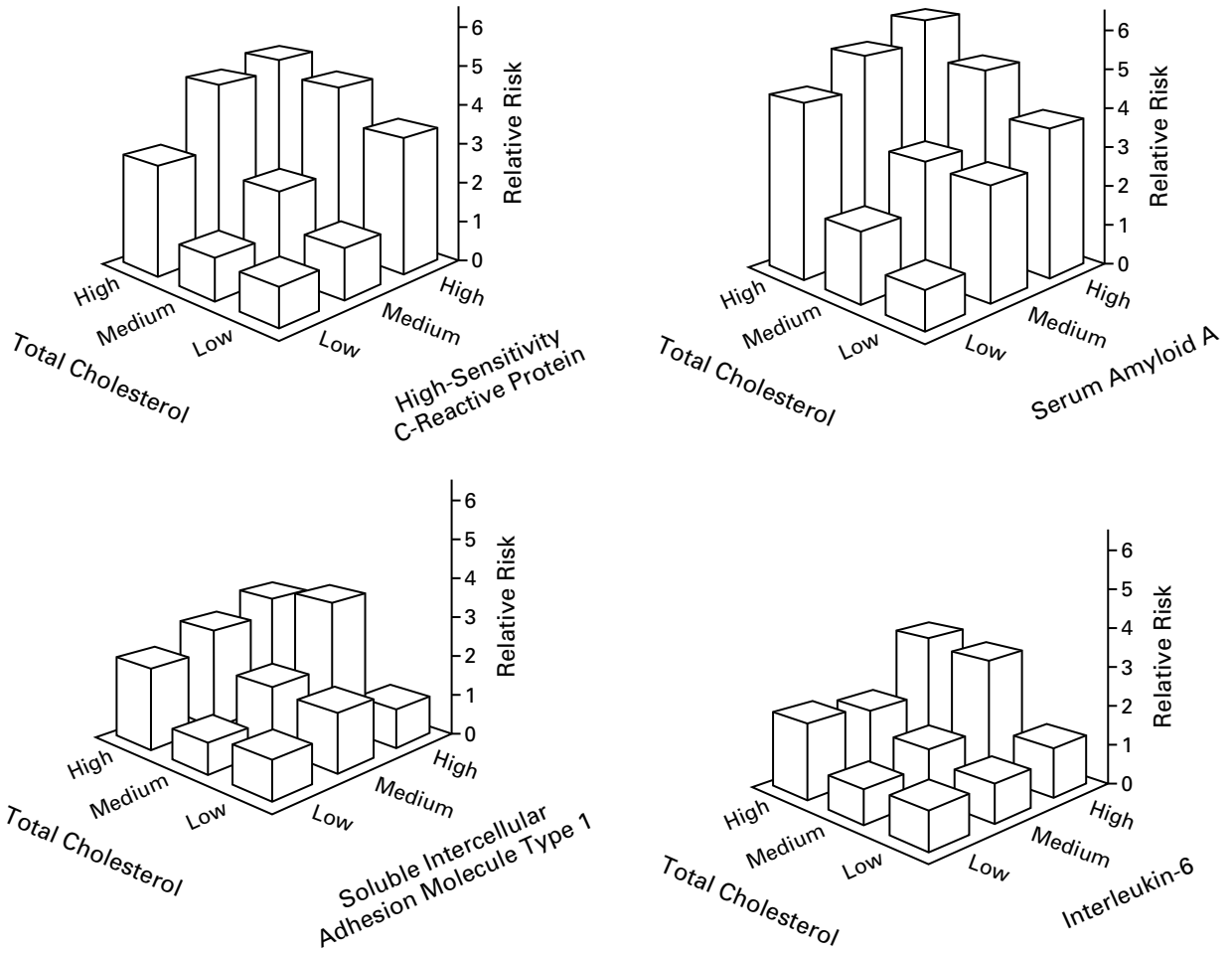


**Figure 1.** Relative Risk of Cardiovascular Events among Apparently Healthy Postmenopausal Women According to Base-Line Levels of Total Cholesterol and Markers of Inflammation.

Each marker of inflammation improved risk-prediction models based on lipid testing alone, an effect that was strongest for hs-CRP and serum amyloid A.

for body-mass index, the presence or absence of hypertension, diabetes, or a parental history of premature myocardial infarction, and the level of HDL cholesterol, the increased risk for women in the highest quartile of hs-CRP at base line remained statistically significant (relative risk, 3.1; 95 percent confidence interval, 1.1 to 8.3; P=0.03). Thus, even among women with "safe" levels of LDL cholesterol, the adjusted relative risk of cardiovascular events increased approximately 39 percent with each increasing quartile for hs-CRP (95 percent confidence interval, 13 to 89 percent; P=0.03). The mean LDL cholesterol level in this subgroup analysis was 104 mg per deciliter (2.7 mmol per liter).

## DISCUSSION

In this prospective study of apparently healthy postmenopausal women, four markers of inflammation — hs-CRP, serum amyloid A, interleukin-6, and sICAM-1 — were found to be significant predictors of the risk of future cardiovascular events. In addition, measurement of these markers increased the predictive value of models based only on standard lipid screening. Of the 12 plasma measures evaluated in this study, hs-CRP was the most significant predictor of the risk of cardiovascular events; when measured with a widely available, standardized commercial assay,[18] this marker distinguished between women at high risk and those at low risk, even in the subgroup of women with LDL cholesterol levels below 130 mg per deciliter (mean, 104 mg per deciliter), the target considered safe in the current guidelines of the National Cholesterol Education Program.[17]

The results of the current study have several important implications. First, the findings confirm that in women, markers of inflammation are important predictors of the risk of cardiovascular events. Previous data on this issue have been derived largely from

studies of middle-aged men.[4-11] Thus, from a pathophysiologic perspective, the current data support the hypothesis that atherosclerosis is, in part, an inflammatory disease.[3]

Second, because we used a commercially available assay to measure plasma hs-CRP,[18] our results provide clinically relevant confirmation of previous findings in this cohort, which were obtained with use of an experimental assay.[13] The commercial assay is inexpensive and can be used with standard hospital and outpatient laboratory equipment; thus, screening for this predictor of cardiovascular risk would be practical in many clinical settings.

Third, we believe the current results have public health implications both in terms of the prediction of the risk of cardiovascular events and in terms of the use of inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase for primary prevention. Although the results of large-scale randomized trials have indicated that HMG-CoA reductase inhibition is effective even among persons at low-to-moderate risk as defined by standard lipid screening,[19,20] the large number of patients who would need to be treated and the high cost of this approach have limited the clinical application of those findings. Thus, our observation that measurement of markers of inflammation such as hs-CRP can significantly improve models for the prediction of cardiovascular risk may lead to better clinical identification of patients who might benefit from primary prevention and for whom the cost-to-benefit ratio for long-term use of statins would be improved. This issue is particularly intriguing because recent data from the Cholesterol and Recurrent Events trial indicate that long-term therapy with pravastatin significantly lowers plasma levels of hs-CRP[21] and that the efficacy of pravastatin in lowering the rate of cardiovascular events is greatest in those with increased levels of hs-CRP.[22] As in the current findings, which indicate that hs-CRP is a potent predictor of risk regardless of the LDL cholesterol level, data from the Cholesterol and Recurrent Events trial indicate that use of pravastatin resulted in decreased levels of hs-CRP in a manner largely independent of LDL cholesterol.[21]

Several limitations of these analyses merit consideration. First, our cohort comprised apparently healthy postmenopausal women, and thus the results may not apply to younger women, who may also be at increased risk for cardiovascular events. Second, we measured each marker of inflammation at study entry and thus could not evaluate the effects of changes in the levels of these markers over time. However, follow-up studies have found that levels of hs-CRP are stable over long periods, as long as measurements are not made within two to three weeks of an acute infection.[21,23] Moreover, with respect to the current results, variation over time in levels of these markers and regression dilution bias would tend, if anything, to lead to an underestimation of net effects. Finally, although base-line levels of several markers of inflammation were greater than normal among women at risk for future cardiovascular events, the mechanisms underlying these elevations remain uncertain. In this study, we did not find significant associations between cardiovascular risk and titers of IgG antibodies against *Chlamydia pneumoniae, Helicobacter pylori,* herpes simplex virus, or cytomegalovirus or between titers of these antibodies and plasma levels of hs-CRP.[24] On the other hand, markers of inflammation, including hs-CRP, interleukin-6, and interleukin-1–receptor antagonist,[25-31] have proved to have predictive value among persons with unstable angina or acute coronary syndromes. Thus, it is also possible that the inflammation that we detected in apparently healthy women who were at risk for future cardiovascular events may be an indirect marker of an enhanced cytokine response to a variety of inflammatory stimuli that ultimately prove critical at the time of acute plaque rupture.[32]

In conclusion, in this prospective evaluation of 12 plasma variables, hs-CRP proved to be the strongest and most significant predictor of the risk of future cardiovascular events. As in previous population-based epidemiologic studies, half of all cardiovascular events in our cohort occurred among women without overt hyperlipidemia. Thus, these data raise the possibility that the addition of hs-CRP to standard lipid screening will generate an improved method for identifying persons at high risk for future cardiovascular events, who would thus be candidates for primary-prevention interventions such as the use of HMG-CoA reductase inhibitors.

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Review Article

*Mechanisms of Disease*

FRANKLIN H. EPSTEIN, M.D., *Editor*

# ATHEROSCLEROSIS — AN INFLAMMATORY DISEASE

RUSSELL ROSS, PH.D.

ATHEROSCLEROSIS is an inflammatory disease. Because high plasma concentrations of cholesterol, in particular those of low-density lipoprotein (LDL) cholesterol, are one of the principal risk factors for atherosclerosis,[1] the process of atherogenesis has been considered by many to consist largely of the accumulation of lipids within the artery wall; however, it is much more than that. Despite changes in lifestyle and the use of new pharmacologic approaches to lower plasma cholesterol concentrations,[2,3] cardiovascular disease continues to be the principal cause of death in the United States, Europe, and much of Asia.[4,5] In fact, the lesions of atherosclerosis represent a series of highly specific cellular and molecular responses that can best be described, in aggregate, as an inflammatory disease.[6-10]

The lesions of atherosclerosis occur principally in large and medium-sized elastic and muscular arteries and can lead to ischemia of the heart, brain, or extremities, resulting in infarction. They may be present throughout a person's lifetime. In fact, the earliest type of lesion, the so-called fatty streak, which is common in infants and young children,[11] is a pure inflammatory lesion, consisting only of monocyte-derived macrophages and T lymphocytes.[12] In persons with hypercholesterolemia, the influx of these cells is preceded by the extracellular deposition of amorphous and membranous lipids.[11,13] By asking questions about arterial inflammation, we may be able to gain insight into the process of atherogenesis.

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## FACTORS THAT INDUCE AND PROMOTE INFLAMMATION OR ATHEROGENESIS

Numerous pathophysiologic observations in humans and animals led to the formulation of the response-to-injury hypothesis of atherosclerosis, which initially proposed that endothelial denudation was the first step in atherosclerosis.[6] The most recent version of this hypothesis emphasizes endothelial dysfunction rather than denudation. Whichever process is at work, each characteristic lesion of atherosclerosis represents a different stage in a chronic inflammatory process in the artery; if unabated and excessive, this process will result in an advanced, complicated lesion. Possible causes of endothelial dysfunction leading to atherosclerosis include elevated and modified LDL; free radicals caused by cigarette smoking, hypertension, and diabetes mellitus; genetic alterations; elevated plasma homocysteine concentrations; infectious microorganisms such as herpesviruses or *Chlamydia pneumoniae*; and combinations of these or other factors. Regardless of the cause of endothelial dysfunction, atherosclerosis is a highly characteristic response of particular arteries.[6-9,14]

The endothelial dysfunction that results from the injury leads to compensatory responses that alter the normal homeostatic properties of the endothelium. Thus, the different forms of injury increase the adhesiveness of the endothelium with respect to leukocytes or platelets, as well as its permeability. The injury also induces the endothelium to have procoagulant instead of anticoagulant properties and to form vasoactive molecules, cytokines, and growth factors. If the inflammatory response does not effectively neutralize or remove the offending agents, it can continue indefinitely. In doing so, the inflammatory response stimulates migration and proliferation of smooth-muscle cells that become intermixed with the area of inflammation to form an intermediate lesion. If these responses continue unabated, they can thicken the artery wall, which compensates by gradual dilation, so that up to a point, the lumen remains unaltered,[15] a phenomenon termed "remodeling." As for the inflammatory cells, granulocytes are rarely present during any phase of atherogenesis.[16] Instead, the response is mediated by monocyte-derived macrophages and specific subtypes of T lymphocytes at every stage of the disease.[17,18]

Continued inflammation results in increased numbers of macrophages and lymphocytes, which both emigrate from the blood and multiply within the lesion. Activation of these cells leads to the release of hydrolytic enzymes, cytokines, chemokines, and

growth factors,[19,20] which can induce further damage and eventually lead to focal necrosis.[21] Thus, cycles of accumulation of mononuclear cells, migration and proliferation of smooth-muscle cells, and formation of fibrous tissue lead to further enlargement and restructuring of the lesion, so that it becomes covered by a fibrous cap that overlies a core of lipid and necrotic tissue — a so-called advanced, complicated lesion. At some point, the artery can no longer compensate by dilation; the lesion may then intrude into the lumen and alter the flow of blood.

#### Hypercholesterolemia and Modified Lipids and Lipoproteins

LDL, which may be modified by oxidation, glycation (in diabetes), aggregation, association with proteoglycans, or incorporation into immune complexes,[22-25] is a major cause of injury to the endothelium and underlying smooth muscle.[25-27] When LDL particles become trapped in an artery, they can undergo progressive oxidation and be internalized by macrophages by means of the scavenger receptors on the surfaces of these cells.[22,24-28] The internalization leads to the formation of lipid peroxides and facilitates the accumulation of cholesterol esters, resulting in the formation of foam cells. The degree to which LDL is modified can vary greatly.[25,27,29] Once modified and taken up by macrophages, LDL activates the foam cells. Removal and sequestration of modified LDL are important parts of the initial, protective role of the macrophage in the inflammatory response[28-30] and minimize the effects of modified LDL on endothelial and smooth-muscle cells. Antioxidants such as vitamin E can also reduce free-radical formation by modified LDL.[31] In addition to its ability to injure these cells,[25,27] modified LDL is chemotactic for other monocytes and can up-regulate the expression of genes for macrophage colony-stimulating factor[32,33] and monocyte chemotactic protein[34] derived from endothelial cells. Thus, it may help expand the inflammatory response by stimulating the replication of monocyte-derived macrophages and the entry of new monocytes into lesions.

The inflammatory response itself can have a profound effect on lipoprotein movement within the artery. Specifically, mediators of inflammation such as tumor necrosis factor $\alpha$, interleukin-1, and macrophage colony-stimulating factor increase binding of LDL to endothelium and smooth muscle and increase the transcription of the LDL-receptor gene.[35,36] After binding to scavenger receptors in vitro, modified LDL initiates a series of intracellular events[36] that include the induction of urokinase[30] and inflammatory cytokines such as interleukin-1.[37-39] Thus, a vicious circle of inflammation, modification of lipoproteins, and further inflammation can be maintained in the artery by the presence of these lipids.

Oxidized LDL is present in lesions of atherosclerosis in humans.[40] In animals with hypercholesterolemia, antioxidants can reduce the size of lesions,[25,41-44] and they reduce fatty streaks in nonhuman primates.[44] The latter observation suggests that the antioxidants have an antiinflammatory effect, perhaps by preventing the up-regulation of adhesion molecules for monocytes.[45] Antioxidants increase the resistance of human LDL to oxidation ex vivo[46] in proportion to the vitamin E content of the plasma. Vitamin E intake is inversely correlated with the incidence of myocardial infarction, and vitamin E supplementation reduced coronary events in a preliminary clinical trial.[47-49] In contrast, other antioxidants, such as beta carotene, have no benefit.[46,50,51]

#### Homocysteine

High plasma homocysteine concentrations were initially thought to be associated with advanced atherosclerosis on the basis of autopsy findings in patients with homozygous defects in enzymes necessary for homocysteine metabolism, such as cystathionine beta-synthase or methylenetetrahydrofolate reductase.[52-56] In patients with such defects, severe atherosclerosis develops in childhood, and many have their first myocardial infarction by the age of 20 years.[55,56] Homocysteine is toxic to endothelium[57] and is prothrombotic,[58] and it increases collagen production[59] and decreases the availability of nitric oxide.[60]

Plasma homocysteine concentrations are slightly elevated in many patients who have no enzymatic defects in homocysteine metabolism.[61] These patients have an increased risk of symptomatic atherosclerosis of the coronary, peripheral, and cerebral arteries.[61] Treatment with folic acid can return their plasma homocysteine concentrations to normal. Trials are under way to determine whether folic acid will prevent the progression or possibly even induce the regression of atherosclerotic lesions.[62]

#### Hypertension

Concentrations of angiotensin II, the principal product of the renin–angiotensin system, are often elevated in patients with hypertension; angiotensin II is a potent vasoconstrictor. In addition to causing hypertension, it can contribute to atherogenesis by stimulating the growth of smooth muscle.[63] Angiotensin II binds to specific receptors on smooth muscle, resulting in the activation of phospholipase C, which can lead to increases in intracellular calcium concentrations and in smooth-muscle contraction,[63] increased protein synthesis, and smooth-muscle hypertrophy.[64] It also increases smooth-muscle lipoxygenase activity, which can increase inflammation and the oxidation of LDL. Hypertension also has proinflammatory actions, increasing the formation of hydrogen peroxide and free radicals such as superoxide anion and hydroxyl radicals in plasma.[27,65,66] These substances reduce the formation of nitric oxide by the

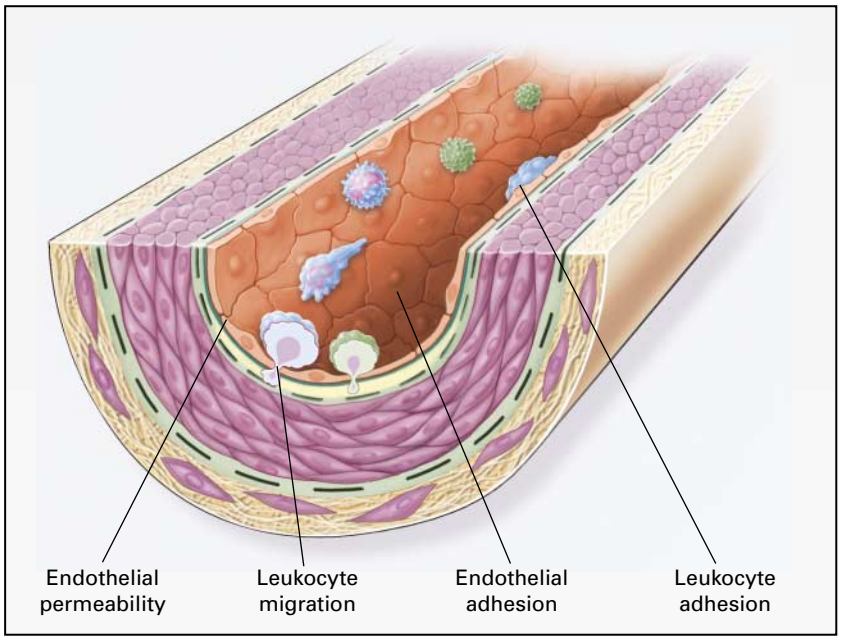


**Figure 1.** Endothelial Dysfunction in Atherosclerosis.

The earliest changes that precede the formation of lesions of atherosclerosis take place in the endothelium. These changes include increased endothelial permeability to lipoproteins and other plasma constituents, which is mediated by nitric oxide, prostacyclin, platelet-derived growth factor, angiotensin II, and endothelin; up-regulation of leukocyte adhesion molecules, including L-selectin, integrins, and platelet–endothelial-cell adhesion molecule 1, and the up-regulation of endothelial adhesion molecules, which include E-selectin, P-selectin, intercellular adhesion molecule 1, and vascular-cell adhesion molecule 1; and migration of leukocytes into the artery wall, which is mediated by oxidized low-density lipoprotein, monocyte chemotactic protein 1, interleukin-8, platelet-derived growth factor, macrophage colony-stimulating factor, and osteopontin.

endothelium,[67] increase leukocyte adhesion,[66] and increase peripheral resistance. Thus, free-radical formation mediates some of the effects of both hypertension and hypercholesterolemia.

### Infection

Several reports have shown a correlation between the incidence of atherosclerosis and the presence of at least two types of infectious microorganisms, herpesviruses and *C. pneumoniae*.[68-70] Both organisms have been identified in atheromatous lesions in coronary arteries and in other organs obtained at autopsy.[69,70] Increased titers of antibodies[71] to these organisms have been used as a predictor of further adverse events in patients who have had a myocardial infarction.[72,73] Nonetheless, there is no direct evidence that these organisms can cause the lesions of atherosclerosis.[68,74,75] Although these organisms are ubiquitous in many tissues and organs, the fact that lesions cannot be induced experimentally in animals by injection of the organisms leaves their role as etiologic agents in question. It is nevertheless possible that infection, combined with other factors, may be responsible for the genesis of the lesions of atherosclerosis in some patients.[68,76]

## THE NATURE OF THE INFLAMMATORY RESPONSE

### Interactions among Endothelial Cells, Monocytes, and T Cells

Specific arterial sites, such as branches, bifurcations, and curvatures, cause characteristic alterations in the flow of blood, including decreased shear stress and increased turbulence.[77] At these sites, specific molecules form on the endothelium that are responsible for the adherence, migration, and accumulation of monocytes and T cells. Such adhesion molecules, which act as receptors for glycoconjugates and integrins present on monocytes and T cells, include several selectins, intercellular adhesion molecules, and vascular-cell adhesion molecules.[78] Molecules associated with the migration of leukocytes across the endothelium, such as platelet–endothelial-cell adhesion molecules,[79] act in conjunction with chemoattractant molecules generated by the endothelium, smooth muscle, and monocytes — such as monocyte chemotactic protein 1, osteopontin,[80] and modified LDL — to attract monocytes and T cells into the artery (Fig. 1).[33]

The nature of the flow — that is, whether shear stress or turbulence is high or low — appears to be

important in determining whether lesions occur at these vascular sites. Changes in flow alter the expression of genes that have elements in their promoter regions that respond to shear stress. For example, the genes for intercellular adhesion molecule 1,[81] platelet-derived growth factor B chain,[82] and tissue factor[83] in endothelial cells have these elements, and their expression is increased by reduced shear stress.[84] Thus, alterations in blood flow appear to be critical in determining which arterial sites are prone to have lesions.[77,85,86] Rolling and adherence of monocytes and T cells occur at these sites as a result of the up-regulation of adhesion molecules on both the endothelium and the leukocytes.

Chemokines may be responsible for the chemotaxis and accumulation of macrophages in fatty streaks (Fig. 2).[87,88] Activation of monocytes and T cells leads to up-regulation of receptors on their surfaces, such as the mucin-like molecules that bind selectins, integrins that bind adhesion molecules of the immunoglobulin superfamily, and receptors that bind chemoattractant molecules.[78] These ligand–receptor interactions further activate mononuclear cells, induce cell proliferation, and help define and localize the inflammatory response at the sites of lesions (Fig. 1).

In genetically modified mice that are deficient in apolipoprotein E (and have hypercholesterolemia), intercellular adhesion molecule 1 is constitutively increased at lesion-prone sites.[86] In fact, it is present on the surface of the endothelium at these sites in normal mice and is increased in mice with apolipoprotein E deficiency. In contrast, vascular-cell adhesion molecule 1 is absent in normal mice but is present at the same sites as intercellular adhesion molecule 1 in mice with apolipoprotein E deficiency.[86] Thus, adherence of monocytes and T cells may occur after an increase in one or more of the adhesion molecules, which may act in concert with chemotactic molecules such as monocyte chemotactic protein 1, interleukin-8, or modified LDL. Would interference with only one of the several adhesion molecules be sufficient to decrease inflammation and thus slow or counteract the process of atherogenesis? In mice that are completely deficient in intercellular adhesion molecule 1, P-selectin, CD18, or combinations of these molecules, lipid

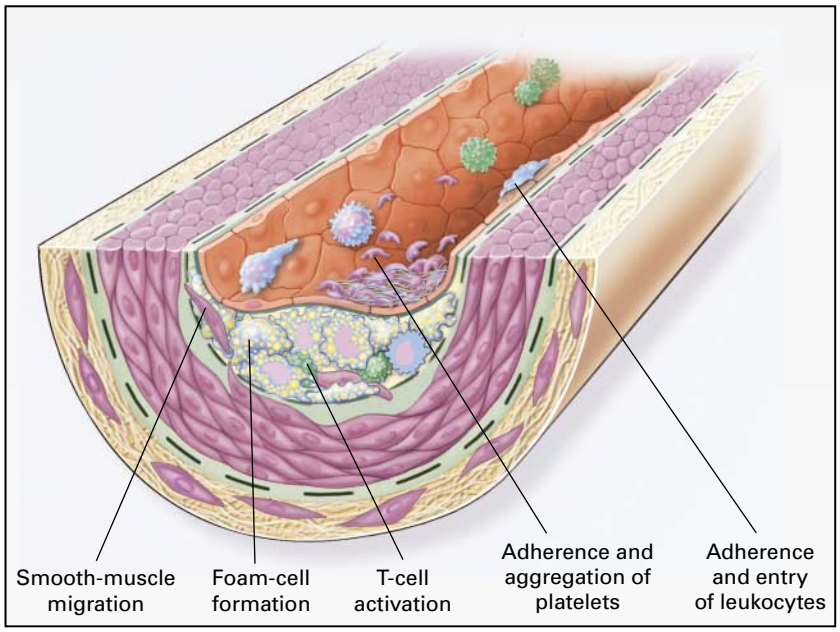


**Figure 2.** Fatty-Streak Formation in Atherosclerosis.

Fatty streaks initially consist of lipid-laden monocytes and macrophages (foam cells) together with T lymphocytes. Later they are joined by various numbers of smooth-muscle cells. The steps involved in this process include smooth-muscle migration, which is stimulated by platelet-derived growth factor, fibroblast growth factor 2, and transforming growth factor $\beta$; T-cell activation, which is mediated by tumor necrosis factor $\alpha$, interleukin-2, and granulocyte–macrophage colony-stimulating factor; foam-cell formation, which is mediated by oxidized low-density lipoprotein, macrophage colony-stimulating factor, tumor necrosis factor $\alpha$, and interleukin-1; and platelet adherence and aggregation, which are stimulated by integrins, P-selectin, fibrin, thromboxane $A_2$, tissue factor, and the factors described in Figure 1 as responsible for the adherence and migration of leukocytes.

feeding leads to smaller lesions of atherosclerosis.[89] Comparison of the relative roles of these molecules in inflammation in the arteries and the microvasculature may provide clues to the relative feasibility of modifying the inflammatory process at these sites, and thus of modifying atherosclerosis.

A recently discovered class of molecules, the disintegrins, sometimes called metalloproteinase-like, disintegrin-like, cysteine-rich proteins (MDCs), has been identified in endothelium, smooth muscle, and macrophages.[90] These transmembrane proteins, which appear to be involved in cell–cell interactions,[90] contain a metalloproteinase sequence in their extracellular segment that permits them to activate molecules such as tumor necrosis factor $\alpha$.[91,92] They are not found in normal arteries, but one of them, MDC15, is present in lesions of atherosclerosis.[90] Adhesion molecules such as L-selectin can be cleaved from the surface of leukocytes by a metalloproteinase (L-selectin sheddase), which suggests that in situations of chronic inflammation it may be possible to measure the "shed" molecules, such as the different adhesion molecules, in plasma, as markers of a sustained inflammatory response.[93,94] Disintegrins may participate in these shedding processes. If shedding occurs, it may be detectable in different types of inflammatory responses. Increased plasma concentrations of shed molecules might then be used to identify patients at risk for atherosclerosis or other inflammatory diseases.

#### Monocytes and Immunity

The ubiquitous monocyte, the precursor of macrophages in all tissues, is present in every phase of atherogenesis. Monocyte-derived macrophages are scavenging and antigen-presenting cells, and they secrete cytokines, chemokines, growth-regulating molecules, and metalloproteinases and other hydrolytic enzymes. The continuing entry, survival, and replication of mononuclear cells in lesions depend in part on factors such as macrophage colony-stimulating factor and granulocyte–macrophage colony-stimulating factor for monocytes and interleukin-2 for lymphocytes. Continued exposure to macrophage colony-stimulating factor permits macrophages to survive in vitro and possibly to multiply within the lesions. In con-

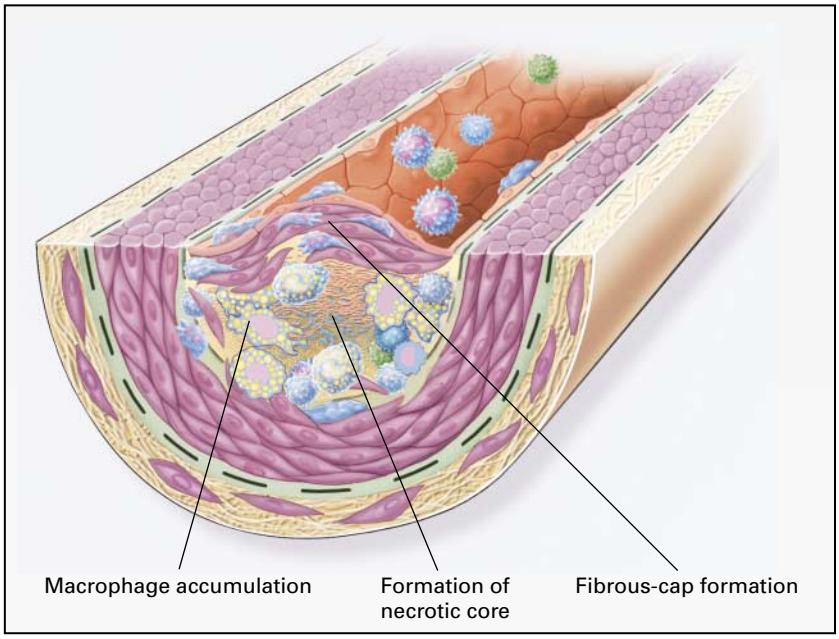


**Figure 3.** Formation of an Advanced, Complicated Lesion of Atherosclerosis.

As fatty streaks progress to intermediate and advanced lesions, they tend to form a fibrous cap that walls off the lesion from the lumen. This represents a type of healing or fibrous response to the injury. The fibrous cap covers a mixture of leukocytes, lipid, and debris, which may form a necrotic core. These lesions expand at their shoulders by means of continued leukocyte adhesion and entry caused by the same factors as those listed in Figures 1 and 2. The principal factors associated with macrophage accumulation include macrophage colony-stimulating factor, monocyte chemotactic protein 1, and oxidized low-density lipoprotein. The necrotic core represents the results of apoptosis and necrosis, increased proteolytic activity, and lipid accumulation. The fibrous cap forms as a result of increased activity of platelet-derived growth factor, transforming growth factor $\beta$, interleukin-1, tumor necrosis factor $\alpha$, and osteopontin and of decreased connective-tissue degradation.

trast, inflammatory cytokines such as interferon-$\gamma$ activate macrophages and under certain circumstances induce them to undergo programmed cell death (apoptosis). If this occurs in vivo, macrophages may become involved in the necrotic cores characteristic of advanced, complicated lesions (Fig. 3).

Initially, the only cells thought to proliferate during expansion of atherosclerotic lesions were smooth-muscle cells. However, replication of monocyte-derived macrophages and T cells is probably of equal importance.[95] The ability of macrophages to produce cytokines (such as tumor necrosis factor $\alpha$, interleukin-1, and transforming growth factor $\beta$), proteolytic enzymes (particularly metalloproteinases), and growth factors (such as platelet-derived growth factor and insulin-like growth factor I) may be critical in the role of these cells in the damage and repair that ensue as the lesions progress (Fig. 2).

Activated macrophages express class II histocompatibility antigens such as HLA-DR that allow them to present antigens to T lymphocytes.[20] Thus, it is not surprising that cell-mediated immune responses may be involved in atherogenesis, since both CD4 and CD8 T cells are present in the lesions at all stages of the process.[96,97] T cells are activated when they bind antigen processed and presented by macrophages. T-cell activation results in the secretion of cytokines, including interferon-$\gamma$ and tumor necrosis factor $\alpha$ and $\beta$, that amplify the inflammatory response.[97] Smooth-muscle cells from the lesions also have class II HLA molecules on their surfaces, presumably induced by interferon-$\gamma$, and can also present antigens to T cells.[97] One possible antigen may be oxidized LDL,[98] which can be produced by macrophages.[99] Heat-shock protein 60 may also contribute to autoimmunity. This and other heat-shock proteins perform several functions, including the assembly, intracellular transport, and breakdown of proteins and the prevention of protein denaturation. These proteins may be elevated on endothelial cells and participate in immune responses.[100]

An immunoregulatory molecule, CD40 ligand,[101] can be expressed by macrophages, T cells, endothelium, and smooth muscle in atherosclerotic lesions in vivo, and its receptor, CD40, is expressed on the same cells. Both are up-regulated in lesions of atherosclerosis, providing further evidence of immune activation in the lesions.[102,103] Furthermore, CD40 ligand induces the release of interleukin-1$\beta$ by vascular cells, potentially enhancing the inflammatory response.[104] Inhibition of CD40 with blocking antibodies reduces lesion formation in apolipoprotein E–deficient mice.[105]

#### Platelets

Platelet adhesion and mural thrombosis are ubiquitous in the initiation and generation of the lesions of atherosclerosis in animals and humans (Fig. 2).[9] Platelets can adhere to dysfunctional endothelium, exposed collagen, and macrophages. When activated, platelets release their granules, which contain cytokines and growth factors that, together with thrombin, may contribute to the migration and proliferation of smooth-muscle cells and monocytes.[106] Activation of platelets leads to the formation of free arachidonic acid, which can be transformed into prostaglandins such as thromboxane $A_2$, one of the most potent vasoconstricting and platelet-aggregating substances known, or into leukotrienes, which can amplify the inflammatory response.

Plaque rupture and thrombosis are notable complications of advanced lesions that lead to unstable coronary syndromes or myocardial infarction (Fig. 4).[9,21,107] Platelets are important in maintaining vascular integrity in the absence of injury and protecting against spontaneous hemorrhage. Activated platelets can accumulate on the walls of arteries and recruit additional platelets into an expanding thrombus. An important component of the platelets is the glycoprotein IIb/IIIa receptor, which belongs to the integrin superfamily of adhesion-molecule receptors and appears on the surface of platelets during platelet activation and thrombus formation. These receptors serve an important hemostatic function, and antagonists to them prevent thrombus formation in patients who have had a myocardial infarction.[108]

## ATHEROSCLEROSIS IN RELATION TO OTHER CHRONIC INFLAMMATORY DISEASES

The cellular interactions in atherogenesis are fundamentally no different from those in chronic inflammatory–fibroproliferative diseases such as cirrhosis, rheumatoid arthritis, glomerulosclerosis, pulmonary fibrosis, and chronic pancreatitis (Table 1). In the examples in Table 1, the response of each particular tissue or organ depends on its characteristic cells and architecture, its blood and lymph supply, and the nature of the offending agents. Thus, the cellular responses in the arteries (atherosclerosis), liver (cirrhosis), joints (rheumatoid arthritis), kidneys (glomerulosclerosis), lungs (pulmonary fibrosis), and pancreas (pancreatitis) are similar yet are characteristic of each tissue or organ.

#### Inflammatory Response

Does the inflammatory response in arteries differ from that in other tissues? Granulocytes are rare in atherosclerosis, and among the other disorders in Table 1, they are present only in rheumatoid arthritis and pulmonary fibrosis. In the case of arthritis, although the early response begins with granulocytes, they are found primarily within the joint cavity. Macrophages and lymphocytes predominate in the synovium, leading to erosion of cartilage and bone, which is replaced by fibrous tissue (pannus). In pulmonary fi-

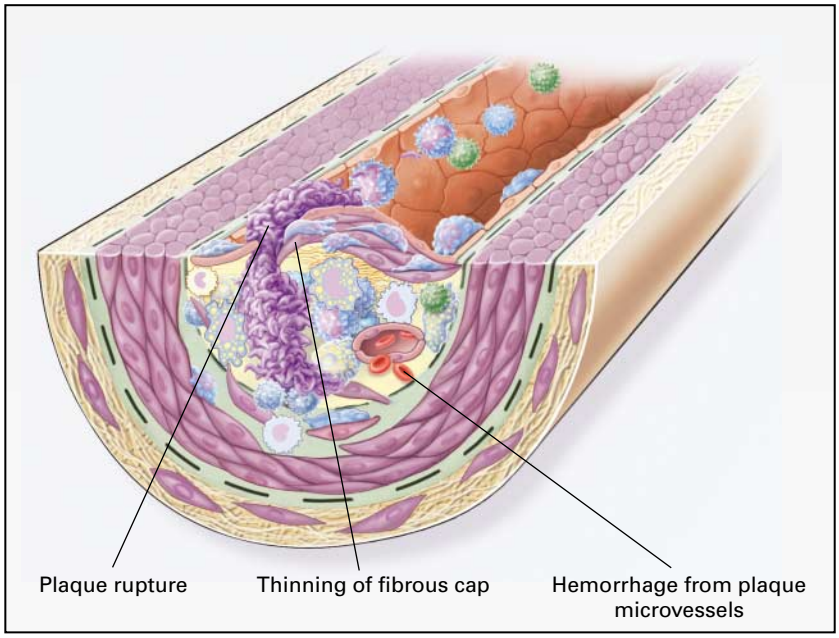


**Figure 4.** Unstable Fibrous Plaques in Atherosclerosis.

Rupture of the fibrous cap or ulceration of the fibrous plaque can rapidly lead to thrombosis and usually occurs at sites of thinning of the fibrous cap that covers the advanced lesion. Thinning of the fibrous cap is apparently due to the continuing influx and activation of macrophages, which release metalloproteinases and other proteolytic enzymes at these sites. These enzymes cause degradation of the matrix, which can lead to hemorrhage from the vasa vasorum or from the lumen of the artery and can result in thrombus formation and occlusion of the artery.

brosis, granulocytes initially appear in the alveolar spaces; however, the lung parenchyma, where fibrosis ultimately occurs, is infiltrated by macrophages and lymphocytes. Thus, there are parallels between atherosclerosis and these other inflammatory diseases.

Are there particular aspects of the chronic inflammatory response in atherosclerosis that can be used to advantage? At least three different types of macrophages, each regulated by different T-cell cytokines (interferon-γ, interleukin-2, interleukin-4, and interleukin-10) have been identified.[122] These differences raise the question whether there are subgroups of monocytes that "home" to a specific tissue or organ. Are there differences in arterial endothelium and microvascular endothelium such that different types of monocytes are attracted to each, and could one take advantage of such differences?[123] One might try to use such differences to modify the inflammatory response so as to emphasize its protective rather than its destructive characteristics.

If the injurious agent or agents are not removed or nullified by the inflammatory response and the inflammation progresses, the response changes from a protective to an injurious response. Such constant or repetitive injury can stimulate each tissue to repair or wall off the damage by means of a fibroproliferative response, which, when excessive, diminishes the functional capacity of the tissue or organ and becomes part of the disease process (Table 1).

#### Instability and Rupture of Plaque

Chronic inflammatory responses are often associated with specific types of injurious or granuloma-inducing agents. In most patients myocardial infarctions occur as a result of erosion or uneven thinning and rupture of the fibrous cap, often at the shoulders of the lesion where macrophages enter, accumulate, and are activated and where apoptosis may occur.[124,125] Degradation of the fibrous cap may result from elaboration of metalloproteinases such as collagenases, elastases, and stromelysins (Fig. 4).[126] Activated T cells may stimulate metalloproteinase production by macrophages in the lesions, which promotes plaque instability and further implicates an immune response.[103] These changes may also be accompanied by the production of tissue-factor procoagulant and other hemostatic factors,[102,127] further increasing the possibility of thrombosis.

Stable advanced lesions usually have uniformly dense fibrous caps. The potentially dangerous lesions are often nonocclusive and thus difficult to diagnose by angiography, yet at autopsy active inflammation is evident in the accumulation of macrophages at sites of plaque rupture.[107] Macrophage accumulation may

**TABLE 1.** CHARACTERISTICS OF ATHEROSCLEROSIS AND OTHER CHRONIC INFLAMMATORY DISEASES.*

| DISEASE | MONOCYTES AND MACROPHAGES | LYMPHOCYTES | GRANULOCYTES | CONNECTIVE-TISSUE CELLS | EXTRACELLULAR MATRIX | PATHOGENETIC MECHANISMS | STUDIES |
|---|---|---|---|---|---|---|---|
| Atherosclerosis | + | + | − | Smooth-muscle cells | Collagen types I, III, and IV, elastin, fibronectin, proteoglycan | Endothelial-cell injury and dysfunction; fibrous cap; new matrix formation and degradation; necrotic core | Ross,[9] Libby and Hansson,[109] Ross and Fuster[110] |
| Cirrhosis | + | + | − | Fibroblasts, Ito cells | Collagen types I and III | Parenchymal-cell injury; new matrix and scarring replacing necrotic parenchyma | Maher,[111] Anthony et al.[112] |
| Rheumatoid arthritis | + | + | +/− | Synovial fibroblasts | Collagen types I and III, fibronectin, proteoglycan | Synovial-cell injury; erosion of cartilage; new matrix scarring (pannus) | Sewell and Trentham,[113] Harris [114] |
| Glomerulosclerosis | + | + | − | Mesangial cells | Collagen types I and IV, fibronectin | Epithelial- and endothelial-cell injury and dysfunction; decrease in glomerular filtration; new matrix formation | Johnson,[115] Magil and Cohen[116] |
| Pulmonary fibrosis | + | + | +/− | Smooth-muscle cells, fibroblasts | Collagen types III and IV, fibronectin | Inflammatory exudate in alveoli and bronchi, organized by extensive matrix deposition and scarring | Kuhn et al.,[117] Lukacs and Ward,[118] Brody et al.[119] |
| Chronic pancreatitis | + | + | − | Fibroblasts | Collagen, fibronectin, proteoglycan | Epithelial (ductal) injury; periductal inflammation; interstitial fat necrosis; new matrix formation | Sarles et al.,[120] DiMagno et al.[121] |

*Plus signs denote the presence of a cell type, and minus signs its absence.

be associated with increased plasma concentrations of both fibrinogen and C-reactive protein,[128-130] two markers of inflammation thought to be early signs of atherosclerosis.[128,131,132] Plaque rupture and thrombosis may be responsible for as many as 50 percent of cases of acute coronary syndromes and myocardial infarction.[21]

## NEW PERSPECTIVES ON THE FORMATION AND PROGRESSION OF LESIONS

### Smooth Muscle

To understand the factors that are important in the proliferative and migratory responses that lead to differences in the organization and enlargement of the lesions in different parts of the arterial tree, it may be helpful to understand the embryonic derivation of the smooth-muscle cells that make up the arteries in different regions. Smooth-muscle cells have different embryonic origins, depending on the segment of the arterial system involved. In some vertebrates, smooth-muscle cells in the upper portion of the thoracic aorta are derived from a neuroectodermal source, whereas those in the abdominal aorta are derived from a mesenchymal source.[133] Although likely, this has not been confirmed in humans. The smooth-muscle cells of coronary arteries appear to originate from a third precursor population in the intracardiac mesenchyme. The existence of these different lineages suggests that smooth muscle in different parts of the arterial tree may respond differently to the stimuli that generate atherosclerotic lesions at each of these sites. To complicate matters further, smooth-muscle cells within the media of large arteries may be heterogeneous, with different proliferative and matrix-producing capabilities.[134]

These differences in the origin of smooth-muscle cells raise questions about whether these cells, on the basis of their lineage, respond differently to different cytokines, mitogens, chemotactic factors, or extracellular matrixes.[135-137] Is there selection of a particular lineage based on the cells' responses to these different substances? Does cell lineage help to explain why lesions in peripheral arteries differ from those in the carotid and coronary arteries?

### The Role of the Matrix

Smooth-muscle cells in the media of arteries, as well as in lesions, are surrounded by different types of connective tissue. In the media of arteries, the matrix consists largely of type I and III fibrillar collagen, whereas in the lesions of atherosclerosis it consists largely of proteoglycan, intermixed with loosely scattered collagen fibrils.

When cultured human arterial smooth-muscle cells are plated on collagen in fibrillar form, the collagen inhibits cell proliferation by up-regulating specific inhibitors of the cell cycle.[137] In vivo degradation of the collagen by collagenase, or migration away from this inhibitory environment, may allow the smooth-muscle cells to respond to mitogenic stimuli and rep-

licate, as they do when they are cultured on nonfibrillar, monomeric collagen. Other matrix molecules, such as fibronectin and heparan sulfate, may be involved, because they can also inhibit the cell cycle, and cell–matrix interactions can lead to the expression of chemokines by macrophages.[138-140] If these interactions were to occur in arteries, they could profoundly influence the inflammatory and fibroproliferative response.[141] Thus, the matrix that surrounds the cells is not neutral and may determine whether they remain quiescent or multiply in response to growth factors.

## CONCLUSIONS

Cells may express different constellations of genes and therefore vary phenotypically, depending on their environment. New techniques have been developed to identify DNA that should yield a vast amount of information about which genes are expressed and in what patterns, information that should help decipher the complex nature of atherogenesis.[142-144] Because atherosclerosis is a multigenic disease, understanding patterns of gene expression may help to explain differences in susceptibility to agents that cause disease. Furthermore, the patterns of gene expression may vary in lesions from different persons and at different sites and may provide clues regarding genetic differences in susceptibility as well as response to therapy.

Advances in molecular genetics have made it possible to remove or insert genes and to determine the roles of their products in disease.[145] Numerous animal models that are useful in studying the genetics of atherogenesis have been produced, such as apolipoprotein E–deficient mice.[146,147] In the absence of apolipoprotein E, lipoprotein remnants are not carried to the liver, where they are normally metabolized, and the mice become hypercholesterolemic and lesions of atherosclerosis develop that are similar to those in humans. To explore the role of monocytes and platelets and of platelet-derived growth factor in atherogenesis, studies are under way in which apolipoprotein E–deficient mice have been made chimeric for a deficiency of platelet-derived growth factor in circulating monocytes and platelets.

Studies in transgenic mice have revealed that Lp(a) lipoprotein, cholesterol ester transfer protein, apolipoprotein A (the principal apoprotein of high-density lipoprotein), and other molecules have little effect on atherogenesis, whereas macrophage colony-stimulating factor appears to be important in the regulation of the numbers of monocytes and macrophages and in lesion formation.[148,149]

Thus, although hypercholesterolemia is important in approximately 50 percent of patients with cardiovascular disease,[5] other factors need to be taken into consideration. Atherosclerosis is clearly an inflammatory disease and does not result simply from the accumulation of lipids. If we can selectively modify the harmful components of inflammation in the arteries and leave the protective aspects intact, we may create new avenues for the diagnosis and management of disease in the 50 percent of patients with cardiovascular disease who do not have hypercholesterolemia.

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# Clinical Application of C-Reactive Protein for Cardiovascular Disease Detection and Prevention

Paul M Ridker, MD

In an attempt to improve global cardiovascular risk prediction, considerable interest has focused on C-reactive protein (CRP), a marker of inflammation that has been shown in multiple prospective epidemiological studies to predict incident myocardial infarction, stroke, peripheral arterial disease, and sudden cardiac death. CRP levels have also been shown to predict risk of both recurrent ischemia and death among those with stable and unstable angina, those undergoing percutaneous angioplasty, and those presenting to emergency rooms with acute coronary syndromes. These highly consistent clinical data are supported by abundant laboratory and experimental evidence that demonstrate that atherothrombosis, in addition to being a disease of lipid accumulation, also represents a chronic inflammatory process. In terms of clinical application, CRP seems to be a stronger predictor of cardiovascular events than LDL cholesterol, and it adds prognostic information at all levels of calculated Framingham Risk and at all levels of the metabolic syndrome. Using widely available high-sensitivity assays, CRP levels of $<1$, 1 to 3, and $>3$ mg/L correspond to low-, moderate-, and high-risk groups for future cardiovascular events. Individuals with LDL cholesterol below 130 mg/dL who have CRP levels $>3$ mg/L represent a high-risk group often missed in clinical practice. The addition of CRP to standard cholesterol evaluation may thus provide a simple and inexpensive method to improve global risk prediction and compliance with preventive approaches.

## Evidence Supporting CRP Use in Primary Prevention

Composed of five 23 kDa subunits, C-reactive protein (CRP) is an hepatically derived pentraxin that plays a key role in the innate immune response. CRP has a long plasma half-life and is now understood to be a mediator as well as a marker of atherothrombotic disease. To date, over a dozen prospective epidemiological studies carried out among individuals with no prior history of cardiovascular disease demonstrate that a single, non-fasting measure of CRP is a strong predictor of future vascular events[1–14] (Figure 1). The relationship between a patient's baseline level of CRP and future vascular risk has been consistent in studies from the United States and Europe, and in most cases has proven independent of age, smoking, cholesterol levels, blood pressure, and diabetes, the major "traditional" risk factors evaluated in daily practice. These effects are present among women as well as men, among the elderly as well as those in middle age, among smokers and non-smokers, and among those with and without diabetes. CRP levels have long-term predictive value. In one recent study, CRP was a strong predictor of risk even 20 years after initial blood samples were obtained.[15]

Very recently, event-free survival data have become available that allow clinicians to interpret CRP levels either in terms of population-based quintiles (Figure 2, left) or in terms of simple clinical cut-points (Figure 2, right).[6] Although the

The opinions expressed in this article are not necessarily those of the editors or of the American Heart Association.

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Dr Ridker is listed as a co-inventor on patents held by the Brigham and Women's Hospital that relate to the use of inflammatory bio-markers in cardiovascular disease.

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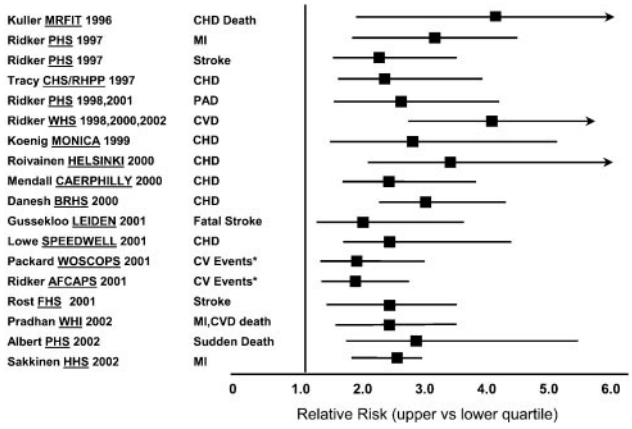


**Figure 1.** Prospective studies relating baseline CRP levels to the risk of first cardiovascular events. CHD indicates coronary heart disease; MI, myocardial infarction; PAD, pulmonary artery disease; CV, cardiovascular; MRFIT, Multiple Risk Factor Intervention Trial; PHS, Physicians' Health Study; CHS, Cardiovascular Health Study; RHPP, Rural Health Promotion Project; WHS, Women's Health Study; MONICA, MONItoring trends and determinants In CArdiovascular disease; HELSINKI, Helsinki Heart Study; CAERPHILLY, Caerphilly Heart Study; BRHS, British Regional Heart Study; LEIDEN, Leiden Heart Study; SPEEDWELL, Speedwell Heart Study; WOSCOPS, West of Scotland Coronary Prevention Study; AFCAPS, Air Force Coronary Atherosclerosis Prevention Study; FHS, Framingham Heart Study; WHI, Women's Health Initiative; and HHS, Honolulu Heart Study.

former approach demonstrates the robust linear relationship between inflammation and vascular disease, the latter approach (in which levels of <1, 1 to 3, and >3 mg/L represent low-, moderate-, and high-risk groups) is likely to have greater clinical appeal.

Prospective data also demonstrate that CRP is a stronger predictor of risk than is low-density lipoprotein (LDL) cholesterol. In the largest study to date, both the area under the receiver operator characteristic (ROC) curve (0.64 versus 0.60) and the population attributable risk percent (40 versus 19) were significantly greater for CRP than for LDL cholesterol.[6]

CRP levels minimally correlate with lipid levels and there is virtually no way to predict CRP levels on the basis of either total cholesterol, high-density lipoprotein cholesterol, or LDL cholesterol. In evaluations including over 25 000 patients, the variance in CRP that can be ascribed to LDL cholesterol has consistently been less than 3% to 5%.[4,6,16] Thus, CRP levels do not supplant lipid evaluation, but must be considered as an adjunct to lipid evaluation. The additive value of CRP to lipid screening in terms of coronary risk prediction has been demonstrated in several settings.[1,3,4,6,17] A simplified clinical approach to this issue based on the Adult Treatment Panel III (ATP III) cut-points for LDL of <130, 130 to 160, and >160 mg/dL and on CRP levels of <1, 1 to 3, and >3 mg/L is shown in Figure 3, as is evidence that CRP adds prognostic information at all levels of the Framingham Risk Score.

## CRP, the Metabolic Syndrome, and Type 2 Diabetes

A unique feature of CRP that further distinguishes it from LDL cholesterol is the fact that inflammation (but not elevated LDL) plays a major role in almost all processes associated with the metabolic syndrome, another group highlighted as being at increased risk according to current ATP III guidelines. That CRP reflects the metabolic syndrome is not surprising, as CRP levels not only correlate with triglycerides, obesity, blood pressure, and fasting glucose (all of which are components of the ATP III metabolic syndrome definition), but also correlate with insulin sensitivity, endothelial dysfunction, and impaired fibrinolysis (factors additionally associated with the metabolic syndrome that are not easily discerned in usual clinical practice).[18] Although cardiac event-free survival is similar for those with CRP levels above or below 3.0 mg/L and for those with and without the metabolic syndrome, it is also clear that CRP adds independent prognostic information on risk at all levels of severity of the metabolic syndrome.[18] Thus, the metabolic syndrome is a heterogenous condition; as shown in Figure 4, CRP levels of <1, 1 to 3, and >3 mg/L differentiate low, moderate, and high risk even when applied to those already defined as having the metabolic syndrome.[18]

Several prospective studies demonstrate that CRP levels additionally predict incident type II diabetes.[19,20] These data further link inflammation, atherothrombosis, and diabetes as tightly interrelated disorders of the innate immune system and may help to explain why diet and exercise are so important to the prevention of both diseases.

## The Population Distribution of CRP

When measured with high-sensitivity assays, the population distribution of CRP has generally been consistent across sex and ethnic groups, and values of 0.3, 0.6, 1.5, 3.5, and 6.6 mg/L have been reported as estimates of the 10th, 25th, 50th, 75th, and 90th percentile cut-points for middle-aged Americans.[6] In 4 major cohort studies performed in the United States, the Physicians Health Study, the Women's Health Study, the Women's Health Initiative, and the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS),[2,3,4,6,10] the quintile distributions of CRP for men and for women not taking hormone replacement therapy (HRT) are remarkably similar, and in practice approximate quintile cut-points of <0.5, 0.5 to 1.0, 1.0 to 2.0, 2.0 to 4.0, and >4.0 mg/L have been suggested for use. An alternative approach, as suggested above, is simply one that emphasizes levels <1, 1 to 3, and >3.0 mg/L as low-, moderate-, and high-risk groups.

Because women taking HRT will have higher levels of CRP,[21,22] risk estimates for such women may need to be calibrated downward. As recently demonstrated in analyses of CRP and HRT in the Women's Health Initiative,[10] however, these effects in terms of actual event prediction are not as large as anticipated. Further, these data suggest that it is the

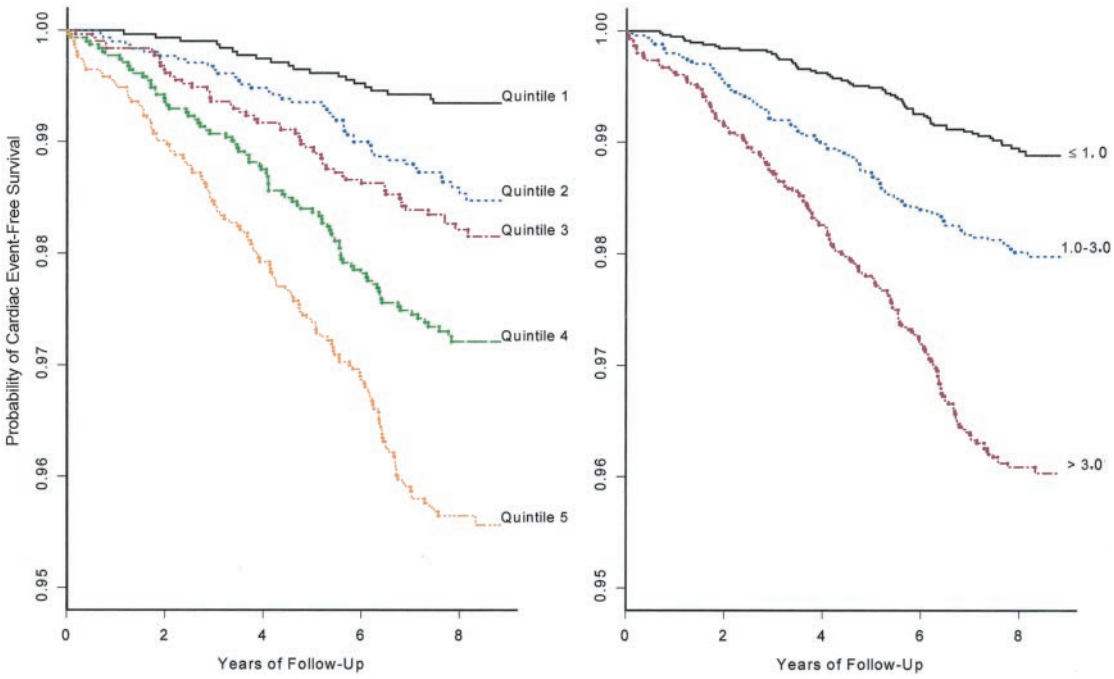


**Figure 2.** Cardiovascular event-free survival among apparently healthy individuals according to baseline CRP levels. Data are shown using population-based quintiles for CRP (left) and using 3 simple clinical cut-points for CRP, <1, 1 to 3, and >3 mg/L (right). Adapted from reference 6.

expressed level of CRP that determines a given woman's vascular risk. Finally, in the Women's Health Study,[3,6] there was no substantive difference in risk estimates for women taking HRT when cut-points were determined among users of HRT rather than non-users. Taken together, these large outcome analyses suggest little value in having separate clinical cut-points for CRP either by sex or by HRT use.

The sparse population data available for blacks is consistent with these findings. However, the total number of individuals evaluated in this group remains small.

## Interpreting CRP Assays, Cost-Effectiveness, and Serial Assessment

In most clinical settings, a single CRP assessment is likely to be adequate as long as levels less than 10 mg/L are observed. Because major infections, trauma, or acute hospitalizations can elevate CRP levels (usually 100-fold or more), levels greater than 10 mg/L should initially be ignored and the test repeated at a future date when the patient is clinically stable. Many investigators have recommended 2 measures of CRP, with the lower value or the average being used to determine vascular risk, a practice consistent with recommendations for cholesterol evaluation. In rare instances where levels of CRP are markedly elevated, alternative sources of systemic inflammation such as lupus, inflammatory bowel disease, or endocarditis should be considered. In such cases, there is usually an accompanying elevation in the erythrocyte sedimentation rate. Accumulated experience in outpatient settings has shown such values to be infrequent.

Because CRP levels are stable over long periods of time, are not affected by food intake, and demonstrate almost no circadian variation, there is no need to obtain fasting blood samples for CRP assessment. Despite being an acute phase reactant, the variability in CRP levels in given individuals is

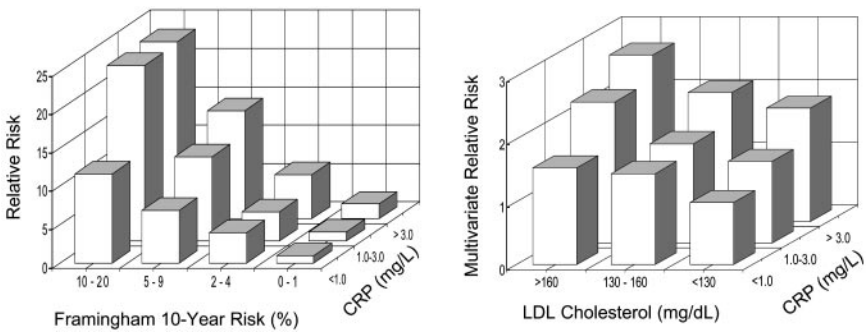


**Figure 3.** CRP provides prognostic information at all levels of LDL cholesterol and at all levels of the Framingham Risk Score. Data adapted from reference 6.

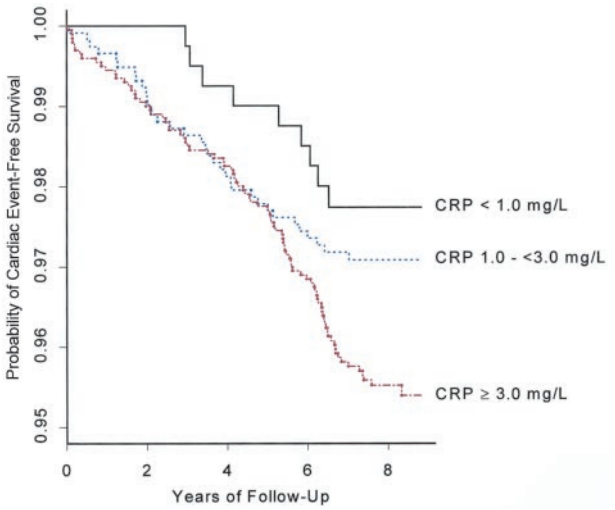


**Figure 4.** Cardiovascular event-free survival according to baseline CRP levels among individuals already defined as having the metabolic syndrome. Adapted from reference 18.

quite similar to that associated with cholesterol screening, as long as the CRP levels are within the clinical range defined above.[23]

Traditional assays for CRP do not have adequate sensitivity to detect levels required for vascular disease prediction. To alleviate this problem, high-sensitivity CRP assays have been developed and are now widely available.[24] The cost of CRP screening is comparable to that of standard cholesterol evaluation and far less than almost all other alternative approaches to cardiovascular screening under consideration. Both in terms of years of life saved and cost-to-benefit ratios, CRP screening seems to be highly effective.[25] In many settings, the inexpensive approach of adding CRP to LDL screening may yield immediate cost-savings in terms of negative predictive value and the subsequent avoidance of unnecessary clinical testing, particularly when compared with far more expensive screening approaches such as electron beam calcium tomography or MRI.

CRP levels within the range detected with high-sensitivity assays have demonstrated specificity for vascular events.[26] Although it has not been determined whether serial CRP assessment provides incremental clinical value, some physicians have elected to use CRP as part of their annual physical examination.

## Comparison of CRP to Other Novel Risk Factors

CRP is not the only inflammatory biomarker that has been shown to predict myocardial infarction and stroke. More sophisticated measures of cytokine activity, cellular adhesion, and immunologic function (such as interleukin-6, intercellular adhesion molecule-1, macrophage inhibitory cytokine-1, and soluble CD40 ligand) have all been shown to be elevated among those at increased vascular risk.[27] These approaches, however, are unlikely to have clinical utility because the assays required for their assessment are either inappropriate for routine clinical use or the protein of interest has too short a half-life for clinical evaluation. Measures for fibrinogen, a biomarker involved in both inflammation and thrombosis, remain poorly standardized, and methodological issues limit use of this parameter despite consistent population-based data. Other broad measures of systemic inflammation, such as the white blood cell count or the erythrocyte sedimentation rate, have proven unreliable in clinical settings. By contrast, high-sensitivity assays for CRP have been standardized across many commercial platforms. Moreover, CRP is highly stable, allowing measures to be made accurately in both fresh and frozen plasma without requirements for special collection procedures. This is due in part to the stable pentraxin structure of CRP and its long plasma half-life of 18 to 20 hours.

In selected patients, such as those with markedly premature and unexplained atherosclerosis, evaluation of other markers, such as lipoprotein(a) and homocysteine, may have clinical utility. In available population-based studies, however, the relative magnitude of these biomarkers has been small in direct comparison to CRP (Figure 5). Recent data also indicate that CRP is a stronger predictor of risk than nuclear magnetic resonance-based evaluation of LDL particle size and concentration.[28]

## Goals of Screening and Therapeutic Options

The primary goal of cardiovascular screening programs should be the identification of high-risk individuals who can be targeted for smoking cessation, diet, exercise, and blood pressure control. It is well established that compliance with lifestyle recommendations is directly related to the absolute risk perceived by individual patients. Thus, because the addition of CRP to lipid evaluation provides an improved prediction tool, consideration of CRP may have usefulness for this reason alone.

There is currently no definitive evidence that lowering CRP will necessarily reduce cardiovascular event rates; studies addressing this issue are only now being designed. However, many interventions known to reduce cardiovascular risk have been

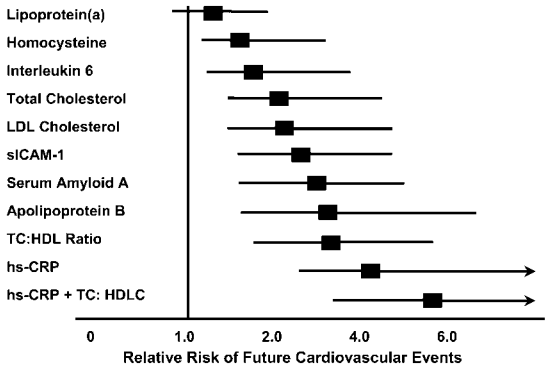


**Figure 5.** Direct comparison of CRP to several other lipid and non-lipid risk factors for cardiovascular disease. SICAM-1 indicates soluble intercellular adhesion molecule-1; hs-CRP, high-sensitivity CRP; HDL, high-density lipoprotein; and HDLC, high-density lipoprotein cholesterol. Adapted from reference 3.

linked to lower CRP levels. In particular, weight loss, diet, exercise, and smoking cessation all lead to both reduced CRP levels and reduced vascular risk.

Several pharmacological agents proven to reduce vascular risk influence CRP levels. Of these, the statin drugs are the most important, and studies with pravastatin, lovastatin, cerivastatin, simvastatin, and atorvastatin have all shown that, on average, median CRP levels decline 15% to 25% as early as 6 weeks after initiation of therapy. As shown in the large-scale Cholesterol And Recurrent Events (CARE)[29] and PRavastatin INflammation/CRP Evaluation (PRINCE)[16] trials and subsequently confirmed in other settings, there is little evidence that the magnitude of LDL reduction predicts the magnitude of CRP reduction. On the other hand, aggressive LDL reduction remains a critical therapeutic goal, and thus serial LDL evaluation should remain the primary method to monitor statin compliance. However, whereas all subjects taking statins achieve a beneficial reduction in LDL levels, there seems to be responders and non-responders for statins in terms of CRP reduction. Whether this latter observation is important in terms of clinical event reduction is currently unknown.

Analyses of 2 randomized trials suggest that the magnitude of risk reduction attributable to statin therapy is particularly large for those with elevated CRP levels. In the CARE trial of secondary prevention, the magnitude of benefit associated with pravastatin use was nearly 55% for those with elevated CRP levels as compared with 30% for those with low CRP levels.[30] Similarly, in the AFCAPS/TexCAPS primary prevention trial, lovastatin use was highly effective among those with elevated CRP levels, even when LDL levels were below thresholds set by the ATP III guidelines.[4] Although performed on a post hoc basis and limited by relatively low event rates, the AFCAPS/TexCAPS analysis suggests that the benefit of statin therapy among those with low LDL but high CRP may be just as large as the benefit observed among those with overt hyperlipidemia.

That patients with elevated CRP but low LDL are at high vascular risk is demonstrated in Figure 6, which shows survival data from the Women's Health Study for those with LDL cholesterol above or below the study median of 124 mg/dL and CRP above or below the study median of 1.52 mg/L.[6] As expected, overall event-free survival was poorest for those with elevated CRP and elevated LDL, whereas the best survival was observed for those with low CRP and low LDL levels. However, event-free survival was actually worse for those with elevated CRP and low LDL when compared with those with elevated LDL and low CRP. Because of the public health implications of these data, a large-scale statin prevention trial of 15 000 patents is scheduled to begin in early 2003 specifically targeting those with native LDL <130 but a CRP above 2.0 mg/L.[31]

Although data are less robust, other lipid-lowering agents reported to reduce CRP include niacin, fibrates, and gemfibrozil. Aspirin also has an intriguing interaction with CRP in

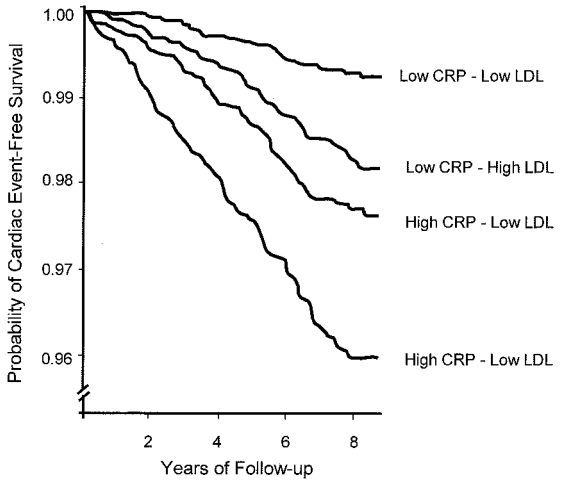


**Figure 6.** Cardiovascular event-free survival according to baseline levels of CRP and LDL. Adapted from reference 6.

that the magnitude of relative risk reduction attributable to aspirin in primary prevention appears to be greatest among those with elevated CRP and declines proportionately in direct relation to CRP levels.[2] Observational data suggest possible differential benefits for clopidogrel and abciximab on the basis of CRP levels before percutaneous coronary interventions.[32–34] Thiazolidinediones also reduce CRP levels.[35]

## Clinical Recommendations

As documented above for primary prevention, CRP is an independent predictor of future cardiovascular events that adds prognostic information to lipid screening, to the metabolic syndrome, and to the Framingham Risk Score.

In outpatient settings, the primary use of CRP should be at the time of cholesterol screening, when knowledge of CRP can be used as an adjunct for global risk assessment.[1] For individuals with LDL levels above 160 mg/dL and for whom the ATP III guidelines already call for therapeutic intervention, an elevated CRP level should aggressively encourage physicians and patients to institute pharmacological therapy in those instances where none is currently being used or where compliance is poor.

For individuals with LDL levels between 130 and 160 mg/dL, the additional finding of an elevated CRP indicates an elevated global risk. In almost all cases, this information should lead to better compliance and adherence with current ATP III treatment guidelines.

For individuals with LDL levels below 130 mg/dL, the finding of an elevated CRP implies substantially higher risk than predicted on the basis of LDL alone. As shown in Figures 3 and 6, such individuals will have risk estimates as high as some individuals with overt hyperlipidemia. Patients with this profile should be advised to adhere carefully with ATP III lifestyle interventions, despite "low" LDL cholesterol levels. Individuals with the low LDL/high CRP phenotype

are at elevated risk of having the metabolic syndrome and should have fasting glucose levels measured. Large-scale, randomized trial evidence is critically needed before such patients should be considered for statin therapy.[31]

An alternative approach in primary prevention is to measure CRP only among those at intermediate risk as defined by the Framingham Risk Score. For example, clinicians might conservatively choose to evaluate CRP only among those with a calculated 10-year Framingham risk between 5% and 20% (see Figure 3). Although this strategy has epidemiological appeal, such an approach requires a second office visit and a second phlebotomy and thus is likely to be less efficient and perhaps less cost-effective.

In secondary prevention, the potential utility of CRP is less certain, as aggressive therapies should already be instituted and LDL evaluation provides an excellent method to assess statin efficacy.

In the setting of acute coronary ischemia and unstable angina, the role of CRP is rapidly evolving. Multiple studies demonstrate that CRP levels predict early and late mortality in acute coronary ischemia and add to the predictive value of cardiac troponin.[36–41] Further, knowledge of inflammatory status has been shown effective in distinguishing patient subgroups more or less likely to benefit from an aggressive versus conservative management approach.[40] However, appropriate clinical cut-points for CRP in the setting of acute ischemia remain uncertain, as does the timing of CRP evaluation in relation to the onset of ischemia. The most foreseeable use of CRP in the emergency room setting is thus likely to be among those with chest pain syndromes who have negative troponin levels. An elevated CRP in this setting is associated with increased short-term as well as long-term risks,[39–41] and thus additional evaluation modalities may be warranted. By contrast, current data suggest that patients with negative troponin and negative CRP levels in the emergency room setting are unlikely to have flow limiting coronary disease.[39,40]

## Acknowledgments

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# PREVENTION OF CORONARY HEART DISEASE WITH PRAVASTATIN IN MEN WITH HYPERCHOLESTEROLEMIA

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**Abstract** *Background.* Lowering the blood cholesterol level may reduce the risk of coronary heart disease. This double-blind study was designed to determine whether the administration of pravastatin to men with hypercholesterolemia and no history of myocardial infarction reduced the combined incidence of nonfatal myocardial infarction and death from coronary heart disease.

*Methods.* We randomly assigned 6595 men, 45 to 64 years of age, with a mean (±SD) plasma cholesterol level of 272±23 mg per deciliter (7.0±0.6 mmol per liter) to receive pravastatin (40 mg each evening) or placebo. The average follow-up period was 4.9 years. Medical records, electrocardiographic recordings, and the national death registry were used to determine the clinical end points.

*Results.* Pravastatin lowered plasma cholesterol levels by 20 percent and low-density lipoprotein cholesterol levels by 26 percent, whereas there was no change with placebo. There were 248 definite coronary events (specified as nonfatal myocardial infarction or death from coronary heart disease) in the placebo group, and 174 in the pravastatin group (relative reduction in risk with pravastatin, 31 percent; 95 percent confidence interval, 17 to 43 percent; $P<0.001$). There were similar reductions in the risk of definite nonfatal myocardial infarctions (31 percent reduction, $P<0.001$), death from coronary heart disease (definite cases alone: 28 percent reduction, $P=0.13$; definite plus suspected cases: 33 percent reduction, $P=0.042$), and death from all cardiovascular causes (32 percent reduction, $P=0.033$). There was no excess of deaths from noncardiovascular causes in the pravastatin group. We observed a 22 percent reduction in the risk of death from any cause in the pravastatin group (95 percent confidence interval, 0 to 40 percent; $P=0.051$).

*Conclusions.* Treatment with pravastatin significantly reduced the incidence of myocardial infarction and death from cardiovascular causes without adversely affecting the risk of death from noncardiovascular causes in men with moderate hypercholesterolemia and no history of myocardial infarction. (N Engl J Med 1995;333:1301-7.)

EARLIER trials of lipid-lowering drugs in the primary prevention of coronary heart disease have demonstrated that lowering cholesterol levels in middle-aged men with hypercholesterolemia reduces the incidence of myocardial infarction.[1-4] However, these studies, because of their design and low rates of observed events, were unable to show a clear effect of therapy on the risk of death from coronary heart disease or death from any cause. A meta-analysis of the trials provided support for the likelihood that therapy lowered the risk of death from coronary heart disease, but it also aroused concern that the risk of death from noncardiovascular causes might be increased by treatment.[5-8] Whether this latter association was due to chance, to the reduction in cholesterol itself, or to an adverse effect of the drugs is not clear.

Recently, a new class of lipid-lowering drug, the 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors, has been introduced into clinical practice. These drugs block endogenous synthesis of cholesterol and reduce the levels of low-density lipoprotein (LDL) cholesterol. They slow the progression of coronary disease and reduce the incidence of death from coronary causes and death from any cause in men with manifest coronary heart disease.[9-15] The present study was designed to evaluate the effectiveness of a reductase inhibitor, pravastatin (Pravachol), in preventing coronary events in men with moderate hypercholesterolemia and no history of myocardial infarction.

## METHODS

### Design

The objective was to enroll approximately 6000 middle-aged men, randomly assigned in a double-blind fashion to receive either pravastatin (40 mg each evening) or placebo and to record their clinical progress over a period of five years. The details of the study design,

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*The members of the West of Scotland Coronary Prevention Study Group are listed in the Appendix.

including the definitions of the end points, have been described previously.[16] Briefly, the primary end point of the study was the occurrence of nonfatal myocardial infarction or death from coronary heart disease as a first event; these two categories were combined. Other principal end points were the occurrence of death from coronary heart disease and nonfatal myocardial infarction. In all categories, the events were classified as either definite or suspected. In addition to the main end points, the effect of treatment on death from cardiovascular causes, death from any cause, and the frequency of coronary revascularization procedures was analyzed.

All subjects provided written informed consent. The study was approved by the ethics committees of the University of Glasgow and all participating health boards.

### Recruitment and Follow-up

Coronary screening clinics were established in primary medical care facilities throughout the West of Scotland district. Approximately 160,000 men ranging in age from 45 to 64 years were invited to attend the clinics to assess their coronary risk factors. A total of 81,161 appeared for the first visit, and those whose nonfasting plasma cholesterol level was at least 252 mg per deciliter (6.5 mmol per liter) but who had no history of myocardial infarction were given lipid-lowering dietary advice[17] and asked to return four weeks later. A total of 20,914 men returned for the second visit, at which time a lipoprotein profile was obtained that measured plasma cholesterol, the cholesterol content of LDL and high-density lipoprotein (HDL), and plasma triglycerides while the subjects were fasting. If on this occasion the LDL cholesterol level was at least 155 mg per deciliter (4.0 mmol per liter) and the subject had no exclusion criteria,[16] he was advised to stay on the lipid-lowering diet for a further four weeks and then to return for a third visit (13,654 attended), at which time a second lipoprotein profile and a 12-lead electrocardiogram (ECG) were obtained. On the fourth visit the patients underwent randomization if they met the following criteria: fasting LDL cholesterol level of at least 155 mg per deciliter during the second and third visits, with at least one value of 174 mg per deciliter or above (4.5 mmol per liter) and one value of 232 mg per deciliter or below (6.0 mmol per liter); no serious ECG abnormalities according to Minnesota code[18] 1 (pathologic Q waves), 4-1, 5-1, or 7-1-1 or arrhythmia such as atrial fibrillation; and no history of myocardial infarction or other serious illness, although men with stable angina who had not been hospitalized within the previous 12 months were eligible. Further details of the inclusion and exclusion criteria were described previously.[16]

The subjects were seen at three-month intervals, and dietary advice was reinforced on each occasion. A fasting lipoprotein profile was obtained every six months, and an ECG was recorded annually or as required clinically. The subjects received a full medical examination by a physician each year.

### Laboratory Analyses

The cholesterol measurement during the first visit was performed on a Reflotron bench-top analyzer (Boehringer–Mannheim, Lewes, Kent, United Kingdom). All subsequent laboratory analyses, including biochemical, hematologic, and lipoprotein profiles, were conducted at the central laboratory at the Glasgow Royal Infirmary. Lipoprotein profiles were determined according to the Lipid Research Clinics protocol[19] with enzymatic cholesterol and triglyceride assays. The laboratory was certified through the Lipid Standardization Program of the Centers for Disease Control and Prevention in Atlanta. Abnormalities in the results of blood tests were identified with the use of published reference ranges.[16]

Siemens Sicard 440 electrocardiographs were used to record the 12-lead ECGs, and the data were transmitted by telephone to the ECG core laboratory at the Glasgow Royal Infirmary for storage on a central Mingocare data base (Siemens Elema, Stockholm, Sweden) and subsequent automated classification according to the Minnesota code, including serial comparisons.[18,20-22] All ECG results were verified by visual inspection.

### Identification and Classification of End Points

At each follow-up visit, adverse events were documented on the basis of the subjects' recall, and if appropriate, further information was obtained from hospital records. All data on randomized subjects were flagged electronically on national computer data bases so that the numbers of deaths, incident cancers, hospitalizations, and cardiac surgeries could be monitored according to previously described methods.[23] Potential end points were reviewed and classified according to predefined criteria[16] by the End-Points Committee, whereas non–coronary heart disease events were reviewed and classified by the Adverse-Events Committee. The progress and conduct of the study were monitored regularly by the independent, unblinded Data and Safety Monitoring Committee. Except for the trial statistician and his assistant, all trial personnel remained unaware of the subjects' treatment assignments throughout the study.

### Statistical Analysis

All data were analyzed according to the intention-to-treat principle. The results of the two fasting lipoprotein profiles obtained during visits 2 and 3 were averaged to produce base-line values. The LDL cholesterol results were analyzed according to both the treatment actually received and the intention-to-treat principle. The analysis based on actual treatment used only the measured lipid levels in subjects who had attended the previous scheduled visit and who had been issued with trial medication at that visit. For the intention-to-treat analysis, all recorded levels were included, without reference to the subjects' degree of compliance at previous visits. In addition, in cases in which no lipid value was available for a scheduled visit and no medication had been issued at the previous visit, the subject's base-line level was used. For each end-point category, the lengths of time to a first event were compared with use of the log-rank test, and the relative reduction in risk resulting from pravastatin treatment, with 95 percent confidence intervals, was calculated with the Cox proportional-hazards model.[24] In addition, Kaplan–Meier time-to-event curves were used to estimate the absolute risk of each event at five years for each treatment group. When a silent myocardial infarction was detected on the basis of serial comparison of ECGs, the event was con-

Table 1. Base-Line Characteristics of the Randomized Subjects, According to Treatment Group.*

| Characteristic | Placebo (N = 3293) | Pravastatin (N = 3302) |
|---|---|---|
| **Continuous variables** | | |
| Age — yr | 55.1±5.5 | 55.3±5.5 |
| Body-mass index† | 26.0±3.1 | 26.0±3.2 |
| Blood pressure — mm Hg | | |
| Systolic | 136±17 | 135±18 |
| Diastolic | 84±10 | 84±11 |
| Cholesterol — mg/dl | | |
| Total | 272±22 | 272±23 |
| LDL | 192±17 | 192±17 |
| HDL | 44±10 | 44±9 |
| Triglycerides — mg/dl | 164±68 | 162±70 |
| Alcohol consumption — units/wk‡ | 11±13 | 12±14 |
| **Categorical variables** — no. of subjects (%) | | |
| Angina§ | 174 (5) | 164 (5) |
| Intermittent claudication§ | 96 (3) | 97 (3) |
| Diabetes | 35 (1) | 41 (1) |
| Hypertension (self-reported) | 506 (15) | 531 (16) |
| Minor ECG abnormality | 259 (8) | 275 (8) |
| Smoking status | | |
| Never smoked | 705 (21) | 717 (22) |
| Exsmoker | 1127 (34) | 1138 (34) |
| Current smoker | 1460 (44) | 1445 (44) |
| Employment status | | |
| Employed | 2324 (71) | 2330 (71) |
| Unemployed | 459 (14) | 430 (13) |
| Retired | 338 (10) | 330 (10) |
| Disabled | 171 (5) | 210 (6) |

*Plus–minus values are means ±SD. To convert values for cholesterol to millimoles per liter, multiply by 0.026, and to convert values for triglycerides to millimoles per liter, multiply by 0.011.

†The weight in kilograms divided by the square of the height in meters.

‡A unit was defined as 1 measure (60 ml) of liquor, 1 glass (170 ml) of wine, or a half pint (300 ml) of beer.

§As indicated by positive responses on the Rose questionnaire.

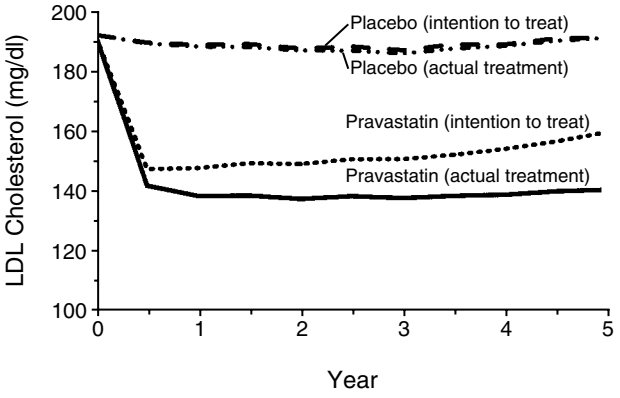



Figure 1. Effects of Pravastatin Therapy on Plasma LDL Cholesterol Levels.
To convert values for cholesterol to millimoles per liter, multiply by 0.026.

sidered to have occurred midway between the first diagnostic ECG and the previous ECG. Two-tailed P values were used throughout.

For the primary end point, an analysis was performed for predefined subgroups[16] characterized at base line according to age (<55 years or ≥55 years), smoking status (smoker or nonsmoker of cigarettes, cigars, or pipes), and whether at least two of the following risk factors were present: smoking, hypertension, a history of chest pain or intermittent claudication (as indicated by positive responses on the Rose questionnaire), diabetes, and a minor ECG abnormality associated with coronary heart disease (Minnesota code 4-2, 4-3, 5-2, or 5-3).

In addition, the effect of treatment was examined in a subgroup with and a subgroup without vascular disease at base line. Vascular disease was considered to be present if there was evidence of angina, intermittent claudication, stroke, transient ischemic attack, and ECG abnormalities according to the Minnesota code. Finally, the influence of base-line lipid levels on the effect of treatment was assessed by dividing the randomized population according to the median plasma cholesterol, LDL or HDL cholesterol, or plasma triglyceride concentration.

The Data and Safety Monitoring Committee conducted annual reviews of the main end points according to the O'Brien and Fleming criteria for stopping the trial prematurely.[25] The overall P value indicating statistical significance was set at 0.01.

## RESULTS

A total of 6595 subjects underwent randomization. The clinical characteristics of the subjects who were screened and those who were randomized have been described previously.[26] The first patient was enrolled on February 1, 1989, and recruitment was completed by September 30, 1991. The final visits were made between February and May 1995, by which time the study population had accrued 32,216 subject-years of follow-up (an average of 4.9 years per subject). At the end of the study, the vital and clinical status of all randomized subjects was ascertained.

The base-line characteristics of the pravastatin and placebo groups are summarized in Table 1. As expected in a trial of this size, the groups were well balanced. For the study population as a whole, the average (±SD) plasma cholesterol level was 272±23 mg per deciliter (7.0±0.6 mmol per liter), the LDL cholesterol level was 192±17 mg per deciliter (5.0±0.5 mmol per liter), and the HDL cholesterol level was 44±9 mg per deciliter (1.14±0.26 mmol per liter). On the basis of positive responses on the Rose questionnaire, evidence of angina was present in 5 percent of the men, whereas 8 percent had ECG ST-T wave changes (Minnesota codes 4-2, 4-3, 5-2, and 5-3). The prevalence of self-reported diabetes mellitus was 1 percent, and that of hypertension was 16 percent; 44 percent of the subjects were current smokers.

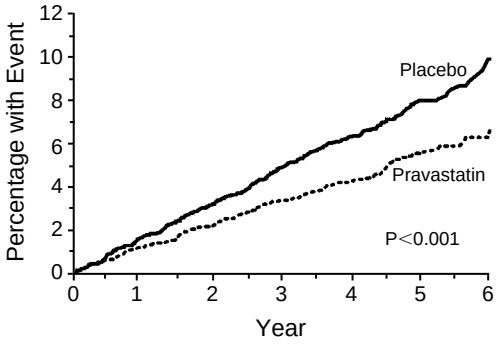
### Withdrawals

The cumulative rates of withdrawal from treatment in the placebo and pravastatin groups were 14.9 percent and 15.5 percent, respectively, at year 1, 19.1 percent and 19.4 percent at year 2, 22.5 percent and 22.7 percent at year 3, 25.2 percent and 24.7 percent at year 4, and 30.8 percent and 29.6 percent at year 5. There was no significant difference in the withdrawal rates between the two groups at any time. The disproportionate increase from year 4 to year 5 can be attributed to the withdrawal from the study of some subjects who had

Table 2. End Points of the Study.*

| VARIABLE | PLACEBO (N = 3293) | PRAVASTATIN (N = 3302) | P VALUE | RISK REDUCTION WITH PRAVASTATIN (95% CI) |
|---|---|---|---|---|
| | *no. of events (absolute % risk at 5 yr)* | | | % |
| **Definite coronary events** | | | | |
| Nonfatal MI or death from CHD | 248 (7.9) | 174 (5.5) | <0.001 | 31 (17 to 43) |
| Nonfatal MI (silent MIs omitted) or death from CHD | 218 (7.0) | 150 (4.7) | <0.001 | 33 (17 to 45) |
| Nonfatal MI | 204 (6.5) | 143 (4.6) | <0.001 | 31 (15 to 45) |
| Death from CHD | 52 (1.7) | 38 (1.2) | 0.13 | 28 (−10 to 52) |
| **Definite + suspected coronary events** | | | | |
| Nonfatal MI or death from CHD | 295 (9.3) | 215 (6.8) | <0.001 | 29 (15 to 40) |
| Nonfatal MI (silent MIs omitted) or death from CHD | 240 (7.6) | 166 (5.3) | <0.001 | 32 (17 to 44) |
| Nonfatal MI | 246 (7.8) | 182 (5.8) | 0.001 | 27 (12 to 40) |
| Death from CHD | 61 (1.9) | 41 (1.3) | 0.042 | 33 (1 to 55) |
| **Other events** | | | | |
| Coronary angiography | 128 (4.2) | 90 (2.8) | 0.007 | 31 (10 to 47) |
| PTCA or CABG | 80 (2.5) | 51 (1.7) | 0.009 | 37 (11 to 56) |
| Fatal or nonfatal stroke | 51 (1.6) | 46 (1.6) | 0.57 | 11 (−33 to 40) |
| Incident cancer | 106 (3.3) | 116 (3.7) | 0.55 | −8 (−41 to 17) |
| **Death from other causes** | | | | |
| Other cardiovascular causes (including stroke) | 12 | 9 | — | — |
| Suicide | 1 | 2 | — | — |
| Trauma | 5 | 3 | — | — |
| Cancer | 49 (1.5) | 44 (1.3) | 0.56 | 11 (−33 to 41) |
| All other causes | 7 | 7 | — | — |
| **Death from all cardiovascular causes** | 73 (2.3) | 50 (1.6) | 0.033 | 32 (3 to 53) |
| **Death from noncardiovascular causes** | 62 (1.9) | 56 (1.7) | 0.54 | 11 (−28 to 38) |
| **Death from any cause** | 135 (4.1) | 106 (3.2) | 0.051 | 22 (0 to 40) |

*The P values are based on the log-rank test. No formal analysis was carried out for events with a low incidence. CI denotes confidence interval, CHD coronary heart disease, MI myocardial infarction, PTCA percutaneous transluminal coronary angioplasty, and CABG coronary-artery bypass graft.




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Placebo | | | | | | | |
| Cumulative events | 0 | 55 | 105 | 159 | 205 | 240 | 248 |
| No. at risk | 3293 | 3230 | 3167 | 3099 | 2714 | 1241 | 83 |
| Pravastatin | | | | | | | |
| Cumulative events | 0 | 40 | 72 | 109 | 138 | 167 | 174 |
| No. at risk | 3302 | 3256 | 3215 | 3162 | 2807 | 1330 | 99 |

Figure 2. Kaplan–Meier Analysis of the Time to a Definite Nonfatal Myocardial Infarction or Death from Coronary Heart Disease, According to Treatment Group.

completed the five years of follow-up and who could have proceeded further but did not wish to do so.

#### Reduction in Lipid Levels

When the data were analyzed according to the treatment actually received, pravastatin was found to have lowered plasma levels of cholesterol by 20 percent, LDL cholesterol by 26 percent (Fig. 1), and triglycerides by 12 percent, whereas HDL cholesterol was increased by 5 percent. There were no such changes with placebo. When the data were analyzed according to the intention-to-treat principle, because such analysis includes subjects who withdrew and noncompliant subjects, there was an apparent reduction in the observed difference in LDL cholesterol levels between treatment groups over time. This result is in contrast to that based on actual treatment, which showed that the differnce was maintained.

#### End Points

As compared with placebo, pravastatin produced a significant reduction in the risk of the combined primary end point of definite nonfatal myocardial infarction and death from coronary heart disease (reduction, 31 percent; 95 percent confidence interval, 17 to 43 percent; $P<0.001$; absolute difference in the risk at five years, 2.4 percentage points) (Table 2 and Fig. 2). The effects of pravastatin on other principal end points are given in Table 2 and Figure 3. The reduction in the risk of nonfatal myocardial infarction was significant ($P\leq0.001$) whether the definite cases of myocardial infarction were considered alone or in combination with suspected cases. Excluding silent myocardial infarctions from the analysis of the primary end point did not affect the outcome (Table 2). For the end point of death from coronary heart disease, there was a significant treatment effect in the analysis of both definite and suspected cases (risk reduction, 33 percent; 95 percent confidence interval, 1 to 55 percent; $P=0.042$), but not in the analysis of definite cases alone, probably because of the smaller number of events in this group. However, there was a similar reduction in risk (28 percent). When the effect of treatment with pravastatin on death from all cardiovascular causes was analyzed, a 32 percent reduction in risk (95 percent confidence interval, 3 to 53 percent; $P=0.033$) was observed. Treatment with pravastatin was associated with similar reductions in the frequency of coronary angiography (31 percent; 95 percent confidence interval, 10 to 47 percent; $P=0.007$)

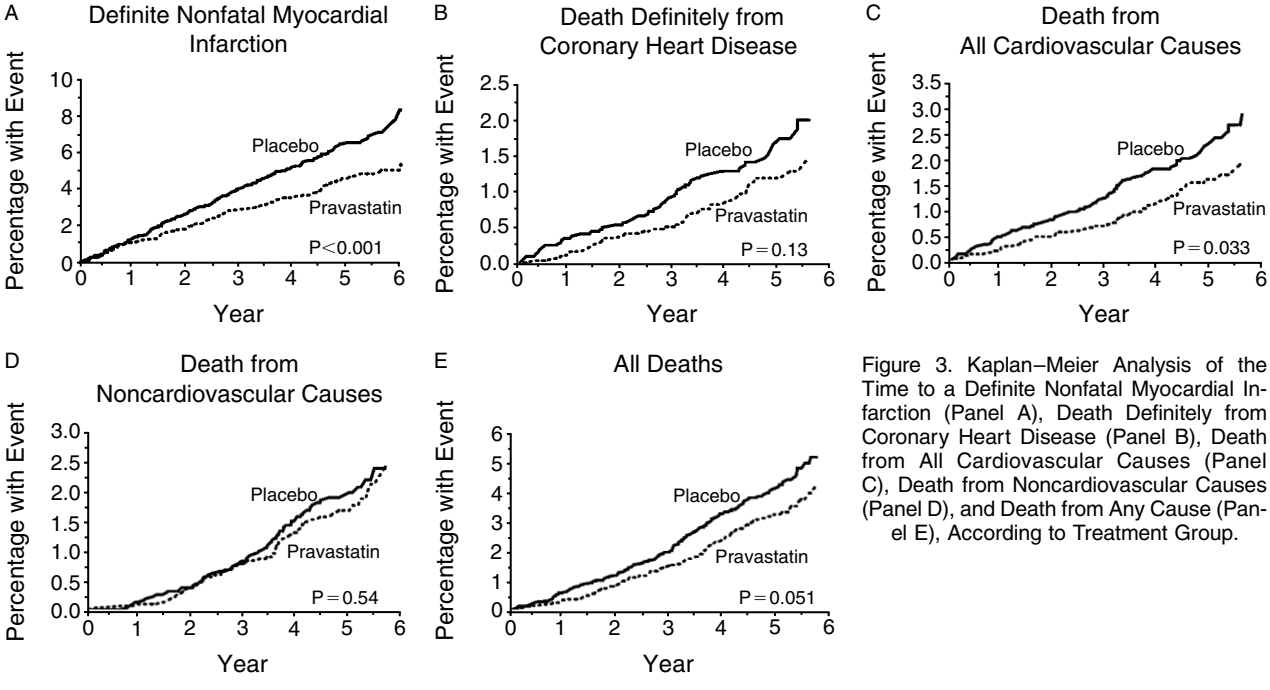



Figure 3. Kaplan–Meier Analysis of the Time to a Definite Nonfatal Myocardial Infarction (Panel A), Death Definitely from Coronary Heart Disease (Panel B), Death from All Cardiovascular Causes (Panel C), Death from Noncardiovascular Causes (Panel D), and Death from Any Cause (Panel E), According to Treatment Group.

and revascularization procedures (37 percent; 95 percent confidence interval, 11 to 56 percent; P = 0.009).

There were 56 deaths from noncardiovascular causes in the pravastatin group and 62 in the placebo group (P = 0.54). There was no significant difference between treatment groups in the numbers of deaths from cancer, suicide, or trauma. There were 46 strokes (6 of which were fatal) in the pravastatin group and 51 (4 fatal) in the placebo group. In the pravastatin group, the reduction in the number of deaths from cardiovascular causes in the absence of any increase in the number of deaths from noncardiovascular causes resulted in a 22 percent reduction in the overall risk of death (95 percent confidence interval, 0 to 40 percent; P = 0.051).

The beneficial effects of pravastatin therapy were evident in all subgroups (Table 3). The numbers of subjects in the subgroups with either multiple risk factors at base line or vascular disease at base line were too small to show a statistically significant effect.

Table 3. Incidence of the Primary End Point, According to Subgroup.

| Variable | Subgroup | No. of Subjects | Placebo | Pravastatin | P Value* | Risk Reduction with Pravastatin (95% CI) |
|---|---|---|---|---|---|---|
| | | | *no. of events (absolute % risk at 5 yr)* | | | % |
| Age | <55 yr | 3225 | 96 (6.1) | 57 (3.5) | 0.0024 | 40 (16 to 56) |
| | ≥55 yr | 3370 | 152 (9.8) | 117 (7.3) | 0.0089 | 27 (8 to 43) |
| Current smoking status | Nonsmoker | 3687 | 104 (6.0) | 74 (4.3) | 0.016 | 31 (6 to 48) |
| | Smoker | 2905 | 144 (10.4) | 100 (7.0) | 0.0035 | 31 (12 to 47) |
| Multiple risk factors† | Absent | 5401 | 178 (6.9) | 114 (4.4) | <0.001 | 37 (20 to 50) |
| | Present | 1194 | 70 (12.7) | 60 (10.2) | 0.20 | 20 (−13 to 43) |
| Cholesterol level‡ | <269 mg/dl | 3192 | 122 (8.1) | 80 (5.4) | 0.0019 | 36 (15 to 51) |
| | ≥269 mg/dl | 3403 | 126 (7.8) | 94 (5.6) | 0.021 | 27 (4 to 44) |
| LDL cholesterol level‡ | <189 mg/dl | 3211 | 110 (7.6) | 71 (4.9) | 0.0025 | 37 (15 to 53) |
| | ≥189 mg/dl | 3384 | 138 (8.3) | 103 (6.1) | 0.016 | 27 (6 to 43) |
| HDL cholesterol level‡ | ≥43 mg/dl | 3304 | 99 (6.2) | 66 (4.3) | 0.011 | 33 (9 to 51) |
| | <43 mg/dl | 3291 | 149 (9.7) | 108 (6.7) | 0.0035 | 31 (11 to 46) |
| Triglyceride level§ | <148 mg/dl | 3239 | 98 (6.3) | 72 (4.4) | 0.024 | 29 (4 to 48) |
| | ≥148 mg/dl | 3356 | 150 (9.4) | 102 (6.6) | 0.0025 | 32 (12 to 47) |
| Prior vascular disease | Absent | 5529 | 183 (7.0) | 125 (4.7) | <0.001 | 33 (15 to 46) |
| | Present | 1066 | 65 (12.8) | 49 (9.6) | 0.075 | 29 (−4 to 51) |

*The P values are based on the log-rank test.

†The presence of two or more of the following risk factors: smoking, hypertension, a history of chest pain or intermittent claudication (as indicated by positive responses on the Rose questionnaire), diabetes, and a minor ECG abnormality associated with coronary heart disease (Minnesota code 4-2, 4-3, 5-2, or 5-3).

‡To convert values for cholesterol to millimoles per liter, multiply by 0.026.

§To convert values for triglycerides to millimoles per liter, multiply by 0.011.

### Other Adverse Events

In the pravastatin group 116 subjects had incident (fatal or nonfatal) cancers, as compared with 106 in the placebo group (P = 0.55). These figures include cases of malignant melanoma but not minor skin cancers. For the placebo and pravastatin groups, respectively, there were 30 and 31 gastrointestinal cancers, 26 and 32 genitourinary cancers, 28 and 27 respiratory tract cancers, and 22 and 26 other cancers. Twenty subjects in the pravastatin group reported myalgia, and 97 muscle aches. The corresponding numbers in the placebo group were 19 and 102 (P not significant). Four subjects (three in the pravastatin group and one in the placebo group) had asymptomatic episodes of elevated creatine kinase concentrations (>10 times the upper reference limit). Elevations in aspartate aminotransferase and alanine aminotransferase values (>3 times the upper reference limits) were recorded for 26 and 16 subjects, respectively, in the pravastatin group, as compared with 20 and 12 subjects in the placebo group (P not significant).

## Discussion

As compared with placebo, pravastatin reduced the risk of fatal or nonfatal coronary events in middle-aged men with hypercholesterolemia and no history of myocardial infarction by approximately 30 percent. The beneficial effects of treatment were remarkably consistent across a variety of coronary end points. In contrast to the results of studies using resins, fibrates, or other 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitors,[1-4,9] the time-to-event curves began to diverge within six months of the initiation of treatment and continued to do so at the same rate throughout the trial. The frequency of the need for coronary angiography and revascularization procedures was significantly lower in the pravastatin group than in the placebo group.

The subjects in this study were representative of the general population in terms of socioeconomic status and risk factors (Table 1). Their plasma cholesterol levels were in the highest quartile of the range found in the British population.[27] A number had evidence of minor vascular disease, and in order to make the findings of the trial applicable to typical middle-aged men with hypercholesterolemia, they were not excluded.

In line with accepted guidelines,[28] the LDL cholesterol level was used as a criterion for entry into the study. As compared with placebo, pravastatin produced a major reduction in this lipoprotein fraction (Fig. 1) and moderate decreases in plasma triglycerides, as well as an increase in HDL cholesterol. These changes are in line with the expected response to pravastatin,[29] and all could potentially result in clinical benefit. The changes in the LDL cholesterol level are more substantial than those observed in earlier primary prevention studies.[1-4]

When the subjects were divided into two groups according to their lipid levels at base line, we found that coronary risk was related to higher plasma LDL cholesterol and triglyceride levels (i.e., levels above the median values) and lower HDL cholesterol levels (i.e., levels below the median value) (Table 3). The plasma cholesterol level was not a significant factor, principally because of the narrow range of cholesterol values used as a criterion for entry into the study. The relative reduc-

tion in risk with pravastatin therapy was statistically significant and of a similar magnitude in subjects with lipid values above and below the median.

The relative reductions in risk attributable to pravastatin therapy were not affected by age (<55 years vs. ≥55 years) or smoking status. Furthermore, a significant treatment effect was seen in the subgroup without multiple risk factors and the subgroup without preexisting vascular disease. Thus, it is possible to conclude that in the subjects who might be considered to fall strictly into the primary-prevention category, pravastatin therapy produced a significant reduction in the relative risk of a coronary event.

Pravastatin therapy was well tolerated and resulted in no more study withdrawals than placebo. In particular, as in an earlier study,[15] there was no evidence that pravastatin adversely affected liver function or caused myopathy. Our results support those of a recent secondary-prevention trial[9] that found that lipid lowering with a 3-hydroxy-3-methylglutaryl–coenzyme A reductase inhibitor is not associated with an increased risk of death from noncardiovascular causes. As in that earlier trial,[9] a comparison of the treatment and placebo groups showed no significant increase in the incidence of fatal or incident cancers or deaths due to suicide or trauma. More data on the adverse-event profile of this class of drugs will become available as the results of other prevention trials are published. In the current study, the benefit of pravastatin therapy with respect to fatal coronary events and the absence of any increase in the number of deaths from other causes led to a 22 percent reduction in the relative risk of death from any cause ($P = 0.051$).

From the data in Table 2, it can be estimated that treating 1000 middle-aged men with hypercholesterolemia and no evidence of a previous myocardial infarction with pravastatin for five years will result in 14 fewer coronary angiograms, 8 fewer revascularization procedures, 20 fewer nonfatal myocardial infarctions, 7 fewer deaths from cardiovascular causes, and 2 fewer deaths from other causes than would be expected in the absence of treatment. Since these figures are based on an intention-to-treat analysis, the magnitude of the benefit in fully compliant subjects is likely to be greater. These findings can be compared favorably with the results of the Medical Research Council trial[30,31] of the treatment of mild hypertension in middle-aged subjects. In that study, it was estimated that five years of active treatment of 1000 men ranging in age from 35 to 64 years would result in six fewer strokes and two fewer cardiovascular events than would be expected. Thus, our results indicate that reducing cholesterol levels with pravastatin reduces the risk of coronary events in asymptomatic subjects with hypercholesterolemia.

## Appendix

The members of the West of Scotland Coronary Prevention Study are as follows: *Executive Committee (Voting Members)* — J. Shepherd (chairman), S.M. Cobbe, A.R. Lorimer, J.H. McKillop, I. Ford, C.J. Packard, P.W. Macfarlane, and G.C. Isles; *Data and Safety Monitoring Committee* — M.F. Oliver (chairman), A.F. Lever, B.W. Brown, J.G.G. Ledingham, S.J. Pocock, and B.M. Rifkind; *End-Points Committee* — S.M. Cobbe, B.D. Vallance, P.W. Macfarlane; *Adverse-Events Committee* — A.R. Lorimer, J.H. McKillop, and D. Ballantyne; *Data-Center Staff* — L. Anderson, D. Duncan, J. McGrath, S. Kean, A. Lawrence, V. Montgomery, and J. Norrie; *Population Screening* — M. Percy; *Clinical Coordination, Monitoring, and Administration* — E. Pomphrey, A. Whitehouse, P. Cameron, P. Parker, F. Porteous, L. Fletcher, and C. Kilday; *Computerized ECG Analysis* — D. Shoat (deceased), S. Latif, and J. Kennedy; *Laboratory Operations* — M.A. Bell and R. Birrell; and *Company Liaison and General Support* — M. Mellies, J. Meyer, and W. Campbell.

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Review

# High-Sensitivity C-Reactive Protein: A Novel and Promising Marker of Coronary Heart Disease

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***Background:*** **Coronary heart disease remains the leading cause of morbidity and mortality in the industrialized world. Clinical and laboratory studies have shown that inflammation plays a major role in the initiation, progression, and destabilization of atheromas. C-Reactive protein (CRP), an acute phase reactant that reflects low-grade systemic inflammation, has been studied in a variety of cardiovascular diseases.**

***Approach:*** **Findings from prospective clinical trials were examined to determine the prognostic utility of CRP in acute coronary syndromes, and observations from epidemiological studies were reviewed to determine the ability of CRP to predict future first coronary events. The analytical considerations of CRP measurement in these clinical applications were also examined.**

***Content:*** **In patients with established coronary disease, CRP has been shown to predict adverse clinical events. In addition, prospective studies have consistently shown that CRP is a strong predictor of future coronary events in apparently healthy men and women. The relative risk associated with CRP is independent of other cardiovascular disease risk factors. High-sensitivity CRP (hs-CRP) assays are needed for risk assessment of cardiovascular disease. Such assays are currently available but may require further standardization because patients' results will be interpreted using population-based cutpoints. Preventive therapies to attenuate coronary risk in individuals with increased hs-CRP concentrations include aspirin and statin-type drugs.**

***Summary:*** **hs-CRP has prognostic utility in patients with acute coronary syndromes and is a strong independent predictor of future coronary events in apparently healthy subjects.**



Coronary heart disease (CHD)[6] is the major cause of death in the developed world. Atherosclerosis, the underlying cause of most CHD, is a process that starts early in life and progresses slowly and silently for decades. The clinical manifestation usually occurs in the form of myocardial infarction (MI), stroke, angina, or sudden death between ages 50 and 60 years in men and between 60 and 70 years in women. Cholesterol screening has been used as a tool to identify individuals who are at increased risk of developing future coronary events. Although this approach has been useful, it fails to identify almost one-half of the 1.3 million individuals who develop MI in the US each year who have either normal or only moderately increased serum cholesterol concentrations.

Laboratory and clinical evidence has demonstrated that atherosclerosis is not simply a disease of lipid deposits. Rather, systemic inflammation also plays a pivotal role in atherothrombotic inception and progression *(1–3)*. Mononuclear cells, macrophages, and T lymphocytes are prominent in atheromatous plaques in the arterial wall *(4–7)*. Furthermore, the shoulder region of a plaque, the most vulnerable site for rupture in acute coronary syndromes, is heavily infiltrated with inflammatory cells *(8–10)*. Cytokines, which cause the de novo hepatic production of acute phase reactants such as C-reactive protein (CRP) *(11)*, have been shown to increase in acute coronary syndromes even in the absence of myocardial necrosis *(12)*. Therefore, CRP has been examined as a surrogate marker of other inflammatory mediators such as interleukin-6 and tumor necrosis factor-$\alpha$ to better understand the inflammatory component of atherosclero-

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[6] Nonstandard abbreviations: CHD, coronary heart disease; MI, myocardial infraction; hs-CRP, high-sensitivity C-reactive protein; RR, relative risk; 95% CI, 95% confidence interval; CARE, Cholesterol and Recurrent Events; MRFIT, Multiple Risk Factors Intervention Trial; PHS, Physicians' Health Study; PVD, peripheral vascular disease; WHS, Women's Health Study; TC, total cholesterol; HDL-C and LDL-C, HDL- and LDL-cholesterol.

sis *(13, 14)*. Current knowledge, however, suggests that the CRP concentration might reflect the vulnerability of the atheromatous lesion and the likelihood of a plaque to rupture *(2, 3, 15)*. This acute phase reactant has been studied over the last several years in a wide variety of atherosclerotic diseases *(12, 16–20)*. Its prognostic utility in acute coronary syndromes *(12, 16–20)* and its ability to predict future coronary events in apparently healthy men and women *(21–30)* have been demonstrated. The development of high-sensitivity CRP (hs-CRP) assays has been instrumental in exploration of the role of this acute phase reactant in predicting first cardiovascular events. Prospective studies have consistently demonstrated a positive association between hs-CRP and future coronary events. For hs-CRP to make the transition from clinical research to the routine clinical setting, however, several important issues must be satisfactorily addressed: (*a*) the availability of population-based cutpoints for interpretation and risk assessment; (*b*) the existence of potential therapeutic modalities; and (*c*) the reliability of the analytical systems used for measurement.

### hs-CRP as a Prognostic Indicator in Acute Coronary Syndromes

Several studies have demonstrated that hs-CRP, measured at either presentation or discharge, may have prognostic value in patients with acute coronary syndromes. Some reports have also examined the risk stratification of patients by hs-CRP alone or in combination with cardiac troponins.

Liuzzo et al. *(12)* showed that in 31 patients with severe unstable angina and no evidence of myocardial necrosis, as documented by the absence of increased cardiac troponin T, hs-CRP concentrations >3 mg/L at admission were associated with an increased incidence of recurrent angina, coronary revascularization, MI, and cardiovascular death. The same group later demonstrated that hs-CRP >3 mg/L at discharge in 53 unstable angina patients was associated with increased readmission for recurrent instability and MI *(16)*. In a similar study of unstable angina, Ferreiros et al. *(18)* concluded that the prognostic value of hs-CRP measured at discharge was better than that determined at admission in predicting adverse outcome at 90 days. Furthermore, hs-CRP was the strongest independent predictor of adverse events in multivariate analysis. Data from the Thrombolysis In Myocardial Infarction 11A (TIMI 11A), a study of unstable angina and non-Q-wave MI, showed that markedly increased hs-CRP (15.5 mg/L) at presentation in 437 patients was a good predictor of 14-day mortality in that population *(19)*. Furthermore, hs-CRP helped to identify those patients with negative cardiac troponin T (qualitative rapid bedside method with cutoff of <0.2 $\mu$g/L) who were at increased risk of mortality *(19)*. Morrow et al. *(19)* concluded from that study that a strategy for risk stratification using both cardiac troponin T and hs-CRP should be considered. Similar conclusions were reported in a follow-up report by the same group using serum amyloid A, another acute phase reactant, instead of hs-CRP *(31)*. A recent report by de Winter et al. *(17)* showed that hs-CRP concentrations >5 mg/L at admission in 150 patients with non-ST-elevation acute coronary syndromes were associated with an increased incidence of major cardiac events within 6 months, regardless of cardiac troponin I values.

### hs-CRP as a Predictor of Future Coronary Events

Over the last 6 years, several prospective studies have demonstrated that hs-CRP is a predictor of future cardiovascular morbidity and mortality among individuals with known cardiovascular disease. Data from the European Concerted Action on Thrombosis and Disabilities (ECAT) Angina Pectoris Study Group, a study of 2121 men and women with stable and unstable angina, demonstrated that each standard deviation increase in hs-CRP was associated with a 45% increase in the relative risk (RR) of nonfatal MI or sudden cardiac death [95% confidence interval (95% CI), 1.15–1.83] *(20)*. Similarly, in the Cholesterol and Recurrent Events (CARE) trial, hs-CRP was a predictor of recurrent coronary events in men and women who had already suffered a MI *(32)*. Those with hs-CRP concentrations in the highest quintile had an 80% higher chance of developing another coronary event within the 5-year study period (RR = 1.77; 95% CI, 1.1–2.9). Therefore, hs-CRP has the potential to be used in the stratification of patients into high- and low-risk groups.

Perhaps of greater clinical importance is the demonstration that hs-CRP concentrations predict first MI and stroke. To date, 10 prospective studies, 6 in the US and 4 in Europe, have consistently shown that hs-CRP is a powerful predictor of future first coronary event in apparently healthy men and women (Fig. 1). Findings from the Multiple Risk Factors Intervention Trial (MRFIT) demonstrated a direct positive association between hs-CRP and CHD mortality in men followed over a 17-year period (RR = 2.8; 95% CI, 1.4–5.4) *(22)*. This relationship, however, was evident only among smokers. A similar association between hs-CRP and future coronary events was noted in the Cardiovascular Health Study and Rural Health Promotion Project, which included men and women over 65 years of age with subclinical cardiovascular disease *(26)*. The Physicians' Health Study (PHS) demonstrated similar positive association between hs-CRP and future coronary events in apparently healthy men *(23)*. Unlike the observation in MRFIT, however, this association was evident in both smokers and nonsmokers. This study showed that those in the highest quartile of hs-CRP had a twofold higher risk of future stroke (RR = 1.9; 95% CI, 1.1–3.3), threefold higher risk of future MI (RR = 2.9; 95% CI, 1.8–4.6), and fourfold higher risk of future peripheral vascular disease (PVD; RR = 4.1; 95% CI, 1.2–6.0) *(23, 28)*. The RRs were stable over a long period of time (≥6 years) and independent of other CHD risk factors. The European MONICA (Monitoring Trends

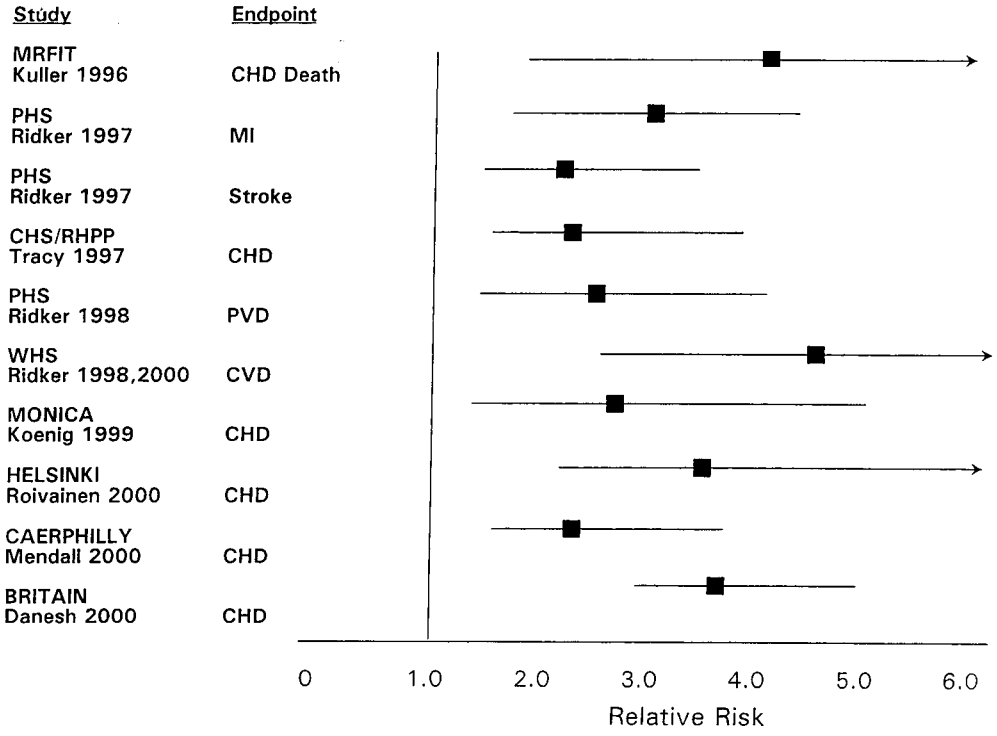


Fig. 1. Prospective studies of hs-CRP as a risk factor for future cardiovascular disease in populations of apparently healthy men and women. RR estimates (■) and 95% CIs (*lines*) are computed for those in the top compared with the bottom quartile. Data from Refs. *(21–30)*.

and Determinants in Cardiovascular Disease) Augsburg study showed that an increase of one standard deviation in the log-transformed value of hs-CRP was associated with a 50% increase in coronary risk and that subjects with hs-CRP concentrations in the highest quintile had a 2.6-fold higher risk of developing future coronary events *(21)*. A recent report from the Helsinki Heart Study confirmed these observations and demonstrated that those in the highest quartile of hs-CRP had a more than threefold higher risk of future MI or cardiac death (RR = 3.56; 95% CI, 1.93–6.57) *(27)*.

Two reports from the Women's Health Study (WHS) showed that hs-CRP is a strong predictor of future cardiovascular events in women (RR = 4.4; 95% CI, 2.2–8.9) *(24, 25)*. In stratified analyses, hs-CRP continued to be a strong predictor of future cardiovascular events even among subgroups of women with no history of hyperlipidemia, hypertension, smoking, diabetes, or family history of CHD *(25)*. The hs-CRP concentrations seen in these postmenopausal women were somewhat higher than those reported previously in men. Although no difference in hs-CRP values was noted between premenopausal women and age-matched males, recent reports showed that hormone replacement therapy (estrogen alone or estrogen and progestin) is associated with increased hs-CRP concentrations *(33, 34)*. These findings suggest that the increased hs-CRP seen in the WHS subjects may reflect the influence of hormone replacement therapy rather than the effect of gender.

## Predictive Value of hs-CRP and Other Biochemical Markers for CHD Risk

The RR estimates derived from most of the above-mentioned prospective studies were independent of other recognized cardiovascular risk factors. Data from both the PHS *(35)* and WHS *(24)* showed that the predictive value of hs-CRP was significantly higher than that associated with traditional biochemical CHD risk markers [total cholesterol (TC), HDL-cholesterol (HDL-C), and LDL-cholesterol (LDL-C)] or novel markers [lipoprotein(a), homocysteine, apolipoproteins AI and B]. In women, for example, the univariate RR of future cardiovascular events presented in Fig. 2 demonstrate that hs-CRP was the single strongest predictor of risk (RR = 4.4; 95% CI, 2.2–8.9). In comparison, LDL-C, a well-established marker of CHD, was a lesser predictor of future risk (RR = 2.4; 95% CI, 1.3–4.6). Furthermore, in a multivariate analysis that accounted for other CHD risk factors (obesity, hypertension, diabetes, family history), only hs-CRP and the ratio of TC to HDL-C had independent predictive value. In the same study of postmenopausal women *(24)*, hs-CRP was shown to predict risk among those with LDL-C values <1300 mg/L, a concentration deemed "desirable" by the current National Cholesterol Education Program guidelines for primary prevention. In this subgroup (mean LDL-C, 1040 mg/L), the RRs of future MI, stroke, and coronary revascularization in the lowest to the highest quartiles of hs-CRP were 1.0, 2.4, 2.9, and 4.1, respectively (95% CI for the 4th vs 1st quartile, 1.7–11.3).

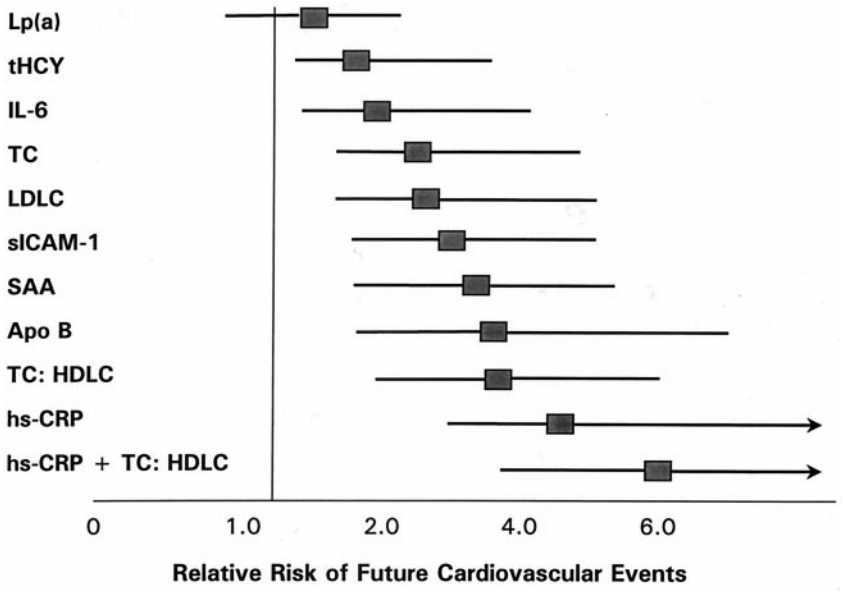


Fig. 2. RRs for future cardiovascular events among apparently healthy women in the WHS according to baseline values of several biochemical markers.

For consistency, risk estimates (■) and 95% CIs (*lines*) are computed for those in the top compared with the bottom quartile for each marker. *Lp(a)*, lipoprotein(a); *tHCY*, total homocysteine; *IL-6*, interleukin-6; *sICAM-1*, soluble intercellular adhesion molecule-1; *LDLC*, LDL-C; *SAA*, serum amyloid A; *Apo B*, apolipoprotein B; *HDLC*, HDL-C. Adapted from Ridker et al. *(24)*.

After adjustment for other CHD risk factors and concentration of HDL-C, the RR associated with hs-CRP remained highly significant (RR = 3.1; 95% CI, 1.1–8.3) and increased ~39% with each increasing quartile of hs-CRP. This study thus demonstrated that hs-CRP can identify individuals at increased risk of developing future coronary events who otherwise would be missed if only lipid measurements were used. Other examined markers of inflammation, e.g., serum amyloid A, interleukin-6, and soluble intercellular adhesion molecule-1, showed consistent association but a slightly weaker RR of future coronary events.

The predictive value of hs-CRP in men and women increased considerably when evaluated in models that included lipid values. Data from the PHS demonstrated that, compared with those with TC and hs-CRP below the 75th percentile, those with increased TC alone had a 2.3-fold increase in risk (95% CI, 1.5–3.7), whereas those with increased hs-CRP alone had a 1.5-fold increase in risk (95% CI, 0.9–2.4) *(35)*. In contrast, the risk of developing coronary events increased 5-fold (95% CI, 2.5–9.8) among those with high concentrations of both TC and hs-CRP. Therefore, the joint effects of both risk factors are greater than the product of the individual effects of each risk factor considered alone. Furthermore, when the study participants were stratified according to quintile of hs-CRP and quintile of TC:HDL-C ratio, the RR of first coronary event in those in the highest quintiles of both hs-CRP and TC:HDL-C ratio was approximately ninefold higher than that of men in the lowest quintiles of these analytes. Data from the WHS demonstrated similar findings such that women in the highest quintile of both hs-CRP and TC:HDL-C ratio had a RR more than eightfold higher than that of women in the lowest quintiles (Fig. 3). In all of these analyses, risk prediction models that incorporated lipids were significantly better ($P < 0.001$) than those based on hs-CRP alone *(24)*.

### Interpretation of hs-CRP Values

For the purpose of assessing risk of future first coronary events, hs-CRP concentration should be interpreted using cut points established by prospective clinical studies. Each patient will be classified into a quintile of risk, depending on the hs-CRP concentration. Therefore, the reporting of hs-CRP results focuses on the quintile of risk and not on the actual mass concentration.

The within-person biologic variability of hs-CRP is low over a long period of time *(36)*. Laboratory measurements on paired samples obtained from 236 subjects at baseline and 5 years later showed that an individual's log-normalized hs-CRP concentrations are highly correlated ($r = 0.60$). Somewhat comparable correlation coefficients were noted for TC ($r = 0.37$), LDL-C ($r = 0.32$), HDL-C ($r = 0.74$), and triglycerides ($r = 0.49$) over the 5-year follow-up period. This finding lends further support to the fact that hs-CRP is a good and biologically stable predictor of future MI despite the fact that it is an acute phase reactant, providing that the patient is not suffering from an active infection or using a drug that affects hs-CRP concentration. hs-CRP values >15 mg/L (~99th percentile of the general population) indicate an active inflammation; patients should be advised to have a repeat measurement in 2–3 weeks or after the infection in resolved.

As indicated earlier, models containing both hs-CRP and TC or the TC:HDL-C ratio were better able to predict future first coronary events than those containing hs-CRP alone. The RRs of future first coronary events for men and women as well as lipid concentrations were computed in quintiles from the PHS and WHS databases, respectively,

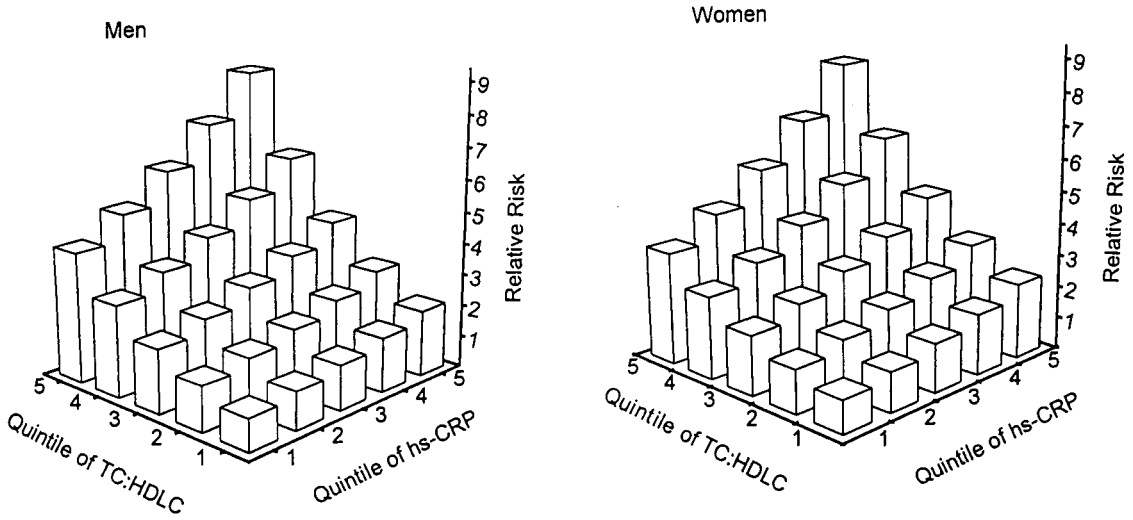


Fig. 3. RRs of first coronary event among apparently healthy men (*left*) and women (*right*) associated with different hs-CRP concentrations and TC:HDL-C ratios.

Adapted from Ridker and co-workers *(23, 24)*.

and are presented in Fig. 3. Because the computed RRs did not vary significantly between men and women, a single risk assessment algorithm is suggested for both genders (Table 1) *(37)*. The hs-CRP concentrations were derived from ongoing population-based surveys. It is important to note that it is not necessary in this case to report the actual hs-CRP concentration to the clinician but only the patient's RR. The clinical laboratory should play an active role in the interpretation and implementation of this clinical application. Providing incomplete information or just the actual hs-CRP concentration will only frustrate and prevent the clinician from correctly interpreting the data and managing the patient.

## Potential Preventive Therapies

Although no specific therapies have been developed to decrease hs-CRP and there is no direct evidence that risk of future cardiovascular events is diminished by reducing hs-CRP, studies have shown that aspirin *(23)* and pravastatin *(32)* are effective in decreasing the incidence of future coronary events in those with increased hs-CRP concentration. These studies suggest that the two examined drugs possess antiinflammatory characteristics.

Among apparently healthy men in the PHS with increased hs-CRP (>2.1 mg/L), aspirin use decreased the risk of future MI by almost 60% *(23)*. In contrast, aspirin use was associated with a much smaller, although statistically significant, 14% decrease in future MI among men with low hs-CRP (<0.55 mg/L). Although the magnitude of reduction in future risk of MI depended on the concentration of hs-CRP, it is important to note that all subjects benefited from aspirin use. These findings suggest that aspirin was acting not only as an antiplatelet agent but also as an antiinflammatory drug.

Similar findings were also noted with pravastatin use in the CARE study *(32)*. As indicated earlier, CARE is a prospective study of men and women with average lipid concentrations who have suffered an MI. Participants

**Table 1. RR estimates for future coronary events in men and women associated with quintiles of hs-CRP and TC:HDL-C ratio.[a]**

| | | | Quintile of hs-CRP, mg/L | | | | |
|---|---|---|---|---|---|---|---|
| | Men | Women | 1 (<0.7) | 2 (0.7–1.1) | 3 (1.2–1.9) | 4 (2.0–3.8) | 5 (3.9–15.0) |
| **Quintile of TC:HDL-C ratio** | | | | | | | |
| **1** | **<3.4** | **<3.4** | 1 | 1.2 | 1.4 | 1.7 | 2.2 |
| **2** | **3.4–4.0** | **3.4–4.1** | 1.4 | 1.7 | 2.1 | 2.5 | 3 |
| **3** | **4.1–4.7** | **4.2–4.7** | 2 | 2.5 | 2.9 | 3.5 | 4.2 |
| **4** | **4.8–5.5** | **4.8–5.8** | 2.9 | 3.5 | 4.2 | 5.1 | 6 |
| **5** | **>5.5** | **>5.8** | 4.2 | 5 | 6 | 7.2 | 8.7 |

[a] RR estimates and TC:HDL-C ratio were derived from the PHS *(23)* and the WHS *(24)* databases. hs-CRP concentrations were derived from ongoing population-based surveys.

were randomized between 40 mg of pravastatin per day and placebo and followed for 5 years *(38)*. Study participants with high hs-CRP (>9.9 mg/L or 90th percentile) at baseline experienced a reduction of 54% in the incidence of recurrent coronary events compared with a reduction of 25% in those with low hs-CRP (<9.9 mg/L or 90th percentile), although baseline lipid values were almost identical in the two groups. Moreover, during the 5-year follow-up, pravastatin lowered mean hs-CRP by almost 40%. This represented a 22% difference at 5 years in median hs-CRP between the pravastatin and placebo groups. Furthermore, the magnitude of change in hs-CRP appeared to be unrelated to that of LDL-C in both the pravastatin and placebo groups. These findings suggest that pravastatin may have antiinflammatory characteristics that are independent from its lipid-lowering property. Clinical trials are currently ongoing to further explore the interaction between pravastatin, aspirin, and the inflammatory response in primary and secondary prevention settings.

### Interrelationships with Other CHD Risk Factors

Several CHD risk factors appear to modulate the inflammatory response and affect hs-CRP concentration. Obesity, for example, is directly associated with increased hs-CRP concentrations, an intriguing observation considering that interleukin-6, the primary stimulant of the de novo hepatic synthesis of CRP, is secreted by adipose tissue *(39, 40)*. Therefore, the attenuation of the inflammatory response may represent a mechanism by which diet and weight loss reduce cardiovascular risk. Cigarette smoking has also been shown to increase the concentration of several inflammatory markers, including hs-CRP, interleukin-6, and soluble intercellular adhesion molecule-1. Increases of both interleukin-6 *(41)* and soluble intercellular adhesion molecule-1 *(42)* were shown to be associated with increased risk of future first coronary events in both men and women. Smoking cessation decreases these markers. Diabetic patients are reported to have increased hs-CRP values *(43)*; In this regard, links between hs-CRP and the insulin resistance syndrome have also been reported *(44)*. In addition, experimental findings suggest that increased blood pressure promotes endothelial expression of cytokines and inflammatory activation *(6, 45, 46)*. These observations suggest that perhaps better control of diabetes and hypertension may attenuate the contribution of the inflammatory response to overall cardiovascular risk. Finally, physical exercise has been shown to have a beneficial effect in terms of reducing the concentration of several inflammatory markers *(47, 48)*. Taken together, the available evidence thus supports the hypothesis that hs-CRP concentrations correlate with endothelial dysfunction *(49)*.

### Analytical Considerations in the Measurement of hs-CRP

Historically, CRP has been measured in clinical laboratories by immunoturbidimetric and immunonephelometric assays designed to detect active inflammation and infection. The dynamic range of these assays spans from 3 mg/L (~90th percentile of the general population) to well over 200 mg/L. Such traditional assays, however, do not have appropriate sensitivity in the range required for the determination of cardiovascular risk in apparently healthy men and women.

To achieve the desired limit of quantification, manufacturers and investigators have continued to use immunochemical techniques in their attempts to measure hs-CRP, but with modifications to increase the detectable signal. Several approaches have been used, including the labeling of anti-CRP antibodies with either an enzyme (ELISA) or a fluorescent compound, and attaching the antibodies, either monoclonal or polyclonal, to polystyrene beads *(50–55)*. The latter approach was popular among manufacturers because it enabled the adaptation to commonly used automated analyzers in clinical chemistry laboratories. Currently, hs-CRP concentrations as low as 0.15 mg/L (<2.5th percentile of the general population) can be reliably measured. It is important to note, however, that not all hs-CRP assays possess a similar sensitivity or lower limit of quantification *(56)*. For practical considerations, it is advisable to have a single CRP assay in the clinical laboratory that is capable of measuring low and high concentrations. However, if that is impossible, clinicians should be aware of the availability of two different CRP assays and request hs-CRP for cardiovascular risk prediction purpose.

Because the hs-CRP value of an individual patient is interpreted in the context of cutpoints established by prospective clinical studies, standardization of hs-CRP assays is crucial. Poor agreement among methods will lead to misclassification and mismanagement of patients. Recent reports have indicated that the measurement of low hs-CRP concentrations is not consistent among various methods, suggesting that standardization efforts are needed *(51, 56)*. In one case where a significant bias was noted between two methods *(51)*, the manufacturers of both reagent systems claimed to have their calibrators traceable to the WHO reference materials. Unfortunately, this is not an unusual occurrence. Although manufacturers attempt to standardize their assays using the WHO calibrators, they often fail to follow the appropriate value transfer protocol from the reference materials to their own calibrators *(57)*. Invariably, this leads to suboptimal standardization.

An in-house hs-CRP ELISA method *(52)*, utilizing polyclonal antibodies from Calbiochem, was used in MRFIT, the Cardiovascular Health Study, and the Rural Health Promotion Project as well as in the early work from the PHS. The analytical performance and clinical efficacy of the ELISA assay were compared with those of an automated and commercially available latex-enhanced method (Dade Behring) *(51)* used at present in several prospective studies, including the PHS, WHS, Women's Health Initiative, Nurses' Health Study, Health Profes-

sionals' Study, and Texas/Air Force Coronary Atherosclerosis Prevention Study *(58)*. The two assays were evaluated using plasma samples from the PVD cohort of the PHS in a nested case-control design. Excellent analytical agreement between the two methods was reported (slope = 0.99; intercept = 0.36 mg/L; $r = 0.95$) *(58)*. In addition, for both methods, the calculated RRs of developing future PVD increased significantly with each increasing quartile of hs-CRP. The calculated interquartile increase in RR of PVD was 31% (95% CI, 5.2–62.2) for the ELISA and 34% (95% CI, 8.2–66.1) for the latex-enhanced method. Furthermore, all but two participants were classified into concordant quartiles or varied by only one quartile. This study demonstrated comparable clinical efficacy of the two methods and linked the earlier and the current data, thus assuring consistency among reported hs-CRP values. On the basis on this report, the US Food and Drug Administration approved the use of this latex-enhanced method in the risk assessment of cardiovascular disease. Therefore, this latex-enhanced method is usually used as the reference procedure when comparison studies of various hs-CRP assays are conducted. At present, only a few hs-CRP methods are commercially available. However, several assays are currently under development or evaluation and are expected to be available for routine clinical use in the very near future.

## CONCLUSION

hs-CRP is a very promising novel biochemical marker for the prediction of future first or recurrent coronary events. American and European prospective studies have been highly consistent regarding the ability of hs-CRP to predict future CHD risk in both men and women. Potential preventive therapeutic modalities to attenuate coronary risk in those with increased hs-CRP concentrations have been suggested. The potential use of hs-CRP as a means to improve the cost-to-benefit ratio in statin therapy is currently under investigation. Although standardization of hs-CRP measurement at the lower concentration range among various methods should be addressed, a robust and Food and Drug Administration-approved method is currently available. Several other sensitive assays are under development and are expected to be commercially available soon.

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# Inflammatory Cytokines Stimulated C-Reactive Protein Production by Human Coronary Artery Smooth Muscle Cells

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***Background***—Serum C-reactive protein (CRP) levels are good predictors of the development of cardiovascular events in apparently healthy men and women. CRP has been believed to be produced exclusively by hepatocytes during the acute-phase response. Several lines of evidence have suggested that atherosclerotic arteries can also produce CRP. However, the cell types that produce CRP locally in the atherosclerotic arterial wall have not been clearly identified.

***Methods and Results***—Human coronary artery smooth muscle cells (HCASMCs) and human umbilical vein endothelial cells (HUVECs) were incubated with interleukin-1$\beta$ (IL-1$\beta$), IL-6, their combination, tumor necrosis factor-$\alpha$ (TNF-$\alpha$), or lipopolysaccharide (LPS) at different concentrations. The supernatants were concentrated and analyzed by a high-sensitivity enzyme-linked immunosorbent assay specific for human CRP. RNA was extracted from the HCASMCs for reverse transcriptase–polymerase chain reaction (RT-PCR) using specific primers for the CRP. Maximal CRP production was observed in HCASMCs after 48 hours of incubation with the combination of 25 ng/mL of IL-1$\beta$ and 10 ng/mL of IL-6, whereas incubation with IL-1$\beta$ or IL-6 alone only modestly induced CRP. Incubation with TNF-$\alpha$ (50 ng/mL) or LPS (1000 EU/mL) resulted in an increase in CRP production comparable to the IL-1$\beta$ and IL-6 combination. The induction of CRP in HCASMCs was independently confirmed by RT-PCR comparing the relative CRP mRNA levels. The induction of CRP production by HCASMCs was not reproduced in HUVECs, however.

***Conclusions***—These results demonstrated that HCASMCs, but not HUVECs, could produce CRP in response to inflammatory cytokines. The locally produced CRP could directly participate in atherogenesis and the development of cardiovascular complications. **(*Circulation*. 2003;108:1930-1932.)**

**Key Words:** atherosclerosis ■ inflammation ■ muscle, smooth ■ interleukins ■ risk factors

C-reactive protein (CRP), a marker of inflammation, is an important predictor of future cardiovascular events in apparently healthy men and women[1–4] and could directly participate in the pathogenesis of atherosclerosis through activation of endothelial cells.[5–9] CRP, named for its capacity to bind to the C-polysaccharide of *Streptococcus pneumoniae*, was the first acute-phase protein to be described.[10] CRP, like other acute-phase proteins, is synthesized by the liver in response to microbial infection, tissue injury, and autoimmune disorders. It had been shown that interleukin-1$\beta$ (IL-1$\beta$) and IL-6 strongly induced the expression of CRP in human hepatocytes[11] and hepatoma cells.[12] Recently, human neuronal cells were found to produce CRP in Alzheimer's disease.[13] In addition, renal cortical tubular epithelial cells were shown to produce CRP after inflammatory stimuli.[14] Interestingly, CRP has also been found in human atherosclerotic plaques,[15] which could be the result of indirect deposit from circulating cells or direct production by cells in the arterial wall. We show that human coronary artery smooth muscle cells (HCASMCs), but not human umbilical vein endothelial cells (HUVECs), can synthesize CRP after stimulation by inflammatory cytokines.

## Methods

### Cell Culture

HCASMCs, HUVECs, and endothelial cell supplements were purchased from Cascade Biologics; penicillin, streptomycin, medium 231, medium 199, and smooth muscle cell growth supplement were from Gibco BRL; and fetal bovine serum, human serum, heparin, and gelatin were obtained from Sigma. HCASMCs were plated onto 0.1% gelatin-coated culture dishes from Corning, Inc, and grown in 231 medium with growth supplement and 1% penicillin/streptomycin; HUVECs were plated onto 0.1% gelatin-coated culture dishes and grown in 199 medium with endothelial growth supplement, heparin, antibiotics, and 15% fetal bovine serum. Cells were used at passage 5 to 7.

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### CRP Assays

CRP level in the cell supernatant was measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit specific for human CRP (Diagnostic System Laboratories) according to the manufacturer's directions. The minimum detectable concentration of the assay was 1.6 ng/mL. All the experiments were performed in triplicate. Cells were cultured in 6-well plates until 80% to 90% confluent and were incubated for 48 hours with recombinant human IL-1$\beta$ (R&D Systems) (25 ng/mL), recombinant human IL-6 (R&D Systems) (10 ng/mL), their combination, recombinant human tumor necrosis factor-$\alpha$ (TNF-$\alpha$) (R&D Systems) (50 ng/mL), or lipopolysaccharide (LPS) derived from *Escherichia coli* O113:H10 (Associates of Cape Cod, Inc) (1000 EU/mL); the culture supernatants were then concentrated ($\approx$10 times) using centrifugal filter units (Millipore) and assayed for CRP.

### CRP mRNA Expression

Cells cultured in 60-mm plates were incubated for 48 hours with 25 ng/mL IL-1$\beta$, 10 ng/mL IL-6, their combination, 50 ng/mL TNF-$\alpha$, and 1000 EU/mL LPS, and total cellular RNA was extracted by Trizol reagent. Reverse-transcriptase polymerase chain reaction (RT-PCR) was performed with the Access RT-PCR System (Promega) according to the manufacturer's directions. For each reaction, 1 $\mu$g of total RNA served as a template. For amplification, a primer pair specific for human CRP (forward, TCGTATGCCACCAAGAGACAAGACA; reverse AACACTTCGCCTTGCACTTCATACT; GenBank accession No. M11725) was used. These primers were designed to yield a product of 440 bp after 40 amplification cycles. In all experiments, control reactions were performed substituting sterile nuclease-free water for the RNA template in the reaction. Glyceraldehyde-3-phosphate dehydrogenase (GADPH) was amplified as a reference for quantification of CRP mRNA. The RT-PCR products were visualized on 1% agarose gel with ethidium bromide.

## Results

### CRP Production by HCASMCs, but Not by HUVECs

The results of CRP released into the media and the CRP mRNA levels in HCASMCs after treatment with inflammatory cytokines were shown in Figures 1 and 2, respectively. As shown in Figure 1A, CRP production was minimal without stimulation, and incubation of HCASMCs with 50 ng/mL of IL-1$\beta$ or 10 ng/mL of IL-6 alone led to a small but significant induction. Maximal CRP production was observed after the combination of the 2 cytokines (Figure 1B). TNF-$\alpha$ or LPS also induced a similar level of CRP production and showed a dose-responsive relationship (Figure 1C). In contrast, CRP production could not be detected in HUVECs after similar stimulation protocols (data not shown). To confirm the results of CRP protein production in HCASMCs, we also assayed the mRNA levels in HCASMCs by RT-PCR. Figure 2 shows CRP mRNA levels in HCASMCs after the different treatments. IL-1 $\beta$ plus IL-6 combination caused a significant increase in CRP mRNA level compared with baseline. Treatment with LPS and TNF-$\alpha$ also upregulates the CRP mRNA levels. The RT-PCR amplified band was confirmed to be authentic CRP by direct sequencing.

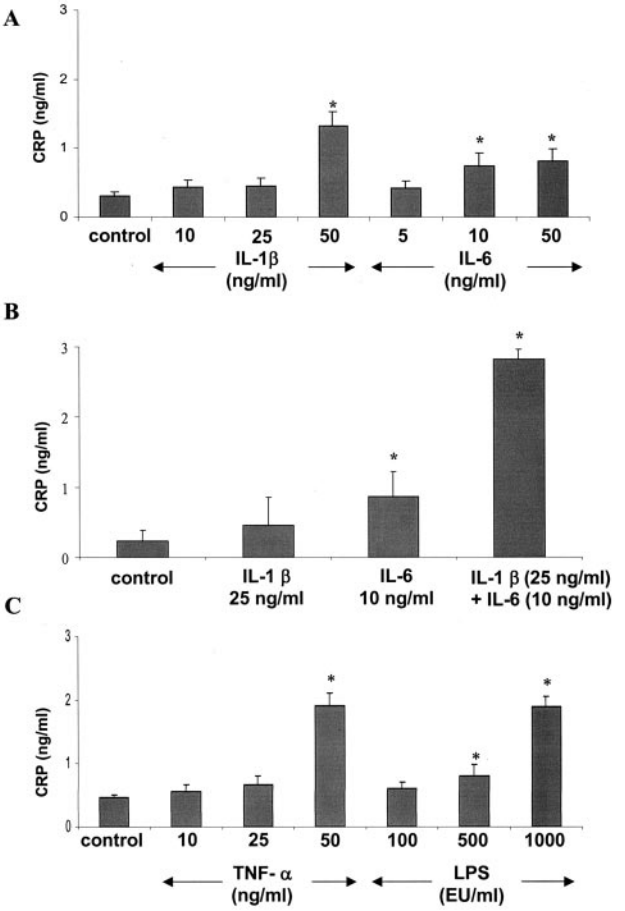


**Figure 1.** Effect of cytokine and LPS treatment on CRP protein production in HCASMCs. HCASMCs were incubated with different stimuli for 48 hours and supernatants were analyzed for CRP. Data represent a mean$\pm$SD. This has also been repeated 3 times. Statistically significant CRP productions ($P<0.05$) were indicated by an asterisk. The data were analyzed using one-way analysis of variance followed by the Scheffe test for multiple comparisons.

## Discussion

CRP has been shown to be an excellent predictor of future cardiovascular events in apparently healthy men and women.[3,4] This could be in part the result of some of the biological properties of CRP such as its stability, lack of diurnal variation, and lack of influence of gender and age.[10] However, accumulating evidence also points to the possibility that CRP is a direct participant in vascular inflammation.[16] One of the outstanding unresolved issues in this field is the source of CRP production in humans. It has been previously assumed that hepatocytes are the only source of CRP production during the acute-phase response. During the acute-phase reaction, serum CRP levels often increase up to 100 or 200 $\mu$g/mL; however, the level of serum CRP that is useful for predicting cardiovascular risk is 1 to 3 $\mu$g/mL. In fact, patients with serum CRP levels $>10$ $\mu$g/mL should have the test repeated at a late date to exclude infection, autoimmune diseases, or malignancy.[17] Thus, we sought to identify another source of CRP production that could help explain the lower level of CRP useful for cardiovascular risk prediction.

We show that CRP is produced by HCASMCs, but not by HUVECs, after exposure to inflammatory cytokines. This locally produced CRP could play an important role in the activation of endothelial cells.[5–9] Two studies have shown that both epithelial cells of the respiratory tract and renal epithelium produce CRP.[14,18] Moreover, neuronal cells also

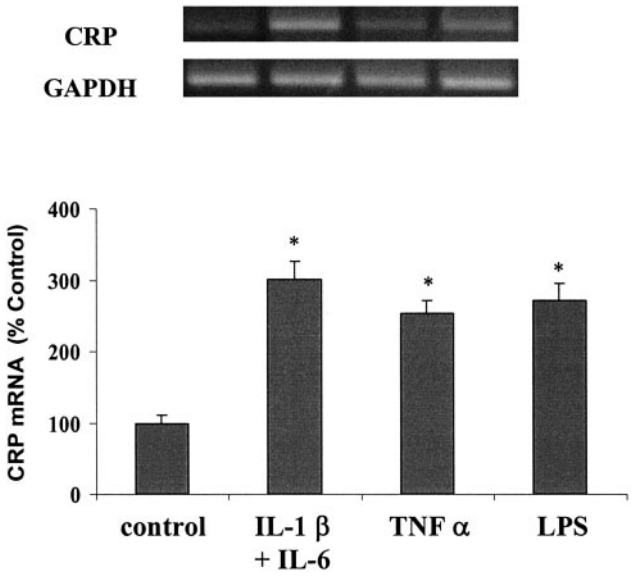


**Figure 2.** Effect of cytokine and LPS treatment on mRNA levels in HCASMCs. HCASMCs were incubated with different stimuli for 48 hours and CRP mRNA expression assessed by RT-PCR. This has also been repeated 3 times. Statistically significant CRP productions ($P<0.05$) were indicated by an asterisk. The data were analyzed using one-way analysis of variance followed by the Scheffe test for multiple comparisons.

seem to be capable of synthesizing acute-phase reactants involved in the pathogenesis of neurodegenerative disease.[13] These studies expand the variety of cell types that could participate in CRP production; however, relevance of these observations to the pathogenesis of atherosclerosis is doubtful. CRP has been observed to colocalize with the terminal complement complex in atherosclerotic plaques.[19] In this report, however, the authors suggested that CRP is deposited from circulating CRP produced by the liver instead of local synthesis. In contrast, work by Yasojima et al.[15] suggested that cells in the arterial wall synthesize CRP. Using in situ hybridization techniques, the authors showed that elongated muscle-like cells inside the atherosclerotic plaque were positive for CRP. Our findings, thus, provide a direct demonstration that HCASMCs, but not HUVECs, are a source of locally produced CRP in the arterial wall. The locally produced CRP can directly participate in atherogenesis and the development of cardiovascular complications.

Isolated human hepatocytes can produce 10 times more CRP compared with control after stimulation.[20] Human hepatoma cell line HepG2 is able to produce CRP ≈4-fold compared with control in conditions similar to those used in our experiments.[21] Another hepatoma cell line Hep3B stimulated with conditioned medium (generated by stimulating peripheral blood mononuclear cell with LPS at a dose of 1 μg/mL for 24 hours) showed an ≈50-fold increase in CRP mRNA compared with unstimulated cells.[14] Thus, CRP productions by human coronary artery smooth muscle cells is less robust than those produced by the liver. This could partly account for the lower serum level of CRP (1 to 3 mg/L) useful for cardiovascular risk prediction and the high level of CRP (>10 mg/L) observed during the acute-phase response.

## Acknowledgment

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# C-Reactive Protein

## A Simple Test to Help Predict Risk of Heart Attack and Stroke

Paul M Ridker, MD, MPH

Of the 1.5 million heart attacks and 600 000 strokes that occur in the United States each year, almost half will affect apparently healthy men and women with normal or even low cholesterol levels. Older age, smoking, diabetes, and high blood pressure all contribute to risk of heart disease. However, you may well have family members or friends who suffer from heart disease yet have few, if any, of these traditional risk factors.

In an effort to better determine risk of heart disease and prevent clinical events, many physicians have begun to measure C-reactive protein (CRP) as a routine part of global risk assessment. This inexpensive and simple approach to heart disease evaluation has recently been endorsed by both the Centers for Disease Control and Prevention and by the American Heart Association. When measured with new "high sensitivity" CRP assays, levels of CRP less than 1, 1 to 3, and greater than 3 mg/L (milligrams per liter) discriminate between individuals with low, moderate, and high risk of future heart attack and stroke. CRP testing, however, is not a replacement for cholesterol evaluation. Rather, CRP testing should be used along with cholesterol and other traditional risk factors to determine individual risk. Evidence also indicates that individuals with high CRP levels are at increased risk of developing diabetes. This Cardiology Patient Page explains the clinical use of CRP and suggests methods for prevention of heart disease for patients found to have elevated levels of CRP.

### What Is CRP?

CRP is a critical component of the immune system, a complex set of proteins that our bodies make when faced with a major infection or trauma. CRP was discovered nearly 70 years ago by scientists exploring the human inflammatory response. The role CRP plays in heart disease, however, has only recently been uncovered.

Everyone makes CRP, but in different amounts depending on a variety of factors, including genetics as well as lifestyle habits. On average, individuals who smoke, have high blood pressure, are overweight, and fail to exercise tend to have high levels of CRP, whereas thin, athletic individuals tend to have lower levels. Nonetheless, almost half of the variation in CRP levels between different people is inherited and thus reflects levels that your parents and grandparents have passed on to you through their genes. This is not surprising given the fundamental role that CRP plays in inflammation, an extremely important process for wound healing, for warding off bacteria and viruses, and for many key processes critical for survival. Research over the past decade has shown that too much inflammation in some circumstances can have adverse effects, particularly on the blood vessels that carry oxygen and nutrients to all the tissues of the body. Scientists now understand that atherosclerosis (the process that leads to cholesterol accumulation in the arteries) is in many ways an inflammatory disorder of the blood vessels, just as arthritis is an inflammatory disorder of the bones and joints.

Many studies have found that blood markers that reflect the inflammatory process are elevated among individuals at high risk for future heart disease. Inflammation is impor-

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Dr Ridker is named as a coinventor on patents filed by the Brigham and Women's Hospital that relate to the use of inflammatory markers in cardiovascular disease. Dr Ridker is supported by grants from the National Heart, Lung, and Blood Institute and receives additional research support from the Leduq Foundation (Paris, France), the Doris Duke Charitable Foundation (New York, NY), and the Donald W. Reynolds Foundation (Las Vegas, Nev).

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tant in all phases of heart disease, including the early initiation of atherosclerotic plaques within the arteries, as well as the acute rupturing of these plaques that results in heart attack and, all too often, sudden death. Until recently, available markers of inflammation were not suitable for use in physicians' offices. By contrast, CRP is very stable and quite easy to measure.

## CRP and the Risk of Cardiovascular Disease

Over a dozen major studies demonstrate that baseline levels of CRP in apparently healthy men and women are highly predictive of future risk of heart attack, stroke, sudden cardiac death, and the development of peripheral arterial disease. Doctors also know that CRP levels predict recurrent coronary events among patients who already suffer from heart disease and that the prognosis of patients in the acute phase of a heart attack is tightly linked to CRP levels. However, the most important current use of CRP is in primary prevention, that is, in the detection of high risk among individuals not yet known to have a problem.

Individuals with elevated levels of CRP have a risk about 2 to 3 times higher than the risk of those with low levels. It is important that your physician request a "high-sensitivity" test for CRP if he or she is using CRP for the purpose of cardiovascular risk assessment. This is because older tests for CRP, which are adequate for monitoring severe inflammatory conditions, do not have the ability to measure levels accurately within the range needed for cardiac risk detection. To remind doctors of this issue, many outpatient laboratories now specifically note on the laboratory request form that the test offered is for "high-sensitivity CRP" or "hs-CRP." Like the cholesterol test, the test for hs-CRP is nothing more than a simple, inexpensive blood test. The easiest way to assess overall risk—and avoid an additional needle stick—is simply to add a CRP evaluation at the time of cholesterol screening.

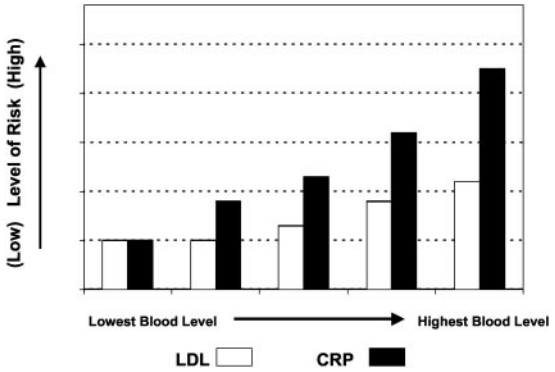


**Figure 2.** hs-CRP is a stronger predictor of heart attack and stroke than LDL cholesterol. Adapted with permission from Ridker et al (*N Engl J Med*. 2002;347:1557–1565).[5] Copyright © 2002 Massachusetts Medical Society. All rights reserved.

## Why Do I Need Both CRP and Cholesterol Measured?

Both cholesterol and CRP predict risk, but you cannot predict your CRP level on the basis of your cholesterol level (or vice versa). That is because each of these blood tests picks up a different component of the disease process. This independent and additive effect is demonstrated in Figure 1, which shows cardiovascular event-free survival for initially healthy individuals according to levels of both CRP and the so-called "bad cholesterol" or LDL cholesterol. As shown, the worst survival (highest risk) is seen among those with high levels of *both* LDL and CRP, while the best survival (lowest risk) is among those with low levels of both markers. However, one person in four will be in the high CRP/low LDL group. Such individuals are at a level of risk greater than that of individuals in the low CRP/high LDL category. Without CRP evaluation, such individuals would be missed if their physicians relied on cholesterol screening alone.

It is important to recognize that high levels of LDL cholesterol remain a critical risk factor and that aggressively lowering LDL cholesterol is a fundamental goal of cardiovascular disease prevention. However, as shown in Figure 2, CRP is actually a stronger overall predictor of heart disease and stroke than is LDL cholesterol. Thus, recent practice recommendations have been to measure cholesterol levels and CRP together and to base

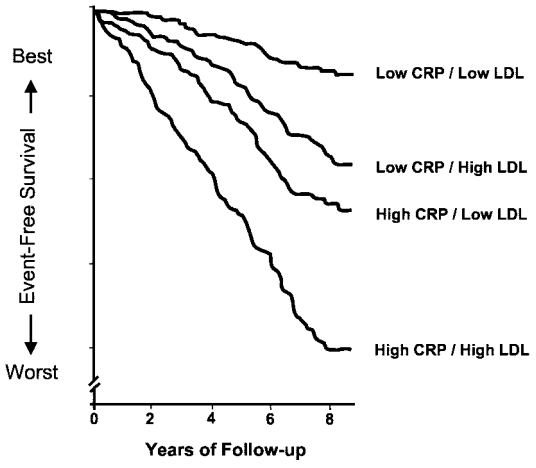


**Figure 1.** Cardiovascular event-free survival based on combined hs-CRP and LDL cholesterol measurements. Adapted from Ridker et al (*N Engl J Med* 2002;347:1557–1565).[5]

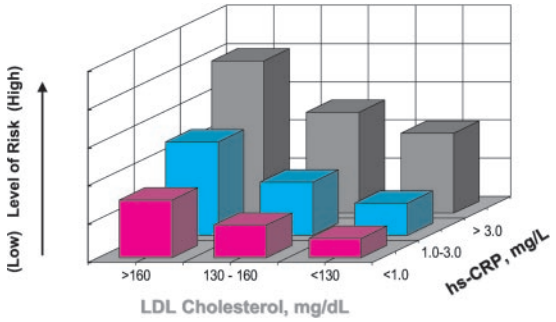


**Figure 3.** hs-CRP improves risk prediction at all levels of LDL cholesterol. Adapted from Ridker et al (*N Engl J Med* 2000;342:836–843).[6]

interventions on the combined information each provides (see below and Figure 3).

In many ways, a decision to test for CRP is similar to the decision to test for cholesterol; knowledge that levels are high should motivate you to lose weight, to diet, to exercise, and to stop smoking. All of these lifestyle changes are well known to reduce the risk of ever getting heart disease, and they all lower CRP levels.

## How Does CRP Compare With Other "Novel Risk Factors"?

CRP is a powerful predictor of risk, particularly when combined with cholesterol evaluation. Some physicians choose to measure CRP along with a panel of other "novel" risk factors including homocysteine and lipoprotein(a). Others may elect to measure CRP along with more expensive tests that measure specific cholesterol subfractions. However, in all direct comparisons, the predictive value for CRP has been substantially greater than that observed for these alternative "novel" markers of risk. Further, only CRP has proven to add important prognostic information to that already available from standard cholesterol screening.

In some communities, imaging techniques including "whole-body scans" that detect calcification in the heart arteries and the aorta have been advocated as screening techniques. While the presence of calcification does increase cardiovascular risk, such scans are not recommended by the American Heart Association and currently are very expensive. An additional concern for these imaging techniques is that results are often misinterpreted by patients and physicians and can lead to unnecessary coronary interventions, including angioplasty and bypass surgery. While CRP levels also have been shown to add prognostic information at all levels of coronary calcium, this information should be used primarily to motivate at-risk individuals to adopt more heart-healthy lifestyles, not to seek aggressive interventional cardiac procedures.

## How Does CRP Affect Diabetes and the Metabolic Syndrome?

Unlike LDL cholesterol, CRP predicts not only heart disease, but also the risk of developing type 2 diabetes. Individuals with CRP levels greater than 3 mg/L have a risk of getting diabetes 4 to 6 times higher than individuals with lower levels of CRP. Part of the link between heart disease and diabetes is due to inflammation, and for many patients that inflammation in turn is the result of obesity, particularly "central obesity" or the tendency to put on weight around the stomach. This is because fat cells or "adipocytes" produce messenger proteins that turn on the production of CRP itself.

The metabolic syndrome is a condition known to predispose patients to diabetes and heart disease. Physicians classify patients as having the metabolic syndrome if they have at least 3 of the following 5 conditions: low HDL cholesterol, central obesity, high triglycerides, increased blood sugar levels, and high blood pressure. However, the metabolic syndrome also entails a number of less easily measured abnormalities that include insulin resistance and problems with blood clotting. CRP levels increase as the number of components of the metabolic syndrome increase. Even among individuals known to have the metabolic syndrome, CRP levels add important prognostic information on risk. Thus, many physicians now also measure CRP as part of the process of defining the metabolic syndrome. This practice is increasingly common among endocrinologists and other physicians interested in the prevention of diabetes as well as heart disease.

## Is CRP Specific for Cardiovascular Disease?

Because CRP is an "acute-phase reactant" and goes up during major trauma and infection, some physicians have worried that CRP testing might be too nonspecific for clinical use. However, multiple studies show that CRP, when measured appropriately with high-sensitivity assays in stable individuals, is quite specific for the prediction of future cardiovascular events. In one recent study, elevated CRP levels were associated with an 8-fold increase in cardiovascular mortality, but had no predictive value for death from other causes. Other studies show that CRP levels predict heart attack and stroke, but not cancer or other major disorders. Thus, a persistently elevated CRP level is indicative of the risk of heart disease and of the accelerated atherosclerosis that affects individuals with diabetes.

## At What Age Should I Be Tested?

The first time to consider CRP evaluation is probably in your mid-30s, the same age that most physicians check cholesterol levels. There is good evidence that CRP levels in your teens and 20s are very predictive of levels later in life. Elevated CRP levels predict risk over the next 30 to 40 years. This is good news from a prevention

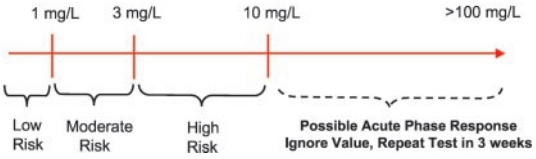


**Figure 4.** Clinical interpretation of hs-CRP for cardiovascular risk prediction. Adapted from Yeh and Willerson (*Circulation* 2003;107:370–372).[9]

perspective because ample time is available to institute lifestyle changes and, where appropriate, initiate pharmacological interventions to prevent first-ever heart attack and stroke.

Unlike cholesterol testing, CRP evaluation does not require you to fast and can be done at any time of the day.

## What Is the Best Way to Lower CRP?

The role of CRP as a predictor of future heart attack and stroke has only recently been described, and it is important to recognize that there is no evidence yet that lowering CRP per se will necessarily lower cardiac risk. However, it took almost 20 years before definitive, randomized clinical trials showed that lowering cholesterol lowered cardiac risk. You and your physician should keep abreast of ongoing studies concerning this important issue.

The good news is that the best ways to lower CRP are already known to lower cardiovascular risk. These include diet, exercise, blood pressure control, and smoking cessation. Thus, an important role for CRP evaluation now is to identify high-risk individuals (even when cholesterol is low) and to motivate them toward heart-healthy interventions.

## What About Aspirin and the "Statin" Drugs?

Aspirin is an antiplatelet drug that, at least in men, has been shown to reduce the risk of first-ever heart attack. Aspirin, however, is also an anti-inflammatory drug, and it has been shown that the magnitude of benefit of aspirin in terms of prevention is greatest among those with high levels of inflammation as defined by elevated CRP levels. Any decision to take aspirin needs to balance potential risks and benefits and should be made in consultation with your physician.

The statin drugs are highly effective at reducing risk of first heart attacks and stroke (primary prevention) as well as reducing recurrent events (secondary prevention). While these drugs work primarily by lowering LDL cholesterol, they also reduce CRP levels in many patients, and it has been suggested that this additional "anti-inflammatory" effect may also have clinical benefit. Currently, statin therapy is warranted for those with known heart disease, those with elevated levels of LDL cholesterol (above 160 mg/dL), and those with diabetes. For more information about statin drugs, please see the Cardiology Patient Page by Gotto (Statins: powerful drugs for lowering cholesterol: advice for patients, *Circulation* 2002;105:1514–1516).

Whether otherwise healthy individuals with low levels of LDL but high levels of CRP should also be on statin therapy is controversial, and a major clinical trial called JUPITER has been designed to address this very question. If you are interested in participating in this study, you can call 1-888-660-8254 or go to http://www.JUPITERstudy.com on the internet.

## Who Should Be Tested for CRP?

The Centers for Disease Control and Prevention and the American Heart Association suggest that CRP evaluation be considered as a part of overall global risk prediction for individuals concerned about vascular risk. The test is most likely to have greatest utility among those at "intermediate" risk where additional prognostic information is likely to change overall risk estimates and motivate lifestyle change. For efficiency of clinical practice and to avoid unnecessary blood draws, many physicians simply add CRP testing to standard cholesterol evaluation. CRP testing is not considered mandatory but rather should be done at the discretion of your physician.

The Centers for Disease Control and Prevention and the American Heart Association also endorsed the use of CRP evaluation for those with a prior history of heart attack and among those admitted to hospital with acute heart disease syndromes. In the Emergency Room setting, patients coming in with chest pain syndromes may also have CRP levels checked in order to identify those at high risk for coronary disease.

## How Do I Interpret CRP Test Results?

Interpreting CRP results is straightforward (Figure 4). All laboratories should report values in mg/L (milligrams per liter). Levels of CRP less than 1 mg/L are desirable and reflect a low overall cardiovascular risk. Levels of CRP between 1 and 3 mg/L are indicative of moderate risk, while levels of CRP in excess of 3 mg/L suggest quite elevated vascular risk. As noted above and described in Figures 1 and 3, this may be true even if your cholesterol levels are low.

It is possible that you will have a CRP level that is very high (above 10 mg/L). In that case, the test should be repeated in about 2 to 3 weeks as levels above 10 mg/L can reflect the presence of an acute infection (this is why it is recommended to have CRP evaluation done when you are feeling well). If on repeat testing the CRP level remains high, you are most likely in the high-risk group.

Postmenopausal women who take standard estrogen or estrogen plus progesterone oral hormone replacement therapy (HRT) tend to have elevated levels of CRP. Women in this group should discuss the relative benefits and risks of HRT since recent studies have not shown HRT to lower cardiovascu-

lar risk. Stopping oral HRT will lower your CRP levels. Topical estrogens and the selective estrogen receptor modulators (SERMS) do not seem to elevate CRP.

Levels of CRP are similar in men and women. The average CRP in middle-aged Americans is about 1.5 mg/L. Approximately 25% of the US population has levels of CRP greater than 3 mg/L, the cut point for high risk.

## Additional Resources


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**C-reactive protein and other inflammatory risk markers in acute coronary syndromes**

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# C-Reactive Protein and Other Inflammatory Risk Markers in Acute Coronary Syndromes

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Markers of myocyte necrosis such as cardiac troponin or creatine kinase-myocardial band are invaluable tools for risk stratification among patients presenting with acute coronary syndromes (ACS). Nonetheless, many patients without any evidence of myocyte necrosis may be at high risk for recurrent ischemic events. In consideration of the important role that inflammatory processes play in determining plaque stability, recent work has focused on whether plasma markers of inflammation may help improve risk stratification. Of these markers, C-reactive protein (CRP) has been the most widely studied, and there is now robust evidence that CRP is a strong predictor of cardiovascular risk among apparently healthy individuals, patients undergoing elective revascularization procedures, and patients presenting with ACS. Moreover, even among patients with troponin-negative ACS, elevated levels of CRP are predictive of future risk. Other, more upstream markers of the inflammatory cascade, such as interleukin (IL)-6, have also been found to be predictive of recurrent vascular instability. A recent report from the second FRagmin during InStability in Coronary artery disease trial investigators suggests that elevated levels of an inflammatory marker such as IL-6 may indicate which patients may benefit most from an early invasive strategy. Other inflammatory markers currently under investigation include lipoprotein-associated phospholipase $A_2$, myeloperoxidase, and pregnancy-associated plasma protein A. Of all these novel markers, CRP appears to meet most of the criteria required for potential clinical application. Furthermore, the benefits of lifestyle modification and drug therapy with aspirin or statins may be most marked among those with elevated CRP levels. (J Am Coll Cardiol 2003;41: 37S–42S) © 2003 by the American College of Cardiology Foundation

Approximately 1.4 million patients with acute coronary syndromes (ACS) without ST-segment elevation are admitted to hospital annually in the U.S. (1). Markers of myocyte necrosis such as creatine kinase-myocardial band (CK-MB) and cardiac troponin are invaluable diagnostic tools for such patients and are routinely used for risk stratification. However, even troponin, a highly specific marker of cardiac myocyte necrosis, has relatively low diagnostic sensitivity for ACS, with only 22% to 50% of patients with unstable angina having positive troponin (I or T) tests (2–5). Moreover, many patients with troponin-negative ACS who have vulnerable coronary plaques remain at high risk for future ischemic events. Thus, an additional test to improve upon risk stratification based on markers of myocyte necrosis alone could prove a valuable aid in clinical practice.

## INFLAMMATORY MARKERS: C-REACTIVE PROTEIN

**Pathophysiology.** The past decade has witnessed an increasing recognition that inflammatory mechanisms play a central role in the pathogenesis of atherosclerosis and its complications (6). Recently, attention has focused on the potential role of plasma markers of inflammation as risk predictors among those at risk for cardiovascular events (7). Of these potential markers, C-reactive protein (CRP) has been the most extensively studied. Produced in the liver in response to interleukin (IL)-6, CRP is an acute phase reactant that serves as a pattern recognition molecule in the innate immune system. It was initially thought of as a downstream bystander marker of vascular inflammation, but recent data suggest that CRP may play an active role in atherogenesis. C-reactive protein opsonization of low-density lipoprotein (LDL) mediates LDL uptake by macrophages (8), and CRP also stimulates monocyte release of pro-inflammatory cytokines such as IL-1b, IL-6, and tumor necrosis factor-alpha (9). Furthermore, CRP mediates monocyte chemotactic protein-1 induction in endothelial cells (10) and causes expression of intercellular adhesion molecule-1 and vascular cellular adhesion molecule-1 by endothelial cells (11). Recent data have shown that CRP co-localizes with the membrane attack complex in early atheromatous lesions, and CRP, complement proteins, and their messenger ribonucleic acid are all substantially upregulated in atheromatous plaque (12).

**CRP as a predictor of risk.** Numerous large-scale epidemiological studies among apparently healthy men and women have found that CRP is a strong independent predictor of future cardiovascular risk (13–22). In the setting of ACS, a landmark study by Liuzzo et al. (23) showed that patients presenting with unstable angina who had elevated

Please refer to the Trial Appendix at the back of this supplement for the complete list of clinical trials.

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**Abbreviations and Acronyms**

ACS = acute coronary syndrome(s)
BNP = B-type natriuretic peptide
CAD = coronary artery disease
CK-MB = creatine kinase-myocardial band
CRP = C-reactive protein
IL = interleukin
LDL = low-density lipoprotein
Lp-$PLA_2$ = lipoprotein-associated phospholipase $A_2$
MI = myocardial infarction
MPO = myeloperoxidase
PAPP-A = pregnancy-associated plasma protein A

plasma levels of CRP (≥3 mg/l) and serum amyloid A had a higher rate of death, acute myocardial infarction (MI), and need for revascularization compared with patients without elevated levels (Table 1).

The Thrombolysis In Myocardial Infarction (TIMI) investigators have since shown that the increased risk associated with high CRP levels may be evident as early as 14 days after presentation with an ACS (24). The Chimeric c7E3 AntiPlatelet Therapy in Unstable angina Refractory to standard treatment (CAPTURE) trial investigators found that, although only troponin T was predictive in the initial 72-h period, both CRP and troponin T were independent predictors of risk at six months (25), while the FRagmin during InStability in Coronary artery disease (FRISC) investigators reported that the risk associated with elevated CRP levels at the time of index event continues to increase for several years (26). In each of the above studies, the predictive value of CRP was independent of, and additive to, troponin (Table 1). Most importantly, CRP has been found to have prognostic value among patients without evidence of myocyte necrosis; specifically, even among patients with negative troponin T, an elevated CRP is predictive of future adverse events (24–26). Recent data also confirm that CRP is a strong independent predictor of short-term and long-term mortality among patients with ACS who are treated with very early revascularization (27).

The exact source of elevated CRP levels among patients with unstable coronary syndromes remains unclear. Data suggest that plaque rupture per se may not be the cause but, rather, that elevated CRP levels may be a marker of the hyper-responsiveness of the inflammatory system to even small stimuli. The CRP levels do not change after balloon angioplasty in patients with stable or unstable angina who have normal pre-procedural levels, but they do increase after angioplasty in unstable patients with elevated CRP at baseline (28). Moreover, even diagnostic angiography without intervention caused an increase in CRP levels among patients with elevated levels at baseline.

**Other inflammatory markers.** Further data regarding upstream mediators of CRP production suggest that this pathway may reflect inflammatory processes that convey increased cardiovascular risk. Elevated levels of IL-1 receptor antagonist and IL-6 at 48 h after presentation are associated with an adverse in-hospital prognosis among patients with ACS, even without a rise in troponin T (29). A recent report from the FRISC II study group has found that circulating levels of IL-6 are a strong independent marker of increased mortality among patients with unstable coronary artery disease (CAD) and may be useful in directing subsequent care (30). For example, randomization to an early invasive strategy led to a 65% relative reduction in 12-month mortality among patients with elevated IL-6 levels. By contrast, among those with low IL-6 levels, an early invasive strategy did not confer any significant benefit over a non-invasive strategy. Furthermore, among patients randomized to the non-invasive arm, the risk associated with elevated IL-6 levels was markedly attenuated if they were assigned to therapy with dalteparin rather than placebo (30). Similar data were observed for CRP. Thus, the use of an inflammatory marker for risk stratification appears to identify patients at high risk for future events, but most importantly, it appears to identify individuals who might benefit most from targeted interventional or intensive medical therapy.

Other novel inflammatory markers have been studied in cardiovascular risk prediction. Lipoprotein-associated phospholipase $A_2$ (Lp-$PLA_2$) circulates in association with LDL-cholesterol and may contribute to atherogenesis by hydrolyzing oxidized phospholipids into pro-atherogenic fragments and by generating lysolecithin, which has pro-inflammatory properties. The West of Scotland study group

**Table 1.** CRP and Cardiovascular Risk in ACS: Results From Recent Trials

| Study/Trial | Results |
|---|---|
| Liuzzo et al. (23)* | Increased rate of death, MI, and revascularization in patients with unstable angina and CRP ≥3 mg/l plus elevated serum amyloid A. |
| TIMI IIa substudy (24)* | Increased risk associated with higher CRP levels, evident as early as 14 days after ACS. |
| CAPTURE (25)* | CRP is an independent predictor of increased risk at 6 months. |
| FRISC (26)* | Increased risk associated with higher CRP levels at index event. |
| Mueller et al. (27) | CRP predictive of short- and long-term mortality among ACS patients treated with early revascularization. |

*The predictive value of CRP was independent of, and additive to, that of troponin.
ACS = acute coronary syndromes; CAPTURE = Chimeric c7E3 AntiPlatelet Therapy in Unstable angina REfractory to standard treatment; CRP = C-reactive protein; FRISC = FRagmin during InStability in Coronary artery disease; MI = myocardial infarction; TIMI = Thrombolysis In Myocardial Infarction.

**Table 2.** Other Novel Inflammatory and Non-Inflammatory Biomarkers of Increased Cardiovascular Risk

| Inflammatory Biomarker | Predictive Value |
|---|---|
| IL-6 | Associated with adverse in-hospital prognosis in ACS patients (29). Independent marker of increased mortality in unstable CAD (30). Associated risk attenuated with dalteparin therapy (30). |
| Lp-$PLA_2$ | Independent predictor of risk for heart disease in a high-risk male population (31). |
| MPO | Significant predictor of CAD risk in case control studies. |
| PAPP-A | Abundantly expressed in unstable plaques and elevated in unstable angina and MI patients; absent or minimally expressed in stable plaques or in stable angina patients or controls (34). |
| Non-inflammatory biomarker | |
| BNP | Baseline levels correlate with risk of death, heart failure, MI in ACS (35). |

ACS = acute coronary syndrome; BNP = B-type natriuretic peptide; CAD = coronary artery disease; IL = interleukin; Lp-$PLA_2$ = lipoprotein-associated phospholipase $A_2$, MI = myocardial infarction; MPO = myeloperoxidase; PAPP-A = pregnancy-associated plasma protein A.

reported that baseline levels of Lp-$PLA_2$ were a strong independent predictor of risk for incident coronary heart disease in a cohort of high-risk hyperlipidemic men (31). Among a lower-risk cohort of normocholesterolemic women, baseline levels of Lp-$PLA_2$ were also higher among cases than controls (32). However, in adjusted analyses, baseline levels of Lp-$PLA_2$ were not a significant predictor of future cardiovascular risk, while CRP remained a strong predictor (32). Lp-$PLA_2$ levels are highly correlated with LDL-cholesterol, which may in part explain these different results. The predictive value of Lp-$PLA_2$ among patients with ACS is currently unknown.

Myeloperoxidase (MPO) levels may be elevated among individuals with CAD (33). Myeloperoxidase is an enzyme secreted by a variety of inflammatory cells, including activated neutrophils, monocytes, and certain tissue macrophages, such as those found in atherosclerotic plaque. The enzyme is not released until leukocyte activation and degranulation. Myeloperoxidase may convert LDL into a high-uptake form for macrophages, leading to foam cell formation, and may also deplete nitric oxide, contributing to endothelial dysfunction. In a recent case-control study, increasing levels of leukocyte-MPO and blood-MPO were significant predictors of the risk for CAD, such that after adjustment for white blood cell count and Framingham risk score, individuals in the highest quartile of blood-MPO had a 20-fold higher risk of CAD than individuals in the lowest quartile (33). Prospective studies are thus needed to test this interesting hypothesis directly.

Recent ACS data have also been presented for pregnancy-associated plasma protein A (PAPP-A) (34). This zinc-binding metalloproteinase enzyme is a specific activator of insulin-like growth factor I, a mediator of atherosclerosis. Among eight patients who died suddenly from cardiac causes, PAPP-A was abundantly expressed in ruptured and eroded unstable plaques, but PAPP-A was absent or minimally expressed in stable plaques. In plaques with large lipid cores and cap rupture, staining for PAPP-A revealed that the enzyme occurred mostly in the inflammatory shoulder region. In a small case-control study, circulating levels of PAPP-A were higher among patients with unstable angina or acute MI than among patients with stable angina and controls (34). Levels of CRP were also higher among those with acute MI and unstable angina than those with stable angina. Among patients with ACS, levels of PAPP-A and CRP were highly correlated ($r = 0.61$), but there was no association between PAPP-A and CK-MB ($r = 0.07$) or troponin I ($r = 0.1$). As with MPO, these data require assessment in larger cohorts.

**Non-inflammatory markers.** de Lemos et al. (35) have also recently reported data regarding the potential prognostic utility of B-type natriuretic peptide (BNP) among patients with ACS in the Orbofiban in Patients with Unstable coronary Syndromes (OPUS)-TIMI 16 study. Unlike inflammatory markers, BNP is a neurohormone synthesized in ventricular myocardium and released in response to pressure overload and ventricular dilation. Baseline levels of BNP, drawn on average 40 h after the onset of ischemic symptoms, correlated with the risk of death, heart failure, and MI at 30 days and 10 months. This association was significant across the full spectrum of ACS, including patients presenting with ST-segment elevation MI, MI without ST elevation, and unstable angina. Although it was statistically significant, the correlation between BNP and CRP was weak ($r = 0.2$; $p < 0.001$). After being adjusted for other independent predictors of risk of death, including the presence or absence of heart failure in patients, the odds ratio for death at 10 months for the top quartile of BNP compared with the lowest was 5.8, BNP also remained a significant predictor of death when analyses were restricted to an investigation of the presence or absence of elevated troponin levels. Non-CRP inflammatory and non-inflammatory biomarker results are summarized in Table 2.

**Clinical utility.** Three major questions must be answered before routine clinical application of inflammatory markers is advocated (36,37). First, does the marker independently predict risk beyond conventional tools? Second, are specific therapies available to reduce levels of the inflammatory

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marker, and third, do therapies that lower plasma levels of inflammatory markers also reduce cardiovascular risk? To this list could also be added the need for a widely available reliable biochemical assay.

Of the inflammatory markers discussed in the previous text, CRP currently meets most, if not all, of these criteria. C-reactive protein has been shown to predict risk in a wide variety of clinical settings; it has incremental value in addition to standard lipid screening for primary prevention (18,19,38) and in addition to cardiac troponin testing among patients with ACS (24–26). Furthermore, a recent analysis by Chew et al. (39) shows that CRP predicts the risk of death or MI at 30 days among patients undergoing percutaneous coronary intervention. In this setting, the risk associated with elevated CRP was independent of, but additive to, the effect of an increased American College of Cardiology/American Heart Association lesion score.

C-reactive protein levels are higher among smokers, diabetics, and obese subjects. Adipose tissue is a potent source of IL-6, the main hepatic stimulus for CRP production. Thus, intensification of dietary measures and exercise programs would seem to be appropriate for these individuals. Statin therapy may have powerful anti-inflammatory effects (40), and in recent clinical studies, statin therapy has been shown to lower CRP levels, an effect that is independent of lipid lowering (19,41–44). Recent data suggest that baseline levels of CRP and IL-6 are strong independent predictors of the risk of developing type II diabetes (45). In this regard, intriguing data from the West of Scotland study suggest that pravastatin therapy, compared with placebo, reduced the risk of development of type II diabetes (46).

Further data suggest that the benefits of statin therapy may be greatest among those with elevated CRP levels, either among post-MI patients (47) or in the primary prevention setting (19). In the Cholesterol And Recurrent Events (CARE) trial population, patients with persistent low-grade vascular inflammation, as evidenced by high CRP and serum amyloid A levels, were at increased risk of recurrent events. Randomization to pravastatin therapy prevented 54% of recurrent events among those with persistent inflammation, compared with 25% among those without (47). Similarly, in the primary prevention Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), individuals with low LDL levels (<149 mg/dl) but high CRP levels (>0.16 mg/dl) were at high risk for future cardiovascular events, and they derived substantial benefit from lovastatin therapy (relative risk compared with placebo = 0.58; 95% confidence interval, 0.34 to 0.98) (19).

Current clinical practice should not be based on these post-hoc analyses (16,43), and there are currently no prospective data that prove that lowering CRP decreases cardiovascular events or improves survival, or that establish defined targets for treatment. Thus, although substantial gains may be made by targeting statin therapy at those with heightened vascular inflammation (48), prospective randomized trials to test these hypotheses directly are needed.

The effect of aspirin on CRP levels is controversial (49,50), but the benefit of aspirin therapy in preventing future MI appears to be greatest among those with elevated CRP levels (16). As noted above, data from the FRISC-II study suggest that the benefits of an early invasive approach may be greatest among those with evidence of a heightened inflammatory response (30). In the absence of an elevated inflammatory response, a less invasive approach may prove equally effective. Again, prospective randomized studies are required to test these hypotheses directly. The possibility of novel anti-inflammatory interventions targeted at specific mediators of vascular inflammation is also appealing.

The optimal cutoff point for defining high CRP levels among patients with ACS remains to be determined. The CAPTURE group found that a threshold of 10 mg/l maximized the predictive value of CRP (25). Several other investigators have used a cutoff point of 3 mg/l for patients with ACS, while the reference ranges for primary prevention populations are lower (16,18,19). The precise cause of these different thresholds remains unclear, but it is probably related to heightened vascular inflammation at the time of presentation with ACS.

## CONCLUSIONS

In summary, the use of CRP and other novel inflammatory markers may significantly add to our ability to correctly identify patients presenting with ACS who are at high risk for future cardiovascular events. The predictive value of CRP appears to be independent of, and in addition to, troponin. Individuals with evidence of heightened inflammation may benefit most from an aggressive modification of lifestyle and an intensification of proven preventive therapies such as aspirin and statins. Moreover, the benefits of an early invasive strategy may also be greatest among those with elevated levels of inflammatory biomarkers.

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# CDC/AHA Workshop on Markers of Inflammation and Cardiovascular Disease

## Application to Clinical and Public Health Practice

## Overview

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The Centers for Disease Control and Prevention/American Heart Association (CDC/AHA) Workshop on Markers of Inflammation and Cardiovascular Disease: Application to Clinical and Public Health Practice was convened on March 14 and 15, 2002, to examine the use of inflammatory markers in patients who are at risk for cardiovascular disease (CVD). The goal of the workshop was to determine which of the available tests, if any, should be used; what results should be used to define high risk; which patients should be tested; and the indications for which the tests would be most useful. To achieve this goal, the workshop participants set down 5 objectives:

1. To review the growing body of scientific evidence from diverse sources and examine the association between several inflammatory markers and CVD, including the strength, consistency, independence, and generalizability of the data
2. To consider the clinical testing and various assays of inflammatory markers and identify which may be the best assay to use in identifying individuals at risk
3. To identify areas in which questions persist to foster additional research
4. To recommend which tests should be performed for which patients and in which clinical settings for the purpose of risk stratification, therapeutic monitoring, and other clinical applications, on the basis of scientific evidence
5. To explore the public health implications of an association between inflammatory markers and CVD

The 1½-day-long workshop consisted of invited lectures by recognized authorities in the field and 3 concurrent discussion groups related to laboratory science, clinical science, and population science. The major results of this workshop were synthesized into a Statement for Healthcare Professionals from the Centers for Disease Control and Prevention and the American Heart Association, which was published in *Circulation* in January 2003.[1] That statement was a distillation of the extensive deliberations of the 3 discussion groups, which continued to examine the evidence in their respective areas and to refine their conclusions. This series of reports presents the findings of the 3 discussion groups in greater detail,[2–4] including information that was not available for the 2003 *Circulation* report, as well as 3 reports from the speakers with background information related to the workshop.[5–7] The references for the latter 3 have been updated.

The purpose of this series is to document for historical purposes the evidence presented at the workshop. It is recognized that this field of research is the focus of intense investigation, and additional relevant studies have been published since the workshop. The workshop co-chairs intend to convene a follow-up conference when additional evidence becomes sufficient to warrant an update of the database and a review of the writing groups' recommendations. Until then, the 2003 statement will serve as an evidence-based guide for the use of inflammatory markers in clinical and public health practice.

A complete list of participants in the discussion groups is included with each report. This conference was jointly sponsored by the CDC and the AHA. Specifically, the National Center for Chronic Disease Prevention and Health Promotion and the National Center for Environmental Health

This paper represents a summary of a scientific conference sponsored by the American Heart Association. The opinions expressed in this paper are those of the authors and do not necessarily represent those of the editor or the American Heart Association. The publication of these proceedings was approved by the American Heart Association Science Advisory and Coordinating Committee on August 4, 2004. All writing group members were required to complete and submit shortly before the workshop a Faculty Disclosure Questionnaire. These disclosures are available as an online appendix at http://www.circulationaha.org (*Circulation*. 2004;110:e577–e578).



**The reports of the Laboratory Science, the Clinical Practice, and the Population Science Discussion Groups and 3 background papers are available online at http://www.circulationaha.org (*Circulation*. 2004;110:e545–e549; e550–e553; e554–e559; e560–e567; e568–e571; and e572–e576).**

**(*Circulation.* 2004;110:e543–e544.)**



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provided financial and organizational support. The AHA, its Expert Panel on Population and Prevention Science, and its Councils on Atherosclerosis, Thrombosis, and Vascular Biology, Clinical Cardiology, and Epidemiology and Prevention coordinated the workshop.[1] The writing groups, endorsed by the Science Advisory and Coordinating Committee of the AHA, included representation from the above-mentioned agencies and organizations, as well as the American Association for Clinical Chemistry and the American College of Cardiology. The workshop was also supported from unrestricted educational grants from industry sponsors (Bristol-Myers Squibb, Merck, Wyeth/Ayerst, and Kos Pharmaceuticals).

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KEY WORDS: AHA Scientific Statements ■ cardiovascular diseases ■ inflammation ■ risk factors ■ epidemiology

# C-Reactive Protein, the Metabolic Syndrome, and Risk of Incident Cardiovascular Events

## An 8-Year Follow-Up of 14 719 Initially Healthy American Women

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***Background***—The metabolic syndrome describes a high-risk population having 3 or more of the following clinical characteristics: upper-body obesity, hypertriglyceridemia, low HDL, hypertension, and abnormal glucose. All of these attributes, however, are associated with increased levels of C-reactive protein (CRP).

***Methods and Results***—We evaluated interrelationships between CRP, the metabolic syndrome, and incident cardiovascular events among 14 719 apparently healthy women who were followed up for an 8-year period for myocardial infarction, stroke, coronary revascularization, or cardiovascular death; 24% of the cohort had the metabolic syndrome at study entry. At baseline, median CRP levels for those with 0, 1, 2, 3, 4, or 5 characteristics of the metabolic syndrome were 0.68, 1.09, 1.93, 3.01, 3.88, and 5.75 mg/L, respectively ($P_{trend}$ <0.0001). Over the 8-year follow-up, cardiovascular event-free survival rates based on CRP levels above or below 3.0 mg/L were similar to survival rates based on having 3 or more characteristics of the metabolic syndrome. At all levels of severity of the metabolic syndrome, however, CRP added prognostic information on subsequent risk. For example, among those with the metabolic syndrome at study entry, age-adjusted incidence rates of future cardiovascular events were 3.4 and 5.9 per 1000 person-years of exposure for those with baseline CRP levels less than or greater than 3.0 mg/L, respectively. Additive effects for CRP were also observed for those with 4 or 5 characteristics of the metabolic syndrome. The use of different definitions of the metabolic syndrome had minimal impact on these findings.

***Conclusions***—These prospective data suggest that measurement of CRP adds clinically important prognostic information to the metabolic syndrome. **(*Circulation*. 2003;107:391-397.)**

**Key Words:** protein, C-reactive ■ risk factors ■ prognosis ■ diabetes mellitus ■ inflammation

Patients with the metabolic syndrome are at increased risk for diabetes and cardiovascular events,[1–3] and the recently released Third Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATP-III) stresses the importance of targeting prevention strategies for such individuals.[4] The ATP-III guideline also suggests a working definition of the metabolic syndrome that includes the presence of at least 3 of the following characteristics: abdominal obesity, elevated triglycerides, reduced levels of HDL cholesterol, high blood pressure, and high fasting glucose. However, all of these parameters are associated with elevated levels of C-reactive protein (CRP), an easily measured inflammatory biomarker that has proven to be a strong, independent predictor of both incident diabetes[5,6] and incident cardiovascular disease.[7–14] CRP levels also correlate with several other components of the metabolic syndrome such as fasting insulin, microalbuminuria, and impaired fibrinolysis that are not easily evaluated in usual clinical practice.[15–20] We therefore sought to evaluate in a large-scale population cohort the potential interrelationships between CRP, the metabolic syndrome, and incident cardiovascular events. We additionally sought evidence as to whether or not CRP might add prognostic information at all levels of severity of the metabolic syndrome.

## Methods

We evaluated the relationship of CRP with components of the metabolic syndrome among apparently healthy women participating in the Women's Health Study (WHS), an ongoing trial of aspirin and vitamin E in primary prevention. Details of the WHS and the methods used to ascertain baseline risk factors and adjudicate clinical outcomes have been described elsewhere.[8,10] In brief, American

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From the Center for Cardiovascular Disease Prevention (P.M.R., J.E.B., N.R.C., N.R.), the Divisions of Preventive Medicine (P.M.R., J.E.B., N.R.C.) and Cardiology (P.M.R.), and the LeDucq Center for Cardiovascular Research (P.M.R., N.R.), Brigham and Women's Hospital, and the Department of Laboratory Medicine, Children's Hospital (N.R.), Harvard Medical School, Boston, Mass.

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Dr Ridker is named as a coinventor on patents filed by the Brigham and Women's Hospital that relate to the use of inflammatory biomarkers in cardiovascular disease and diabetes.

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women aged 45 years and over with no prior history of cardiovascular disease or cancer were enrolled between November 1992 and July 1995, at which time they provided detailed information on demographic, lifestyle, and behavioral risk factors. Of these women, 28 345 provided baseline blood samples collected in EDTA, which were stored in liquid nitrogen. Since enrollment, all study participants have been followed up for incident cardiovascular events, including nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization procedures, and cardiovascular death.

Because recent randomized trial evidence indicates a net hazard in association with hormone replacement therapy (HRT), we elected to increase the generalizability of our data by limiting our analysis to the 15 745 WHS participants not using HRT at study entry. Of these, 14 719 were also free of diabetes at study entry and contributed complete data for all 5 components of the metabolic syndrome. Baseline blood samples from these women were thawed and assayed for CRP by a validated high-sensitivity assay (Denka Seiken), whereas triglyceride and HDL cholesterol levels were ascertained with direct measurement assays (Roche Diagnostics).

Women with 3 or more of the following attributes are typically defined as having the metabolic syndrome: (1) triglycerides $\geq$150 mg/dL; (2) HDL cholesterol $<$50 mg/dL; (3) blood pressure $\geq$135/85 mm Hg; (4) obesity as defined by a waist circumference $>$88 cm; and (5) abnormal glucose metabolism as defined by a fasting glucose $\geq$110 mg/dL. In the WHS, triglycerides, HDL cholesterol, and blood pressure were directly ascertained as outlined above. However, waist circumference was not measured until year 6 of follow-up. As such, we elected to use as our cutpoint for obesity a body mass index (BMI) $>$26.7 kg/m$^2$, a value that corresponded to the same percentile cutpoint for BMI at year 6 as did a waist circumference of 88 cm measured at that time. To address whether this choice of BMI affected our results, we repeated our analyses using a BMI cutpoint of 30 kg/m$^2$ as suggested in recent European guidelines.[21] Because fasting glucose levels were not available, we elected to conservatively use the diagnosis of incident type II diabetes during study follow-up as an alternative measure of baseline impairment of glucose metabolism. To address how closely these definitions represented the metabolic syndrome, we compared the proportion of women in the present study categorized according to characteristics of the metabolic syndrome as defined above to that previously published for American women in the National Health and Nutrition Survey (NHANES)[22] using categories defined by the ATP-III guideline.

To evaluate for evidence of association between baseline CRP levels and the metabolic syndrome, we first compared the distribution of CRP levels among individuals with or without each of the individual components of the syndrome as defined above. Because levels of CRP are skewed, we evaluated the significance of any differences in median values between groups using the Wilcoxon rank-sum test. We then classified all study subjects as having 0, 1, 2, 3, 4, or 5 components of the metabolic syndrome and assessed for evidence of a relation of median CRP levels across these groups using the Jonckheere-Terpstra test. We then used logistic regression analysis to discern whether elevated CRP levels added prognostic information on risk of subsequent cardiovascular events across the full spectrum of severity of the metabolic syndrome. Consistent with recent recommendations from the Centers for Disease Control and Prevention, a CRP cutpoint of 3 mg/L was used to differentiate high-risk and low-risk groups.[23]

To directly compare the clinical utility of CRP alone to that of the metabolic syndrome alone, we constructed 8-year cardiovascular event-free survival curves for those with CRP levels above or below 3.0 mg/L and compared these to survival curves based on the presence or absence of 3 or more components of the metabolic syndrome. Age-adjusted c statistics, analogous to the area under the receiver operator characteristic (ROC) curve, were used to assess the discrimination of cardiovascular prediction models based on CRP alone versus those based on having 3 or more characteristics of the metabolic syndrome. These analyses were then repeated with continuous rather than dichotomous definitions used for components of the metabolic syndrome. Finally, in analysis stratified by those with and without the metabolic syndrome, we sought evidence in terms of cardiovascular event-free survival that CRP levels might have additional prognostic value in the prediction of incident cardiovascular end points.

**TABLE 1. Prevalence of Metabolic Syndrome Abnormalities Among Participants in the Women's Health Study**

| No. of Metabolic Abnormalities | Women's Health Study (n=14 719) | NHANES Survey (n=4549) |
|---|---|---|
| $\geq$1 | 72.2(0.4) | 70.9(1.2) |
| $\geq$2 | 45.9(0.4) | 42.7(1.3) |
| $\geq$3 | 24.4(0.4) | 23.4(0.9) |
| $\geq$4 | 8.9(0.2) | 9.6(0.5) |
| 5 | 1.2(0.1) | 2.9(0.3) |

Data are % (SEM). For comparison, data are also shown from the NHANES survey. See Methods for a description of the criteria used for each component of the metabolic syndrome.

## Results

Mean age of the 14 719 women evaluated in the present study was 54$\pm$7.6 years. As defined by the proportion of individuals with increasing numbers of characteristics of the metabolic syndrome, the women participating in the present study were almost identical to those evaluated in the recent NHANES report[22] (Table 1). Specifically, the proportion of women in the present cohort with 3 or more characteristics of the metabolic syndrome was 24.4% compared with 23.4% in NHANES.

Table 2 presents median CRP values (with interquartile ranges) for those study participants with and without each individual component of the metabolic syndrome. Consistent with prior cross-sectional data, CRP levels were significantly

**TABLE 2. Median CRP Levels (Interquartile Range) Among 14 719 American Women According to the Presence or Absence of Each Component of the Metabolic Syndrome**

| Characteristic | Present | Absent | *P* |
|---|---|---|---|
| Obesity (n=5158) | 3.13 (1.57–5.63) | 0.95 (0.43–2.04) | $<$0.0001 |
| Hypertriglyceridemia (n=4297) | 2.56 (1.24–4.84) | 1.11 (0.48–2.57) | $<$0.0001 |
| Low HDL cholesterol (n=7572) | 2.02 (0.88–4.26) | 1.00 (0.44–2.31) | $<$0.0001 |
| High blood pressure (n=4859) | 2.38 (1.06–4.78) | 1.14 (0.48–2.56) | $<$0.0001 |
| Abnormal glucose metabolism (n=568) | 4.30 (2.51–7.48) | 1.39 (0.57–3.16) | $<$0.0001 |
| $\geq$3 Characteristics (n=3597) | 3.38 (1.76–6.01) | 1.08 (0.47–2.37) | $<$0.0001 |

See Methods for a description of the criteria used for each component of the metabolic syndrome.

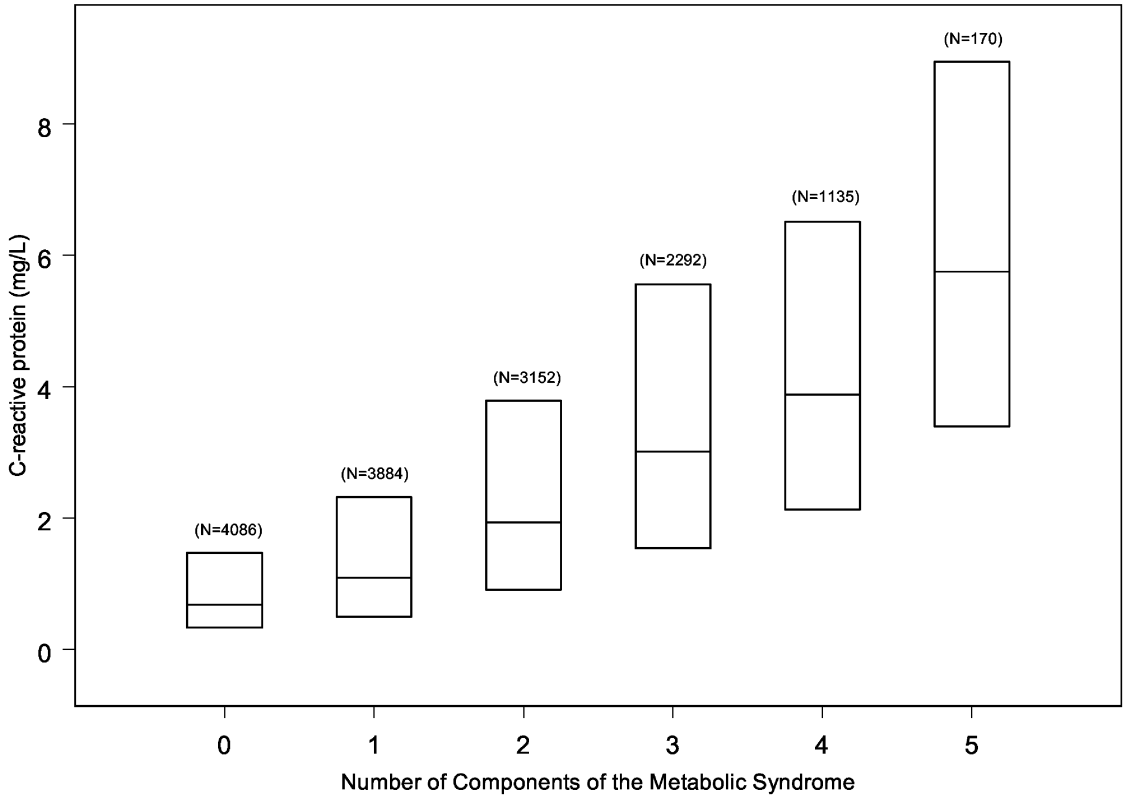


**Figure 1.** Distribution of CRP levels among 14 719 American women according to presence of 0, 1, 2, 3, 4, or 5 components of metabolic syndrome. Box plots demonstrate median, 25th, and 75th percentile values for CRP.

higher among women who had each component of the metabolic syndrome than among women who did not (all $P<0.0001$).

Figure 1 displays the distribution of CRP levels after women were classified according to their total number of components of the metabolic syndrome. As shown, there was a strong linear increase in CRP levels as the number of components of the metabolic syndrome increased; median CRP levels for those with 0, 1, 2, 3, 4, or 5 characteristics of the metabolic syndrome were 0.68, 1.09, 1.93, 3.01, 3.88, and 5.75 mg/L, respectively ($P_{trend}<0.0001$).

As shown in Figure 2, CRP levels >3 mg/L at baseline added prognostic information at all levels of severity of the metabolic syndrome. This additive effect was particularly apparent among those with 3, 4, or 5 characteristics of the metabolic syndrome (all $P<0.001$).

Figure 3 presents results of the survival analyses directly comparing CRP with the metabolic syndrome. As shown, the predictive value of CRP levels above or below 3.0 mg/L in terms of the development of first-ever cardiovascular events was quite similar to the predictive value associated with having or not having 3 or more characteristics of the metabolic syndrome. In age-adjusted analyses, the area under the ROC curve associated with CRP alone was 0.77 versus 0.78 for the metabolic syndrome.

As prespecified, we additionally sought evidence that CRP might have prognostic utility among those with and without the metabolic syndrome. We therefore first performed an analysis limited to the 3597 study participants classified as having 3 or more characteristics of the metabolic syndrome at study entry. Among these women, we observed significant increases in rates of future cardiovascular disease as levels of baseline CRP increased. Specifically, age-adjusted incidence rates were 3.4 and 5.9 events per 1000 person-years of exposure for those with baseline CRP levels less than or greater than 3.0 mg/L, respectively ($P<0.001$).

To further explore these interrelationships, we divided the study cohort into 4 groups on the basis of the presence or absence of the metabolic syndrome and on the basis of CRP levels less than or greater than 3.0 mg/L. As shown in Figure 4 (left), CRP evaluation provided additional prognostic information both for those with and without the metabolic syndrome. The age-adjusted relative risks of future cardiovascular events for women in the low-CRP/no metabolic syndrome, high-CRP/no metabolic syndrome, low-CRP/yes metabolic syndrome, and high-CRP/yes metabolic syndrome groups were 1.0 (referent), 1.5 (95% CI 1.0 to 2.2), 2.3 (95% CI 1.6 to 3.3), and 4.0 (95% CI 3.0 to 5.4), respectively.

We performed several additional analyses to address the robustness of these findings. First, because the concept of the metabolic syndrome was developed in part to reflect a secondary target population without hyperlipidemia, we repeated our analyses for the 12 453 women with baseline LDL cholesterol levels <160 mg/dL and for the 8500 women with LDL cholesterol <130 mg/dL. As shown in Figure 4 (middle and right), CRP provided prognostic information in addition to the metabolic syndrome in both of these latter analyses. The relative risks and associated CIs for these analyses are presented in Table 3.

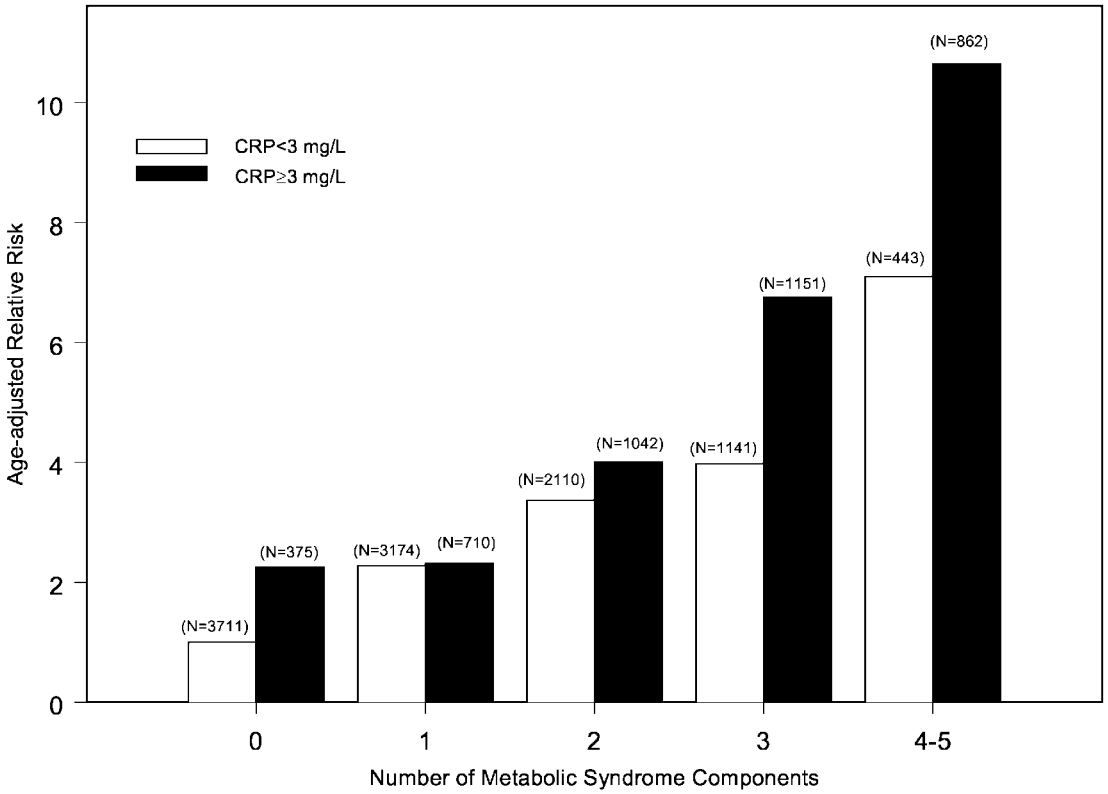


**Figure 2.** Relative risks of future cardiovascular events according to number of components of metabolic syndrome and according to CRP levels above or below 3.0 mg/L.

Second, and as also shown in Table 3, we repeated our analyses using only the end point of coronary heart disease. For this end point, overall effects were, if anything, larger than that observed with the a priori combined end point that also included thromboembolic stroke.

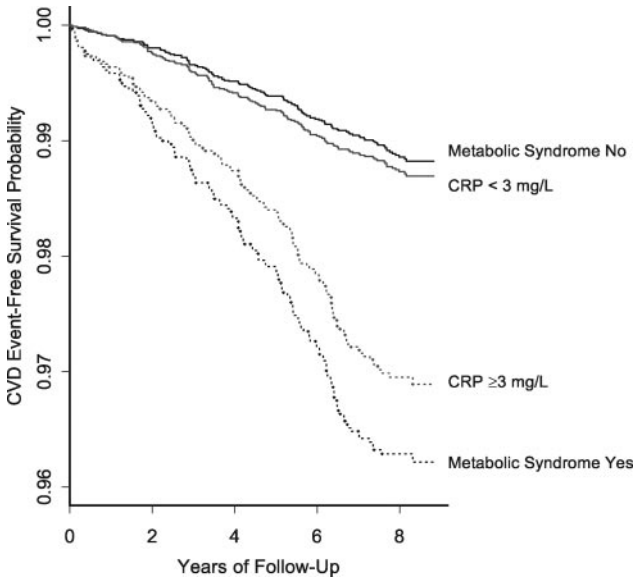


**Figure 3.** Comparison of cardiovascular event-free survival for those with and without metabolic syndrome to those with baseline CRP levels above or below 3.0 mg/L. CVD indicates cardiovascular disease.

Third, we repeated our analyses using continuous rather than dichotomous variables and found similar effects. In the continuous variable models, the relative risk of future cardiovascular events associated with CRP levels >3.0 mg/L was 1.5 ($P=0.006$), and the area under the ROC curve was 0.82. By contrast, when dichotomous definitions for each component of the metabolic syndrome were used, the corresponding relative risk was 1.6 ($P=0.0003$), and the corresponding area under the ROC curve was 0.79.

Fourth, we repeated our primary analyses using a BMI cutpoint of 30 kg/m$^2$ and again found almost identical results in terms of additive predictive value. Use of this cutpoint, however, classified only 17% of the present cohort as obese. By contrast, the use of a BMI cutpoint of 26.7 kg/m$^2$ (as done in our primary analyses) classified 32% of the cohort as obese, a value closer to that observed in the NHANES survey.

Finally, we performed an additional analysis limited to those 3597 participants with the metabolic syndrome at study entry and found that CRP levels <1, 1 to 3, and >3 mg/L stratified the population into 3 risk groups such that those with the metabolic syndrome and the highest CRP levels had a relative risk 2.1 times that of those with the metabolic syndrome who had the lowest CRP levels (95% CI 1.1 to 4.2, $P=0.001$; Figure 5). In all these analyses, virtually identical results were observed when we excluded incident diabetes as part of the definition of the metabolic syndrome.

## Discussion

Recent guidelines stress the importance of identifying individuals with the metabolic syndrome as a high-risk group for

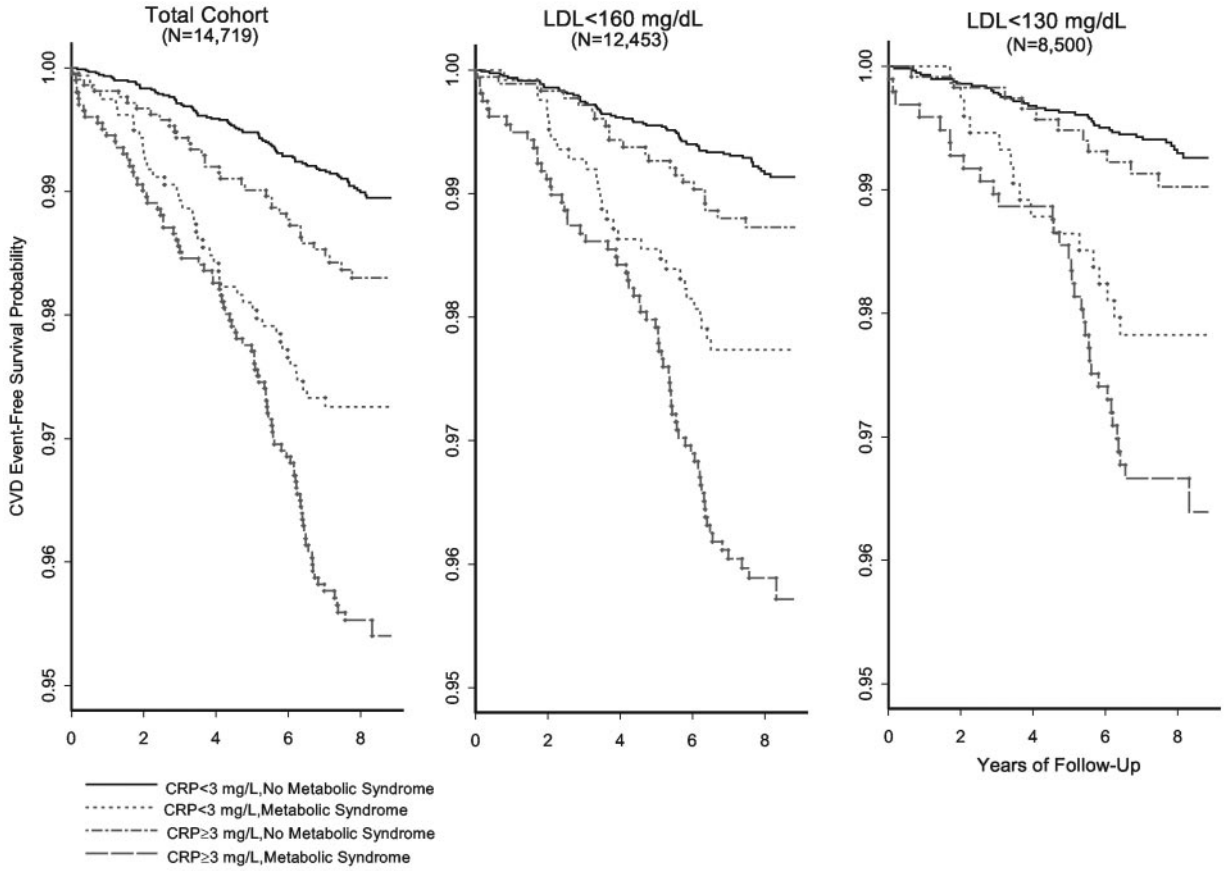


**Figure 4.** Cardiovascular event-free survival in analyses stratified by both CRP and metabolic syndrome. CVD indicates cardiovascular disease.

the development of cardiovascular disease.[4] The present prospective cohort of 14 719 initially healthy women confirms this association, because those with the metabolic syndrome had significantly worse cardiovascular event-free survival than did those without the metabolic syndrome. However, the present data also demonstrate that at all levels of severity of the metabolic syndrome, CRP added important and independent prognostic information in terms of future cardiovascular risk. This additive effect was present in all study groups evaluated and was robust to the several methods used to define the metabolic syndrome.

That CRP levels correspond with individual components of the metabolic is consistent with work of other investigators[15–20] and the hypothesized role of inflammation in several processes critical to the development of both diabetes and atherothrombosis.[24,25] Indeed, in this cohort, we have previously shown baseline CRP levels to be a strong predictor not only of myocardial infarction and stroke[8,10] but also of incident type 2 diabetes.[5] Rapidly evolving work now demonstrates that in addition to being a marker of innate immunity, CRP also has several direct effects at the level of the vessel wall.[26,27] These observations, along with basic research into the inflammatory mechanisms of both diabetes and vascular dysfunction, provide strong evidence that insulin resistance and atherosclerosis share a common inflammatory basis.[28] CRP, however, is also associated with several aspects

**TABLE 3. Relative Risks (95% CIs) of Future Cardiovascular Events According to CRP Levels Greater Than or Less Than 3.0 mg/L and According to the Presence or Absence of the Metabolic Syndrome**

| | All Cardiovascular Events | | | |
|---|---|---|---|---|
| | Total Cohort (n=14 719) | LDL <160 mg/dL (n=12 453) | LDL <130 mg/dL (n=8500) | Coronary Events Total Cohort (n=14 719) |
| CRP <3 mg/L, no metabolic syndrome | 1.0 | 1.0 | 1.0 | 1.0 |
| CRP >3 mg/L, no metabolic syndrome | 1.5 (1.0–2.2) | 1.3 (0.8–2.2) | 1.2 (0.6–2.3) | 1.6 (0.9–2.7) |
| CRP <3 mg/L, yes metabolic syndrome | 2.3 (1.6–3.3) | 2.2 (1.4–3.5) | 2.5 (1.4–4.4) | 3.1 (2.0–4.9) |
| CRP >3 mg/L, yes metabolic syndrome | 4.0 (3.0–5.4) | 4.4 (3.1–6.3) | 4.4 (2.8–7.1) | 5.5 (3.8–8.0) |

Data are shown for all cardiovascular events (n=255) and for coronary events only (n=163).

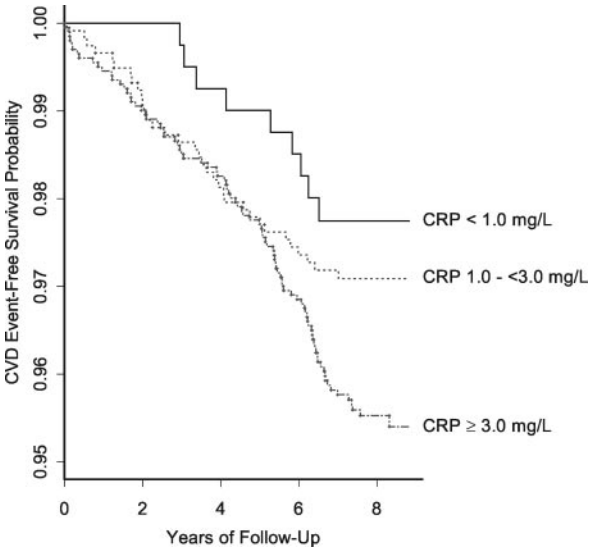


**Figure 5.** Cardiovascular event-free survival in analyses stratified by CRP levels <1, 1 to 3, and ≥3 mg/L. Data are shown for 3597 study participants with metabolic syndrome at study entry. CVD indicates cardiovascular disease.

of the metabolic syndrome not easily ascertained in usual clinical practice, including fasting insulin, hypofibrinolysis, and microalbuminuria.[15–20] Our finding that CRP measurement adds important prognostic information to clinical definitions of the metabolic syndrome is thus consistent with this hypothesis.

Limitations of this study must be considered. First, the study included only women. We believe, however, that these data are likely to generalize to men because other studies have linked markers of inflammation to individual components of the metabolic syndrome in men, and many cohort studies have already shown CRP to independently predict vascular events in men.[7,11–14] Second, because we did not have fasting glucose levels in all participants, we elected instead to use the diagnosis of incident diabetes during follow-up as a surrogate for abnormal baseline glucose metabolism. We believe this choice to be valid because other work has shown CRP levels to correlate with fasting glucose level[29] and predict incident type 2 diabetes.[5,6] Moreover, as shown in Table 1, this choice was, if anything, conservative, because it resulted in only 1.2% of the present cohort being defined as having all 5 characteristics of the metabolic syndrome versus 2.9% in the NHANES survey. We also believe it unlikely that this decision affected validity, because elevated fasting glucose is by far the least common abnormality used to define those with the metabolic syndrome. Finally, these analyses do not make adjustment for other factors that may affect CRP levels, such as smoking status.

We recognize that these data have broad implications for the development of therapies targeting insulin resistance, diabetes, and atherothrombosis. We have previously shown that aspirin and statins are relatively more effective in reducing vascular risk among those with elevated CRP levels,[7,9,30] and we have hypothesized on that basis that CRP is likely to have utility in the targeting of therapies for the primary prevention of cardiovascular disease. At the same time, weight reduction and exercise, the first-line therapies stressed by ATP-III for the management of the metabolic syndrome, also reduce CRP levels. Furthermore, a recent report suggests that rosiglitazone directly reduces CRP levels, an intriguing observation because this PPAR-$\gamma$ inhibitor is already established as standard therapy for those with type II diabetes.[31]

In sum, these data provide clear evidence that the presence of at least 3 of 5 components of the metabolic syndrome predicts incident cardiovascular events in apparently healthy women. However, these data also indicate that among those with and without the metabolic syndrome, baseline CRP levels add clinically relevant prognostic information concerning future vascular risk.

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# Plasma Concentration of C-Reactive Protein and the Calculated Framingham Coronary Heart Disease Risk Score

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***Background***—Although C-reactive protein (CRP) predicts vascular risk, few data are available evaluating the relation between CRP and the Framingham Coronary Heart Disease Risk Score (FCRS).

***Methods and Results***—CRP levels were compared with calculated 10-year FCRS in a cross-sectional survey of 1666 individuals free of cardiovascular disease. Among men and women not using hormone replacement therapy (HRT), CRP levels were significantly related to 10-year Framingham Coronary Heart Disease Risk categories [total cholesterol (TC) score for men and women: $r=0.29$ and $r=0.22$, respectively; LDL cholesterol score for men and women: $r=0.29$ and $r=0.22$, respectively, all probability values $<0.01$]. However, CRP levels correlated minimally with individual components of the FCRS, which included age ($r_{men}=0.17$, $r_{women}=-0.003$), TC ($r_{men}=-0.02$, $r_{women}=-0.006$), HDL-C ($r_{men}=0.13$), LDL-C ($r_{men}=-0.0002$, $r_{women}=0.012$), blood pressure ($r_{men}=0.18$, $r_{women}=0.22$), diabetes ($r_{men}=0.10$, $r_{women}=0.07$), and smoking ($r_{men}=0.16$, $r_{women}=0.14$) status. For women taking HRT, no significant relation was observed between CRP and the FCRS, although the power to detect effects in this subgroup is limited.

***Conclusions***—Our data demonstrate that CRP levels significantly correlate with calculated 10-year Framingham Coronary Heart Disease Risk in men and women not taking HRT but correlate minimally with most individual components of the FCRS. These data provide additional support for continued evaluation of CRP as a potential adjunct in the global prediction of cardiovascular risk. **(*Circulation*. 2003;108:161-165.)**

**Key Words:** prevention ■ inflammation ■ risk factors

The Framingham Coronary Heart Disease Risk Score (FCRS) is a simplified coronary prediction tool developed to enable clinicians to estimate cardiovascular risk in middle-aged individuals.[1,2] Although it is an important clinical tool, it is recognized that not all persons at high coronary heart disease risk are identified by the FCRS. For example, recent evidence indicates that the *c* statistic for the area under the receiver operator characteristic curve associated with the FCRS varies between 0.63 to 0.83 in different populations.[3] In an effort to improve coronary heart disease risk prediction, several novel cardiovascular risk markers have been evaluated as potential adjuncts to lipid screening in primary prevention. Of these, C-reactive protein (CRP), a marker of low-grade inflammation, has been extensively studied in several large, prospective, epidemiological studies.[4–7] However, few data are available directly comparing CRP levels with calculated FCRS. Although both CRP and the FCRS each predict vascular risk, the extent to which CRP reflects any individual component of the FCRS is unclear.

## Methods

We measured CRP levels and calculated the FCRS among 932 men and 734 women participating in the primary prevention arm of the Pravastatin Inflammation/CRP Evaluation (PRINCE) Study,[8,9] a multicenter, community-based study of the effect of 40 mg pravastatin or placebo on CRP levels over a 6-month follow-up period. At study entry, in addition to providing a blood sample for CRP and lipid evaluation, 1666 of a total of 1702 participants provided data on age, gender, smoking status, and diabetes history. Data on weight, height, and blood pressure were measured by the participant's physician at study entry. None of the participants had a history of myocardial infarction, stroke, or coronary revascularization. Participants all provided written informed consent, and all procedures followed were in accordance with institutional guidelines.

Plasma samples were assayed for CRP by using a clinically validated high-sensitivity assay[10]; total cholesterol, HDL cholesterol, and LDL cholesterol levels were determined in a Centers for Disease Control and Prevention standardized laboratory. Framingham Coronary Heart Disease risk was calculated by using previously published algorithms that used baseline cardiac risk factors including age, HDL cholesterol, LDL cholesterol, total cholesterol, smoking status, blood pressure, and diabetes history.[2]

To assess the relation between CRP and individual components of the FCRS, we first calculated the scores corresponding to the individual components of the FCRS as well as the total score. Next, Pearson correlation coefficients relating these individual risk factor scores and the total score to the natural log of baseline CRP levels were calculated. Additionally, biserial correlation coefficients were computed for diabetic and smoking status because both of these

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**TABLE 1. Baseline Characteristics of Study Participants**

| | Men (n=932) | Women (n=734) |
|---|---|---|
| Age, y | 53.0 (47.0, 63.0) | 59.0 (52.0, 69.0) |
| Aspirin use, % | 29.6 | 26.6 |
| Smoking status, % | | |
| Never | 45.6 | 54.3 |
| Current | 15.2 | 15.3 |
| Past | 39.2 | 30.4 |
| Body mass index, kg/m$^2$ | 28.5 (26.0, 31.5) | 28.6 (25.0, 33.4) |
| Blood pressure, mm Hg | | |
| Systolic | 130.0 (120.0, 140.0) | 130.0 (120.0, 140.0) |
| Diastolic | 80.0 (76.0, 86.0) | 80.0 (70.0, 84.0) |
| Diabetes, % | 9.3 | 12.3 |
| hs-CRP, mg/L | 1.50 (0.80–3.20) | 2.90 (1.30–5.80) |
| HRT use (n=275) | ... | 3.80 (2.00, 6.80) |
| No HRT use (n=459) | ... | 2.40 (1.10, 5.00) |
| Alcohol use, % | | |
| Daily | 14.2 | 3.8 |
| Weekly | 27.9 | 10.3 |
| Monthly | 19.2 | 17.5 |
| Never/rarely | 38.7 | 68.4 |
| Triglycerides, mg/dL | 162.5 (117.0, 235.0) | 158.0 (113.0, 231.0) |
| Cholesterol, mg/dL | | |
| Total | 222.5 (205.0, 245.0) | 235.0 (214.0, 256.0) |
| LDL-C | 139.4 (125.4, 156.4) | 143.6 (126.9, 160.5) |
| HDL-C | 35.8 (30.8, 41.5) | 43.4 (36.3, 50.4) |
| Exercise activity, % | | |
| Daily | 6.9 | 4.8 |
| ≥2 times/wk | 33.0 | 27.7 |
| Once/wk | 12.3 | 6.5 |
| <1 time/wk | 10.1 | 9.5 |
| Never/rarely | 37.7 | 51.5 |

*Values are percentage or median and associated interquartile range.

variables are binary. The components of the FCRS were also divided into different categories, and median CRP levels were calculated and plotted per category.

The FCRS was also divided into 5 clinically meaningful categories to reflect increasing 10-year coronary heart disease risk. Median CRP levels were then computed for each coronary heart disease risk category. Separate FCRS calculations were performed for total cholesterol and LDL cholesterol and for men and women. Additionally, as CRP levels are known to be elevated by estrogen therapy use,[11,12] we performed stratified analyses for women on this basis. To assess the relation between increasing Framingham Coronary Heart Disease risk categories and median CRP levels, a linear regression analysis was performed. All probability values are 2 tailed.

## Results

The baseline characteristics of the study participants are shown in Table 1. Compared with men, women were older (59.0 versus 53.0 years), more likely to have diabetes (12.3% versus 9.3%), and had higher total cholesterol (235.0 versus 222.5 mg/dL), HDL cholesterol (43.4 versus 35.8 mg/dL), and LDL cholesterol levels (143.6 versus 139.4 mg/dL). As expected, median CRP levels were significantly higher among women (2.90 mg/L; interquartile range, 1.30 to 5.80 mg/L) than among men (median CRP=1.50 mg/L; interquartile range, 0.80 to 3.20 mg/L), an effect largely the result of HRT use. Specifically, those women who reported current estrogen therapy use (HRT) had higher baseline CRP levels (median=3.80 mg/L; interquartile range, 2.00 to 6.80 mg/L) than those women who were not taking HRT (median=2.40 mg/L; interquartile range, 1.10 to 5.00 mg/L).

**TABLE 2. Pearson Correlation Coefficients Relating Baseline C-Reactive Protein to Individual Components of the Framingham Coronary Risk Score**

| | | Women | |
|---|---|---|---|
| Variable | Men (n=932) | HRT Use (n=275) | No HRT Use (n=459) |
| Age score | 0.17 | −0.09* | −0.003* |
| Total cholesterol score | −0.02* | 0.06* | −0.006* |
| HDL cholesterol score | 0.13 | 0.0006* | 0.24 |
| LDL cholesterol score | −0.0002* | 0.011* | 0.012* |
| Blood pressure score | 0.18 | 0.13 | 0.22 |
| Diabetes score | 0.10 | 0.11* | 0.07* |
| Smoking score | 0.16 | −0.003* | 0.14 |

All $P$ values<0.01.
*$P$ values >0.05.

We found a modest correlation between CRP levels and the FCRS in men and women not taking HRT by using both the total cholesterol ($r_{men}$=0.29, $P$<0.01; $r_{women}$=0.22, $P$<0.01) and LDL cholesterol ($r_{men}$=0.29, $P$<0.01; $r_{women}$=0.22, $P$<0.01) scoring algorithms. As shown in Table 2, although we also noted modest associations between CRP and HDL cholesterol ($r$=0.24, $P$<0.01) and blood pressure scores ($r$=0.22, $P$<0.01) in women not taking HRT, we found minimal additional evidence of association between CRP levels and the individual components of the FCRS. For example, in men and women taking HRT, CRP had the largest correlation with baseline blood pressure ($r_{men}$=0.18, $r_{women}$=0.13). Furthermore, except for the relation between smoking and CRP among men, biserial correlation coefficients assessing the relation between diabetic status and CRP ($r_{men}$=0.11, $r_{women\ hrt}$=0.11, $r_{women\ no\ hrt}$=0.08) as well as between smoking status and CRP ($r_{men}$=0.22, $r_{women\ hrt}$=-0.004, $r_{women\ no\ hrt}$=0.14) were almost identical to the corresponding Pearson correlation coefficients noted in Table 2. Likewise, plots showing median CRP levels versus individual components of the FCRS among men and women not taking HRT also demonstrate minimal association between CRP levels and the components of the FCRS (Figures 1 and 2). Specifically, among men, plots of HDL-C and CRP demonstrate a small decrease in median CRP levels with increasing HDL-C levels, whereas there is small increase in CRP concentrations at the highest levels of systolic blood pressure (Figure 1). Plots for women not taking HRT demonstrate similar findings (Figure 2).

Figure 3 shows that median CRP levels increased in men with each increasing calculated Framingham 10-year coronary risk category. This significant positive trend between increasing CRP levels and progressively higher FCRS was

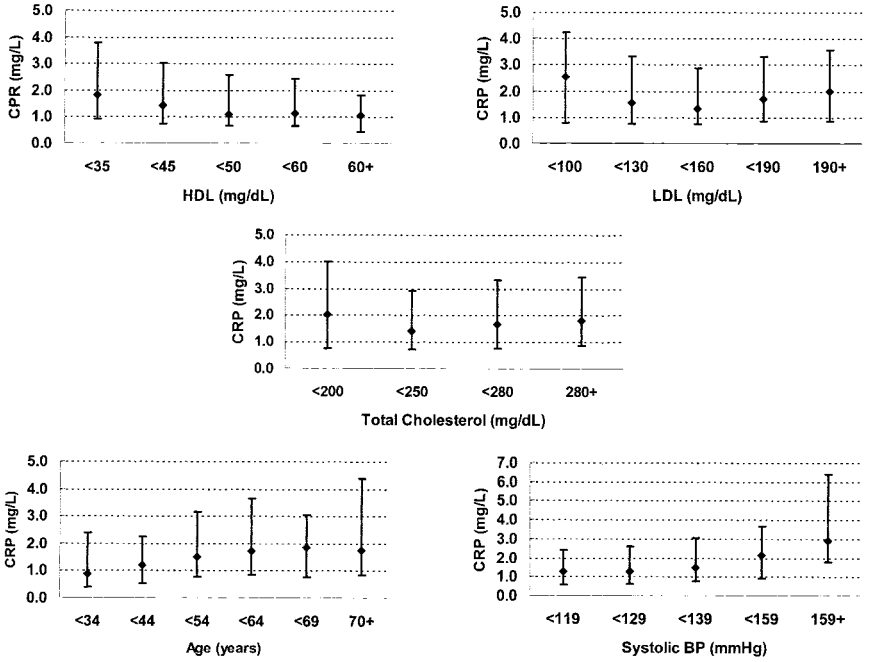


**Figure 1.** Comparison of components of the FCRS with CRP in the PRINCE Primary Prevention Cohort: Men.

noted with the use of both the total cholesterol ($P_{trend}<0.01$) and LDL cholesterol ($P_{trend}<0.01$) scoring algorithms. A similar pattern was observed for women, but this effect was attenuated in magnitude as the result of an apparent modification effect by HRT use. As shown in Figure 4 (top), among women not taking HRT, the relation between CRP and FCRS was similar to that noted in men ($P_{trend}<0.01$). By contrast, among HRT users where as reported, CRP levels were higher, the relation between CRP and FCRS was not statistically significant (total cholesterol score computation, $P_{trend}=0.18$; LDL cholesterol score computation, $P_{trend}=0.28$; Figure 4, bottom).

## Discussion

These cross-sectional data indicate that plasma concentration of CRP is significantly associated with calculated FCRS among middle-aged men and women not taking HRT. Overall, individuals in the lowest cardiovascular risk category had CRP levels that were at least half those of individuals in the highest CHD risk category. However, despite this positive association, CRP levels correlated minimally with most individual components of the FCRS.

The dichotomy observed in our data is intriguing and suggests that whereas CRP is related to the FCRS, CRP and the individual components of the FCRS might be reflecting

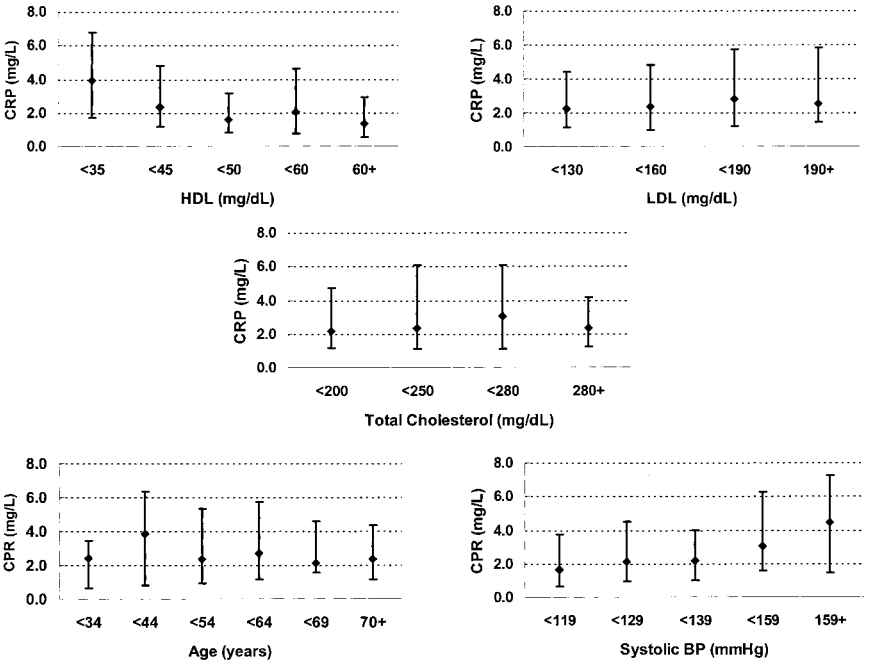


**Figure 2.** Comparison of components of the FCRS with CRP in the PRINCE Primary Prevention Cohort: Female non-HRT users.

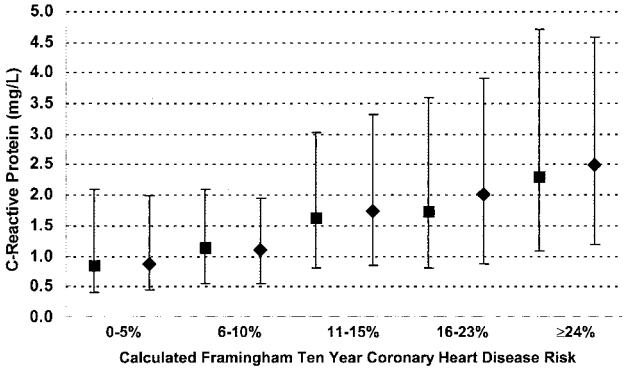


**Figure 3.** Comparison of Framingham Coronary Heart Disease Risk with CRP in the PRINCE Primary Prevention Cohort: Men.

different aspects of cardiovascular risk. In support of this hypothesis are previous data from several large prospective cohorts[5–7,13,14] that indicate that CRP predicts risk of incident cardiovascular events, even after adjustment for other traditional risk factors. Furthermore, recent data from the Women's Health Study (WHS) Cohort[15] demonstrate that after adjustment for all components of the FCRS, CRP remained an independent predictor of future cardiovascular risk. Therefore, the current data are consistent with the hypothesis that the addition of CRP to the FCRS might be useful in the context of overall cardiovascular risk determination.

As previously described,[11,12] we also observed in our women that median CRP levels were twice as high in HRT users as compared with non-HRT users. Our data extend this observation by further demonstrating a discordance between CRP and FCRS in women taking HRT. The underlying mechanism for this effect modification by HRT is uncertain but may relate to first-pass effects of HRT on hepatic CRP production.[16] These issues have clinical importance and require evaluation in experimental settings.

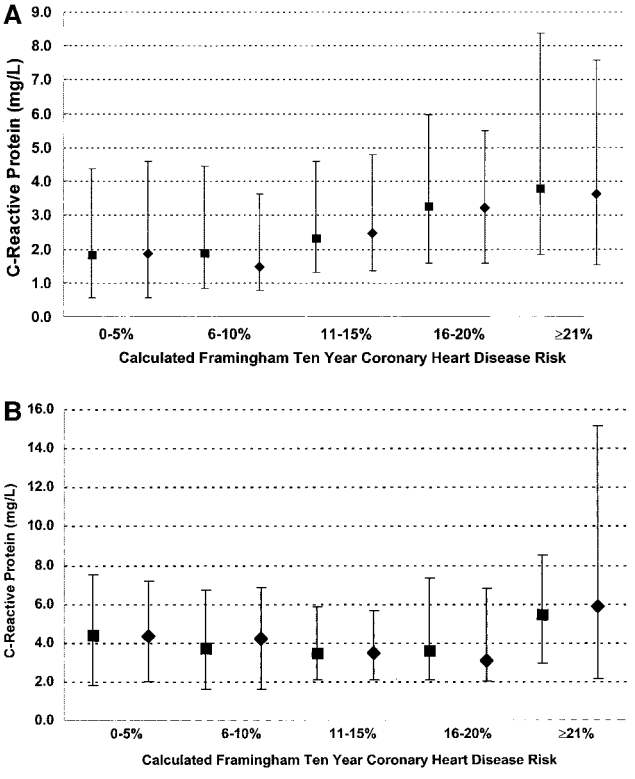


**Figure 4.** Top, Comparison of Framingham Coronary Heart Disease Risk with CRP in the PRINCE Primary Prevention Cohort: Female non-HRT users. Bottom, Comparison of Framingham Coronary Heart Disease Risk with CRP in the PRINCE Primary Prevention Cohort: Female HRT users.

These data are also important because they have implications for the design of future trials of statin therapy in the primary prevention of cardiovascular disease. Previous data demonstrate that by lowering LDL levels, HMG CoA reductase inhibitors decrease the risk of future cardiovascular events.[17,18] However, traditional LDL screening, a critical component of the FCRS, misses many individuals in primary prevention who are at high risk for coronary events. Because statins lower CRP levels in an LDL-independent manner,[9,14,19] CRP screening in conjunction with lipid screening might help identify those individuals who may benefit from prophylactic statin therapy. For example, in AFCAPS/TexCAPS (Air Force/Texas Coronary Atherosclerosis Prevention Study), individuals with below-median LDL and above-median CRP levels had a similar risk of future vascular events as did those with overt hyperlipidemia.[14] In addition, lovastatin was as effective in decreasing cardiovascular event rates among individuals in the below-median LDL/above-median CRP group as it was in participants with above-median LDL levels. Furthermore, assessment of the ability of CRP and LDL-C to predict cardiovascular risk in the WHS cohort revealed that CRP was a better predictor than LDL-C in risk prediction.[15] On the basis of these data, we have initiated a large-scale primary prevention trial of statin therapy among patients with low LDL but high CRP to directly test this hypothesis.[20] As shown in the current analysis, such a study must include large numbers of women and detailed knowledge of HRT status at study initiation and during follow-up.

In summary, in this cross-sectional survey, whereas CRP levels were significantly associated with the level of coronary heart disease risk as calculated by the FCRS in men and women not taking HRT, CRP levels correlated only minimally with most individual components of the FCRS. These data imply that CRP may capture different components than the traditional components of coronary risk reflected in the FCRS and support the hypothesis that CRP may have an adjunctive role in the global risk prediction of cardiovascular disease.[4]

## Appendix

Components of the Framingham Cardiovascular Risk Score include age, blood pressure, total cholesterol/LDL cholesterol, HDL cholesterol, diabetes, and smoking status.

## Acknowledgments

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# Clinical Usefulness of Very High and Very Low Levels of C-Reactive Protein Across the Full Range of Framingham Risk Scores

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***Background***—High-sensitivity C-reactive protein (hsCRP) is a strong independent risk factor for cardiovascular events, and levels of hsCRP of <1, 1 to <3, and ≥3 mg/L have been suggested to define low-, moderate-, and high-risk groups. However, the positive predictive value of very low (<0.5 mg/L) and very high levels of hsCRP (>10.0 mg/L) is uncertain.

***Methods and Results***—Baseline levels of hsCRP were evaluated among 27 939 apparently healthy women who were followed up for myocardial infarction, stroke, coronary revascularization, or cardiovascular death. Crude and Framingham Risk Score (FRS)–adjusted relative risks (RRs) of incident cardiovascular events were calculated across a full range of hsCRP levels. Cardiovascular risks increased linearly from the very lowest (referent) to the very highest levels of hsCRP. Crude RRs for those with baseline hsCRP levels of <0.5, 0.5 to <1.0, 1.0 to <2.0, 2.0 to <3.0, 3.0 to <4.0, 4.0 to <5.0, 5.0 to <10.0, 10.0 to <20.0, and ≥20.0 mg/L were 1.0, 2.2, 2.5, 3.1, 3.7, 4.2, 4.9, 6.3, and 7.6, respectively (*P* for trend <0.001). After adjustment for FRS, these risks were 1.0, 1.6, 1.6, 1.7, 1.9, 2.2, 2.3, 2.8, and 3.1 (*P* for trend <0.001). All risk estimates remained significant in analyses stratified by FRS and after control for diabetes. Of the total cohort, 15.1% had hsCRP <0.50 mg/L, and 5.4% had hsCRP >10.0 mg/L.

***Conclusions***—Both very low (<0.5 mg/L) and very high (>10 mg/L) levels of hsCRP provide important prognostic information on cardiovascular risk. hsCRP is clinically useful for risk prediction across a full range of values and across a full range of FRS. **(*Circulation*. 2004;109:1955-1959.)**

**Key Words:** risk factors ■ prevention ■ epidemiology ■ inflammation ■ C-reactive protein

High-sensitivity C-reactive protein (hsCRP) has emerged as a strong independent risk factor for future cardiovascular events that adds prognostic information at all levels of LDL cholesterol, at all levels of the Framingham Risk Score (FRS), and at all levels of the metabolic syndrome.[1] On the basis of published data from large, prospective cohorts,[2–9] the Centers for Disease Control and Prevention and the American Heart Association (CDC/AHA) in January of 2003 issued the first set of clinical guidelines for hsCRP as a part of global risk prediction and suggested that levels of hsCRP of <1, 1 to <3, and ≥3 mg/L be used to represent low, moderate, and high vascular risk.[10] However, as clinicians have begun using hsCRP on a regular basis, questions about the usefulness of both very high and very low levels of hsCRP have emerged. In particular, some physicians have raised concern that very high levels of hsCRP (>10 mg/L) may represent nonspecific inflammation and therefore lack positive predictive value. At the same time, others have voiced concern that very low levels of hsCRP might give patients a false sense of security, particularly when other traditional risk factors are present. We addressed these clinical issues in the large-scale Women's Health Study, in which baseline levels of hsCRP as well as FRS were measured among 27 939 apparently healthy women who were followed up over a 9-year period for the occurrence of first cardiovascular events.

## Methods

The Women's Health Study is an ongoing trial of aspirin and vitamin E in primary prevention being conducted among American women age ≥45 years with no previous history of cardiovascular disease or cancer. Participants were enrolled between November 1992 and July 1995, at which time they provided detailed information on demographic, lifestyle, and behavioral risk factors. Among women enrolled, 28 345 provided a baseline blood sample, of which 27 939 underwent successful measurement of LDL cholesterol, HDL cholesterol, and hsCRP.[9] As described elsewhere, all women have been followed up for incident cardiovascular events, including nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization procedures, and cardiovascular death.[9]

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**TABLE 1. Crude and FRS-Adjusted Relative Risks of First Cardiovascular Events According to hsCRP Cutpoints of <1, 1 to <3, and ≥3 mg/L**

| | Total Cohort (n=27 939) | | | | No HRT (n=15 745) | | | |
|---|---|---|---|---|---|---|---|---|
| hsCRP, mg/L | Events, n | Crude RR | FRS-Adjusted RR | FRS+DM-Adjusted RR | Events, n | Crude Adjusted RR | FRS-Adjusted RR | FRS+DM-Adjusted RR |
| <1.00 | 105 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) | 75 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 1.00–<3.00 | 202 | 1.7 (1.4–2.2) | 1.2 (1.0–1.5) | 1.2 (0.9–1.5) | 120 | 1.8 (1.4–2.5) | 1.2 (0.9–1.6) | 1.1 (0.9–1.6) |
| ≥3.00 | 391 | 3.0 (2.4–3.7) | 1.7 (1.3–2.2) | 1.5 (1.2–1.9) | 223 | 3.9 (3.0–5.0) | 1.9 (1.4–2.5) | 1.6 (1.2–2.2) |
| *P* for trend | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 |

RR indicates relative risk; FRS, adjusted for the Framingham Risk Score age; and FRS+DM, adjusted for FRS and diabetes mellitus. Values represent RR (95% CI) compared with the referent (ref) group.

Following guidelines issued by CDC/AHA,[10] we initially classified all study participants into 3 groups on the basis of baseline hsCRP levels of <1, 1 to <3, and ≥3 mg/L. Cox proportional-hazards models were then used to compute relative risks of future cardiovascular events across these 3 study groups. We then addressed the issue of whether very high or very low levels of hsCRP have clinical relevance for risk prediction in 2 stages. First, to avoid the possibility of data-derived findings, we initially reclassified all participants into 1 of 10 groups based on increasing deciles of the distribution of hsCRP. Second, to increase clinical usefulness, we repeated these analyses after classifying all participants into 1 of the following categories of baseline hsCRP: <0.5, 0.5 to <1.0, 1.0 to <2.0, 2.0 to <3.0, 3.0 to <4.0, 4.0 to <5.0, 5.0 to <10.0, 10.0 to <20.0, and ≥20.0 mg/L. In each instance, Cox proportional-hazards models were used to compute relative risks across the full spectrum of hsCRP levels. For all models, we computed both crude relative risks and relative risks adjusted for the FRS and additionally for diabetes. Because hormone replacement therapy (HRT) is known to elevate hsCRP levels, we repeated all analyses for the subgroup of women not using these agents at study entry.

## Results

The risk factor profile of participants in the Women's Health Study is similar to that of the general population in terms of both lipid levels and the proportion having metabolic syndrome.[11] Among the 27 939 women evaluated in this analysis, 12% were smokers at study entry, 2.5% had diabetes, and 25% had a history of hypertension. The mean body mass index was 25.9 kg/m$^2$. Between study initiation and time of this analysis, 698 first cardiovascular events were reported and confirmed by the end-points committee.

Table 1 presents the crude and FRS-adjusted relative risks of future cardiovascular events according to the clinical cutpoints set by the CDC/AHA guidelines. Compared with women with baseline levels of hsCRP <1 mg/L, the crude relative risk for those with baseline hsCRP levels between 1 and <3 mg/L was 1.7 (95% CI, 1.4 to 2.2), whereas the relative risk for those with baseline hsCRP levels ≥3 mg/L was 3.0 (95% CI, 2.4 to 3.7) (*P* for trend across groups <0.001). As expected, these risks were attenuated but remained statistically significant in models adjusted for FRS and additionally for diabetes. As also shown in Table 1, these effects remained statistically significant in the subgroup analysis of those 15 745 women not taking HRT at study entry (*P* for trend across groups <0.001).

Table 2 presents crude and FRS-adjusted relative risks of future cardiovascular events in analyses in which hsCRP levels were classified into 10 groups based on exact decile cutpoints. As shown, there is a strong and highly significant linear association between baseline hsCRP and future cardiovascular risk across the full spectrum of hsCRP levels. Specifically, crude relative risks from the very lowest (referent) to very highest deciles of baseline hsCRP were 1.0, 1.3, 2.6, 2.2, 3.0, 3.4, 3.6, 4.2, 5.1, and 6.3 (*P* for trend across groups <0.001). After adjustment for FRS, these risk estimates were 1.0, 0.9, 1.7, 1.3, 1.7, 1.6, 1.7, 1.9, 2.1, and 2.4 (*P* for trend across groups <0.001). Almost identical findings were observed in the subgroup not taking HRT at study entry (*P* for trend <0.001).

Table 3 presents crude and adjusted relative risks of future cardiovascular events in analyses in which baseline hsCRP values were defined according to clinically useful cutpoints of hsCRP rather than strict deciles. Again, in analyses of both the total cohort and those not taking HRT, a highly significant relationship between hsCRP and risk was observed across the full spectrum of hsCRP values. Specifically, the very lowest risk was observed among those in the referent group with hsCRP levels <0.5 mg/L, whereas risk was almost 8-fold higher among those with levels of hsCRP in excess of 20 mg/L (crude relative risk, 7.6; 95% CI, 4.7 to 12.1). These effects were even stronger in the non–HRT-using subgroup, in which the crude relative risk for those with hsCRP levels ≥20 mg/L was increased nearly 10-fold. All findings remained statistically significant after adjustment for FRS and additionally for diabetes (*P* for trend across groups <0.001 for both the total cohort and non–HRT users).

Figure 1 presents the relative impact of both very high and very low levels of hsCRP on future vascular risk using clinically relevant cutpoints for hsCRP. For comparison, the CDC/AHA cutpoints of <1, 1 to <3, and ≥3 mg/L used to determine low, moderate, and high risk are also shown. Figure 2 shows the predictive value of hsCRP levels among those with calculated 10-year Framingham Risks above and below 10%.

Finally, because diabetes is often considered a coronary risk equivalent, we repeated our analyses for those women free of diabetes at study entry. Among such women, the relative risks for those with baseline hsCRP levels <0.5, 0.5 to <1.0, 1.0 to <2.0, 2.0 to <3.0, 3.0 to <4.0, 4.0 to <5.0, 5.0 to <10.0, 10.0 to <20.0, and ≥20.0 mg/L were 1.0, 2.1, 2.6, 3.0, 3.6, 4.0, 4.6, 5.0, and 7.4, respectively (*P* for trend <0.001).

**TABLE 2. Crude and FRS-Adjusted Relative Risks of First Cardiovascular Events According to Increasing Deciles of hsCRP With Cutpoints Also Provided**

| | | Total Cohort (n=27 939) | | | | No HRT (n=15 745) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Decile | hsCRP, mg/L | Events, n | Crude RR | FRS-Adjusted RR | FRS+DM-Adjusted RR | Events, n | Crude-Adjusted RR | FRS-Adjusted RR | FRS+DM-Adjusted RR |
| 1 | <0.36 | 22 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) | 11 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 2 | 0.36–<0.64 | 28 | 1.3 (0.7–2.2) | 0.9 (0.5–1.6) | 0.9 (0.5–1.6) | 10 | 0.9 (0.4–2.2) | 0.6 (0.2–1.4) | 0.6 (0.2–1.4) |
| 3 | 0.64–<1.00 | 55 | 2.6 (1.6–4.3) | 1.7 (1.0–2.8) | 1.7 (1.0–2.8) | 22 | 2.1 (1.0–4.2) | 1.1 (0.5–2.4) | 1.1 (0.5–2.4) |
| 4 | 1.00–<1.46 | 49 | 2.2 (1.4–3.7) | 1.3 (0.8–2.2) | 1.3 (0.8–2.2) | 34 | 3.3 (1.7–6.4) | 1.7 (0.9–3.5) | 1.7 (0.9–3.5) |
| 5 | 1.46–<2.02 | 65 | 3.0 (1.9–4.9) | 1.7 (1.0–2.7) | 1.7 (1.0–2.7) | 31 | 2.9 (1.5–5.7) | 1.3 (0.6–2.6) | 1.3 (0.6–2.6) |
| 6 | 2.02–<2.74 | 72 | 3.4 (2.1–5.5) | 1.6 (1.0–2.6) | 1.6 (1.0–2.6) | 38 | 3.6 (1.8–7.0) | 1.6 (0.8–3.1) | 1.5 (0.8–3.0) |
| 7 | 2.75–<3.71 | 76 | 3.6 (2.2–5.7) | 1.7 (1.0–2.7) | 1.6 (1.0–2.6) | 47 | 4.4 (2.3–8.6) | 1.7 (0.9–3.2) | 1.6 (0.8–3.1) |
| 8 | 3.71–<5.17 | 90 | 4.2 (2.6–6.7) | 1.9 (1.2–3.0) | 1.8 (1.1–2.9) | 54 | 5.1 (2.7–9.8) | 1.8 (0.9–3.4) | 1.7 (0.9–3.3) |
| 9 | 5.17–<7.73 | 108 | 5.1 (3.2–8.0) | 2.1 (1.3–3.4) | 1.9 (1.2–3.1) | 77 | 7.3 (3.9–13.8) | 2.4 (1.2–4.5) | 2.0 (1.1–3.9) |
| 10 | ≥7.73 | 133 | 6.3 (4.0–9.8) | 2.4 (1.5–3.9) | 2.1 (1.3–3.2) | 94 | 9.0 (4.8–16.9) | 2.8 (1.5–5.2) | 2.3 (1.2–4.3) |
| *P* for trend | | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | <0.001 |

Abbreviations as in Table 1. Values represent RR (95% CI) compared with the referent (ref) group.

hsCRP cutpoints shown are for the total cohort. Decile cutpoints for hsCRP for the group not taking HRT are <0.29, 0.29–<0.49, 0.49–<0.75, 0.75–<1.08, 1.08–<1.52, 1.52–<2.09, 2.09–<2.93, 2.93–<4.19, 4.19–<6.61, and ≥6.61 mg/L.

In all analyses, virtually identical results were obtained when individual components of the FRS were used.

## Discussion

These prospective data indicate that the predictive value of hsCRP for future cardiovascular events is linear across a full range of values. Most importantly, these data demonstrate that both very high (>10 mg/L) and very low (<0.5 mg/L) levels of hsCRP provide important prognostic information on vascular risk across a full range of FRS. These observations were consistent in analyses using deciles of hsCRP as well as clinically relevant cutpoints and were present in the total cohort as well as in the subgroups of those not taking HRT and those without diabetes.

The present data have both clinical and pathophysiological relevance. From a clinical perspective, these data demonstrate that the predictive value of hsCRP is strongly linear across the full range of values. Thus, not only is there no evidence in these data of any threshold effect, but there is also no evidence that unusually low or unusually high values represent false-positive findings. Quite to the contrary, these data indicate that there is considerable predictive value of hsCRP levels beyond the ranges suggested by the recent CDC/AHA guidelines for use of hsCRP.[10] Thus, in addition to the "high-risk" group defined by the CDC/AHA as having levels of hsCRP between 3 and 10 mg/L, there appears to be a "very-high-risk" group with levels of hsCRP in excess of 10 mg/L (which in our study represented 5.5% of the total

**TABLE 3. Crude and FRS-Adjusted Relative Risks of First Cardiovascular Events Across a Full Range of Clinically Set hsCRP Cutpoints**

| | Total Cohort (n=27 939) | | | | No HRT (n=15 745) | | | |
|---|---|---|---|---|---|---|---|---|
| hsCRP, mg/L | Events, n | Crude RR | FRS-Adjusted RR | FRS+DM-Adjusted RR | Events, n | Crude Adjusted RR | FRS-Adjusted RR | FRS+DM-Adjusted RR |
| <0.50 | 34 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) | 21 | 1.0 (ref) | 1.0 (ref) | 1.0 (ref) |
| 0.50–<1.0 | 71 | 2.2 (1.4–3.2) | 1.6 (1.1–2.4) | 1.6 (1.1–2.5) | 54 | 3.0 (1.8–5.0) | 2.1 (1.3–3.6) | 2.1 (1.3–3.5) |
| 1.0–<2.0 | 111 | 2.5 (1.7–3.7) | 1.6 (1.1–2.4) | 1.6 (1.1–2.4) | 68 | 3.2 (1.9–5.1) | 1.8 (1.1–3.0) | 1.8 (1.1–3.0) |
| 2.0–<3.0 | 91 | 3.1 (2.1–4.6) | 1.7 (1.1–2.5) | 1.7 (1.1–2.5) | 52 | 4.2 (2.5–7.0) | 2.1 (1.2–3.5) | 1.9 (1.2–3.3) |
| 3.0–<4.0 | 79 | 3.7 (2.5–5.6) | 1.9 (1.3–2.9) | 1.9 (1.2–2.8) | 47 | 5.6 (3.3–9.3) | 2.4 (1.4–4.1) | 2.3 (1.4–3.9) |
| 4.0–<5.0 | 63 | 4.2 (2.8–6.4) | 2.2 (1.4–3.3) | 2.0 (1.3–3.1) | 42 | 7.5 (4.4–12.6) | 3.3 (1.9–5.6) | 2.9 (1.7–5.1) |
| 5.0–<10.0 | 169 | 4.9 (3.4–7.1) | 2.3 (1.5–3.3) | 2.0 (1.4–3.0) | 94 | 7.9 (4.9–12.7) | 3.1 (1.9–5.1) | 2.6 (1.6–4.3) |
| 10.0–<20.0 | 44 | 6.3 (4.0–9.8) | 2.8 (1.7–4.4) | 2.4 (1.5–3.8) | 24 | 10.4 (5.8–18.7) | 4.0 (2.2–7.4) | 3.3 (1.8–6.1) |
| ≥20 | 36 | 7.6 (4.7–12.1) | 3.1 (1.9–5.1) | 2.4 (1.5–4.0) | 16 | 9.3 (4.8–17.9) | 3.9 (2.0–7.5) | 2.9 (1.5–5.6) |
| *P* for trend | | <0.001 | <0.001 | <0.001 | | <0.001 | <0.001 | 0.002 |

Abbreviations as in Table 1. Values represent RR (95% CI) compared with the referent (ref) group. Data are shown for the total cohort (n=27 939) and for those women not taking HRT (n=15 745).

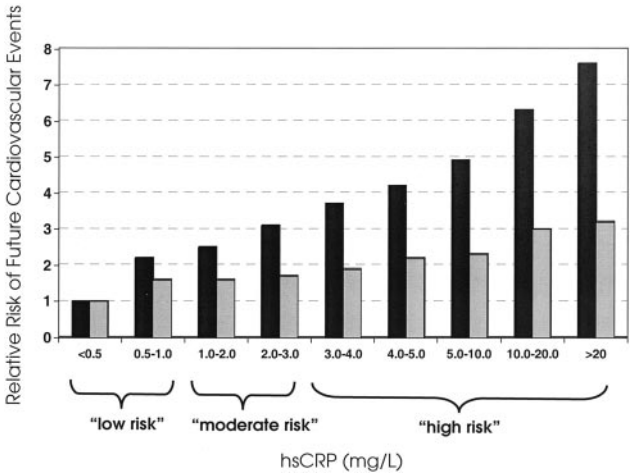


**Figure 1.** Relative risks of future cardiovascular events across a full clinical range of hsCRP values. Black bars represent crude relative risks; gray bars, risks adjusted for FRS.

population). Moreover, although levels of hsCRP ≥20 mg/L were rare (2.2% of the total population), these individuals were observed to have the very highest risk of future vascular events. By contrast, risk appeared to be very low for individuals at the other end of the spectrum with hsCRP levels <0.5 mg/L (15.1% of the study population). Indeed, this group appeared to have very low risk even when compared with those with hsCRP levels between 0.5 and 1.0 mg/L. As shown in our multivariate analyses, this was true even when other risk factors were present and after adjustment for the FRS and additionally for diabetes.

From a pathophysiological perspective, these analyses also raise several intriguing issues. First, the observation that individuals with exceptionally low levels of hsCRP have very low risks of future cardiovascular events provides clinical support for the concept that CRP itself may have a direct role in atherothrombosis and raises the possibility that a virtual absence of CRP may in fact be protective. For example, mice transgenic for human CRP not only begin to express elevated CRP levels for the first time but also have increased rates of arterial thrombosis, at least compared with wild-type mice that minimally express CRP.[12] Recent work further indicates that CRP can be produced within the vascular smooth muscle of diseased coronary arteries[13,14] and that this production may directly lead to the expression of several mediators of the atherothrombotic process, including adhesion molecule induction, reduced NO production, and altered fibrinolytic function.[15] Thus, individuals without expressed CRP levels may largely be free of these proatherogenic responses. Conversely, our observation that individuals with very high levels of hsCRP are at very high vascular risk is consistent with the hypothesis that CRP may have direct arterial effects or be a surrogate for these effects. In this regard, rather than suggesting that markedly elevated levels of hsCRP represent a false-positive response, the current clinical data raise the possibility that chronic inflammation from any of several causes may well increase vascular risk. As such, these data are consistent with reports suggesting that several chronic conditions including arthritis, periodontal disease, and chronic low-grade infection may all predispose to atherothrombotic events.[16]

Our data also reinforce the need to use high-sensitivity assays for the evaluation of CRP. Although older assays for CRP might be able to reliably detect levels in excess of 10 mg/L (the very-high-risk group), it is only with use of hsCRP assays that clinical detection across a full range can be assessed. As demonstrated in these data, that range must include those at high risk (hsCRP between 3 and 10 mg/L) as well as those at very low risk (<0.5 mg/L) and intermediate risk (hsCRP between 1.0 and 3.0 mg/L), all levels undetectable without high-sensitivity assays.

An important limitation of our study is that we evaluated hsCRP levels only once at baseline and thus cannot eliminate the possibility that some of the marked elevations observed might well reflect a clinically silent acute-phase response. However, this potential misclassification bias among those with high levels of hsCRP can lead only to an underestimation of true effects, not a falsely high risk estimate. Thus, the magnitude of predictive values found here for hsCRP are, if anything, likely to be underestimates of true effects. Clinicians can largely avoid this difficulty by simply measuring hsCRP twice whenever levels are in excess of 10 mg/L. This

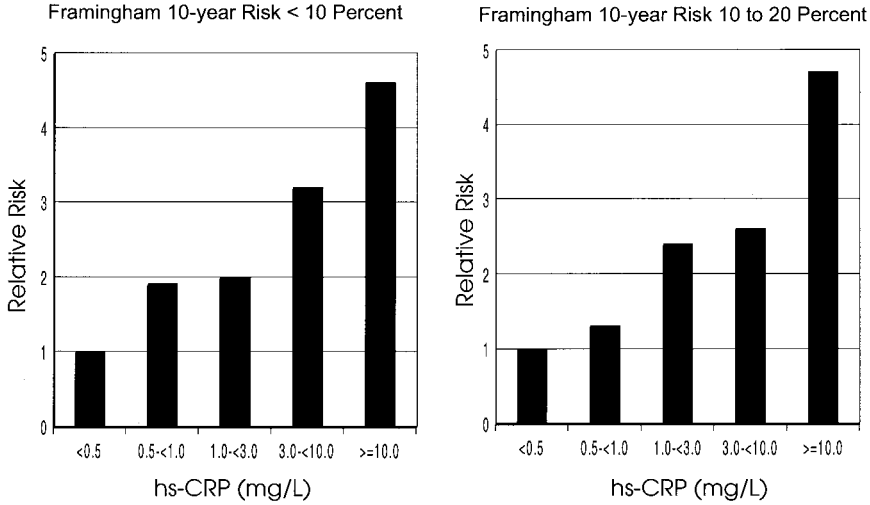


**Figure 2.** Relative risks of future cardiovascular events among those with calculated 10-year Framingham risks <10% (left) and between 10% and 20% (right).

practice is consistent with the recent CDC/AHA guidelines and, as has been found in several reports, greatly reduces any residual variation in levels that may be observed in outpatient clinical use.[17,18] Finally, absolute event rates within the Women's Health Study are low in comparison to the general population because of the "healthy cohort effect" and the fact that our participants are healthcare providers. However, the fact that hsCRP has been shown to predict vascular risk with similar magnitude in multiple other studies of men and women suggests that the relative risks described here are generalizable.

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# Should C-Reactive Protein Be Added to Metabolic Syndrome and to Assessment of Global Cardiovascular Risk?

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***Abstract***—Of novel risk factors for cardiovascular disease currently under investigation, high-sensitivity C-reactive protein (hsCRP) is the most promising. To date, more than 20 prospective epidemiologic studies have demonstrated that hsCRP independently predicts vascular risk, 6 cohort studies have confirmed that hsCRP evaluation adds prognostic information beyond that available from the Framingham Risk Score, and 8 cohort studies have demonstrated additive prognostic value at all levels of metabolic syndrome or in the prediction of type 2 diabetes. In contrast to several other biomarkers that also reflect biological aspects of inflammation, hypofibrinolysis, and insulin resistance, hsCRP measurement is inexpensive, standardized, widely available, and has a decade-to-decade variation similar to that of cholesterol. Given the consistency of prognostic data for hsCRP and the practicality of its use in outpatient clinical settings, we believe the time has come for a careful consideration of adding hsCRP as a clinical criterion for metabolic syndrome and for the creation of an hsCRP-modified coronary risk score useful for global risk prediction in both men and women. Toward this end, we believe experts in the fields of epidemiology, prevention, vascular biology, and clinical cardiology should be convened to begin discussing the merits of this proposal. **(*Circulation*. 2004;109:2818-2825.)**

**Key Words:** inflammation ■ risk factors ■ prevention ■ diabetes mellitus ■ atherosclerosis

The identification of individuals who are at high risk for developing cardiovascular disease but who currently lack symptoms is a critical issue in primary prevention. For more than 30 years, cardiovascular risk prediction algorithms have relied on blood pressure, smoking status, hyperlipidemia, and the presence or absence of diabetes. These core traditional risk factors for heart disease and stroke derive largely from the groundbreaking Framingham Heart Study that first provided the conceptual basis for cardiovascular risk factors in the early 1960s.[1] With corroborating evidence from major cohort studies performed worldwide, these risk factors and their interactions with age and sex were formally codified in the 1980s into the Framingham Risk Score.[2,3] This scoring system, along with its European counterpart,[4] has been highly successful and forms the basis for most coronary risk detection and prevention programs.[5] In current practice, those with 10-year Framingham coronary heart disease (CHD) risk estimates that are less than 5% are considered to be at low risk, those with 10-year estimates between 6% and 20% are considered at intermediate risk, and those with 10-year risks of 20% and higher (or who have diabetes) are considered to be coronary risk equivalents.[6,7]

Despite the success of the Framingham Risk Score, there are limitations to this approach. First, it is widely recognized that a fifth of all events occur among individuals in whom traditional risk factors have not been identified.[8] Moreover, the specificity of traditional risk factors is limited.[9] Multiple studies additionally confirm that most vascular events occur among individuals without evidence of very high cholesterol levels[10] and that the intermediate-risk group is large, heterogeneous, and in need of better methods for risk stratification.[11] Finally, the relationship between Framingham scores and absolute risk for CHD varies across populations.[12–14]

For all of these reasons, there has been considerable interest in developing novel risk factors that might improve global risk prediction. To be useful in a clinical setting, the biomarker of interest should provide information on risk or prognosis beyond that available from standard global assessment tools. Successful screening techniques should also be inexpensive and available to primary care practitioners to ensure appropriate interpretation and follow-up. Thus, imaging techniques, including MRI, carotid ultrasonography, and coronary calcium detection, are unlikely to be useful as first-level screening tools. Similarly, metabolic evaluations, such as oral glucose tolerance testing, may be impractical given the time constraints of daily clinical practice. By contrast, simple blood tests that can be sent at the time of cholesterol evaluation are more likely to succeed.

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Of potential novel risk factors presently available, high-sensitivity C-reactive protein (hsCRP), a marker of low-grade vascular inflammation, is among the most promising. Prospective epidemiologic studies consistently demonstrate that hsCRP adds independent prognostic information at all levels of LDL cholesterol and at all levels of the Framingham Risk Score.[15] The Centers for Disease Control and Prevention (CDC) and the American Heart Association (AHA) published in January of 2003 the first set of guidelines to endorse use of hsCRP as an adjunct to traditional risk factor screening.[16] The CDC/AHA report also endorsed hsCRP as the only inflammatory biomarker currently available with adequate standardization and predictive value to justify use in outpatient clinical settings. On the basis of data from available investigations, levels of hsCRP $<1$, 1 to 3, and $>3$ mg/L have been defined as lower, moderate, and higher cardiovascular risk. Taking a conservative approach, the CDC/AHA report suggested that the best use of hsCRP was in patients at intermediate Framingham risk.

In the year since publication of the CDC/AHA report, abundant data have emerged not only confirming the ability of hsCRP to add prognostic information to the Framingham Risk Score but also linking hsCRP to metabolic syndrome and the development of incident type 2 diabetes. Moreover, accumulating data suggest that both very low and very high levels of hsCRP seem to provide independent prognostic information across a full spectrum of Framingham risk.[17] At the same time, cost-effectiveness studies have found that, given the low cost of screening for hsCRP, simultaneous evaluation of hsCRP at the time of lipid screening may be more efficient than a selective policy of hsCRP use that requires a return visit to the primary care physician as well as an additional outpatient phlebotomy.[18,19]

All of these new data raise the possibility that hsCRP testing may improve CHD risk assessment, and clinicians within the prevention community have begun considering the use of hsCRP as a core part of global risk assessment, both in terms of Framingham risk evaluation and in terms of a modified metabolic syndrome evaluation. We thus review here evidence for hsCRP as a potential adjunct to both the Framingham Risk Score and as an additional clinical criterion for diagnosis of metabolic syndrome.

## Evidence That hsCRP Is Independent of and Adds Predictive Value to the Framingham Risk Score

To date, 22 prospective studies of hsCRP and risk of future cardiovascular disease have been presented, and all are positive. Furthermore, 6 major prospective studies have demonstrated that hsCRP adds prognostic information on cardiovascular risk beyond that available using the Framingham Risk Score alone. Four investigations—the Physicians' Health Study (PHS),[20] the Women's Health Study (WHS),[10,21] the Atherosclerosis Risk in Communities Study (ARIC),[22] and the Air Force/Texas Atherosclerosis Prevention Study (AFCAPS/TexCAPS)[23]—were performed in the United States, and 2 studies, the Monitoring of Trends and Determinants of Cardiovascular disease (MONICA) study[24] and the Reykjavik Study,[25] were performed in Europe. In addition, the Framingham Heart Study itself has provided

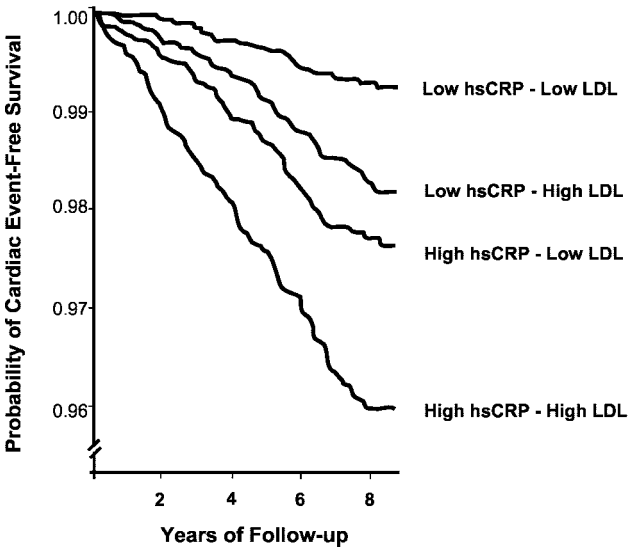


**Figure 1.** Cardiovascular event-free survival among apparently healthy individuals according to baseline levels of hsCRP and LDL cholesterol. Reprinted with permission from Reference 10. Copyright © 2002 Massachusetts Medical Society. All rights reserved.

evidence that hsCRP independently predicts thrombotic events in the cerebral circulation,[26] and the Pravastatin Inflammation/CRP Evaluation (PRINCE) database has provided evidence that hsCRP picks up risk information that cannot be gleaned from the individual Framingham covariates.[27]

The largest of the American cohorts is the WHS, a prospective evaluation of 27 939 initially healthy American women who underwent hsCRP evaluation along with a full lipid panel and Framingham risk assessment and were monitored over a period of 8.3 years for the occurrence of first-ever cardiovascular events.[10,21] When this study was first presented, 571 first-ever nonfatal myocardial infarctions, nonfatal strokes, coronary revascularizations, or cardiovascular deaths had accrued. Following the National Cholesterol Education Program Adult Treatment Panel III (ATP III) guidelines, the WHS emphasized hard cardiovascular end points and did not include angina pectoris.[10]

Overall, baseline hsCRP levels in the WHS were a strong predictor of future vascular events; the relative risks for those with lowest to highest quintiles of hsCRP at baseline were 1.0, 1.8, 2.3, 3.2, and 4.5 ($P<0.001$). After adjustment for age, smoking, diabetes, blood pressure, and hormone replacement therapy, the risk in the top quintile of hsCRP was 2.3 (95% CI, 1.6 to 3.4). The hsCRP levels minimally correlated with LDL in the WHS, and a combined approach, using both lipids and hsCRP, provided improved prediction of cardiovascular event-free survival (Figure 1).

Most importantly, hsCRP levels remained a highly significant predictor of risk in the WHS after adjustment for the Framingham Risk Score.[10] After taking into account all components of the Framingham Risk Score, the relative risks for those with lowest to highest hsCRP quintiles at baseline were 1.0, 1.3, 1.4, 1.7, and 1.9 ($P<0.001$) for all participants and 1.0, 1.6, 1.5, 1.8, and 2.2 ($P<0.001$) for those not taking

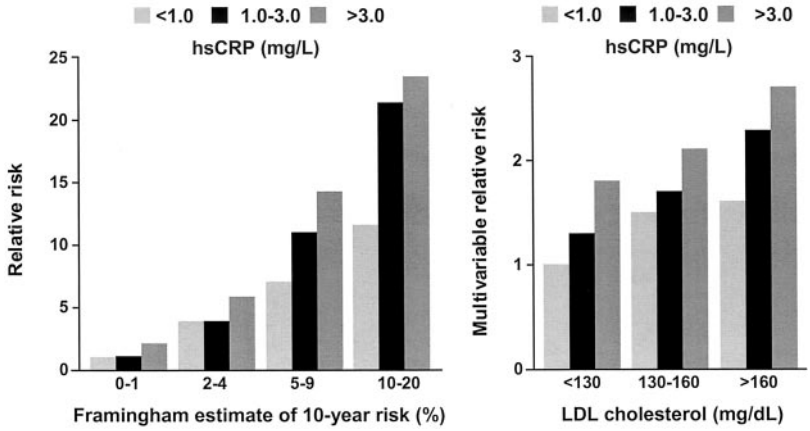


**Figure 2.** hsCRP adds prognostic information at all levels of LDL cholesterol (right) and at all levels of the Framingham Risk Score (left). Reprinted with permission from Reference 10. 

hormone replacement therapy. The addition of data on hsCRP provided qualitatively important information on risk at all levels of LDL cholesterol after adjustment for usual risk factors (Figure 2, right) and at all levels of estimated 10-year risk based on the Framingham Risk Score (Figure 2, left). These latter analyses were based on levels of hsCRP <1, 1 to 3, and >3 mg/L, the cut points suggested for use by the CDC/AHA guidelines.

Since publication of these results, there has been continued accrual of cardiovascular end points within the WHS as well as ongoing analysis of the utility of hsCRP as a risk predictor. For example, within the WHS, evidence is now available that demonstrates predictive value both for extremely low levels of hsCRP (<0.5 mg/L) and for extremely high levels of hsCRP (>10 mg/L).[17] This new analysis is important because it shows that dividing hsCRP levels into five categories (<0.5, 0.5 to <1.0, 1.0 to <3.0, 3.0 to <10.0, and ≥10.0 mg/L) may improve risk discrimination at both low and high levels of the Framingham Risk Score, potentially leading to a superior way to code hsCRP for use in CRP-modified algorithms (Figure 3). These data are also consistent with the hypothesis that very low levels of hsCRP may protect against acute vascular events. On the other hand, chronic inflammation from any source leads to excess risk, a hypothesis consistent with evidence about direct mechanisms by which CRP may affect both atherosclerotic development and acute thrombosis.

Data from the WHS demonstrate the additive value of hsCRP to the Framingham Risk Score and provide confirmation of data that had been presented earlier for men in the PHS.[20] In that cohort of healthy middle-aged men, baseline levels of hsCRP were independently predictive of future myocardial infarction and thromboembolic stroke but not of venous thrombosis. Furthermore, the PHS demonstrated that the relative benefit of aspirin was greatest in preventing vascular events among those with the highest hsCRP levels, an intriguing observation given the antiinflammatory properties of aspirin.[20] That hsCRP is an independent predictor beyond the Framingham Risk Score is also evident in those early original data. Specifically, after adjustment for all components of the Framingham Risk Score, the relative risks of future myocardial infarction in the PHS for those with hsCRP levels <1, 1 to 3, and >3 mg/L were 1.0, 1.7, and 2.2 (95% CI for those with hsCRP >3.0 mg/L, 1.2 to 3.8).

Data on hsCRP from the PHS and WHS have been corroborated by similar analyses from other large cohorts from the United States and Europe. In a case-cohort analysis of 12 819 apparently healthy middle-aged men and women participating in the ARIC study over a 6-year follow-up period, the relative risks of incident coronary heart disease for those with baseline hsCRP levels <1.0, 1.0 to 3.0, and >3.0 mg/L were 1.0, 1.6, and 2.5 after adjusting for age, sex, and ethnicity.[22] After full adjustment for the Framingham covariates and additionally for diabetes, these risk estimates were

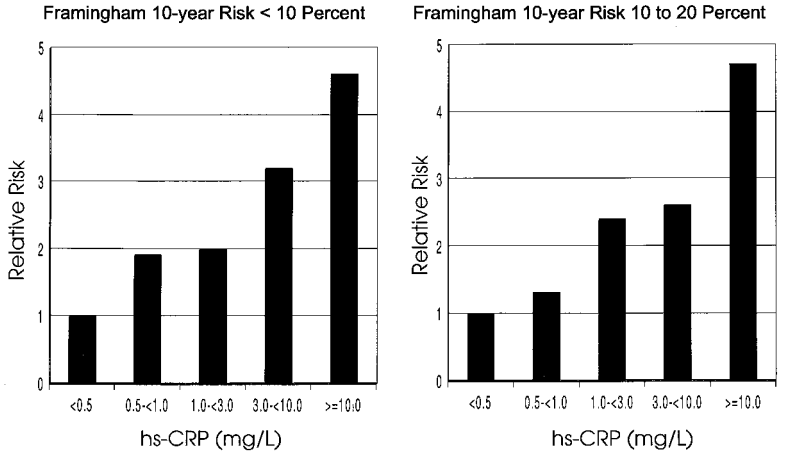


**Figure 3.** Clinical utility of very high (>10 mg/L) as well as very low (<0.5 mg/L) levels of hsCRP among those with 10-year Framingham estimated risks <10% (left) and between 10% and 20% (right). Data from Reference 17.

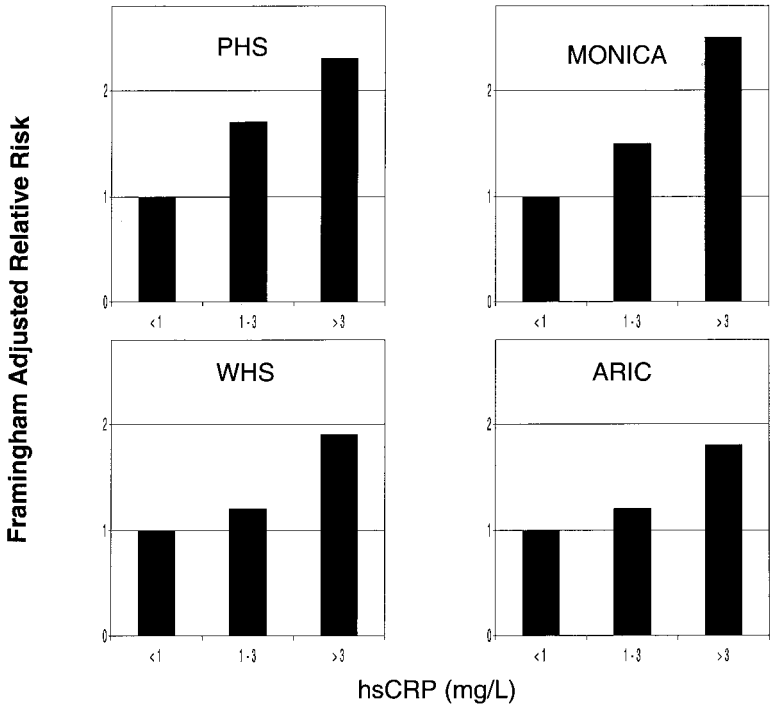


**Figure 4.** Framingham-adjusted relative risks of future coronary events according to baseline levels of hsCRP <1, 1 to 3, and >3 mg/L in 4 major cohort studies. Data from References 10, 20–22, 24, and 51.

1.0, 1.2, and 1.8, respectively (95% CI for those with hsCRP >3.0, 1.0 to 3.0). Almost identical data derive from a prospective evaluation of 3435 German men participating in the MONICA-Augsberg Cohort Study in which 191 incident coronary events occurred during 6.6 years of follow-up.[24] In this study of men, as in the WHS study of women, hsCRP levels at baseline were independently associated with incident coronary events. These effects remained significant ($P<0.001$) after adjustment for the Framingham Risk Score, such that persons with hsCRP levels of <1, 1 to 3, and >3 mg/L had fully adjusted relative risks of 1.0, 1.5, and 2.5, respectively (95% CI for those with hsCRP >3.0, 1.8 to 3.7). The exceptional consistency of these Framingham-adjusted findings for hsCRP across the PHS, WHS, ARIC, and the MONICA studies using the AHA/CDC established cut points for hsCRP of <1, 1 to 3, and >3 mg/L are shown in Figure 4.

Strong supportive evidence for the addition of hsCRP to Framingham risk evaluation also comes from the large Reykjavik Study that included 2459 incident events during an 18-year follow-up period.[25] Although this prospective study used an hsCRP cut point of 2.0 rather than 3.0 and thus would tend to underestimate effects compared with other cohorts, a highly significant fully adjusted odds ratio of 1.5 was nonetheless observed. In fact, this 50% increase in risk associated with hsCRP was observed not only after control for typical Framingham covariates but also after additional control for diabetes, triglycerides, body mass index, and indices of pulmonary function. Moreover, the odds ratio for hsCRP observed in the Reykjavik Study was exactly the same as the adjusted odds ratio observed for hypertension and statistically similar to that of smoking. Furthermore, the fully adjusted odds ratio for hsCRP during the initial 10 years of follow-up was 1.84, a risk estimate consistent with all prior studies.

Although there has been controversy about the relative importance of hsCRP compared with cholesterol in the Iceland analysis, it is important to recognize that the Reykjavik population studied had a mean total cholesterol of 247 mg/dL compared with the United States average of 213 mg/dL. Thus, the Icelandic data not only confirm prior reports that hsCRP significantly predicts risk after adjustment for Framingham covariates but also demonstrate the additive clinical value of hsCRP in a population with much higher baseline cholesterol levels than those observed in contemporary American and European studies.

In addition to these 5 major cohorts, supportive evidence for the addition of hsCRP to Framingham risk evaluation also comes from other sources. Within the AFCAPS/TexCAPS analysis of 5742 apparently healthy individuals enrolled in a randomized primary prevention trial of lovastatin versus placebo, each quartile increase in baseline hsCRP was associated with a 21% increase in the risk of a first cardiovascular event (95% CI, 4% to 41%), an effect that again persisted after control for all individual components of the Framingham Risk Score.[23] Similarly, in an analysis of 1666 individuals free of cardiovascular disease enrolled in the PRINCE study, hsCRP levels correlated modestly with 10-year Framingham Risk Scores yet showed minimal relation to any individual component of the score itself.[27] Thus, as in the prospective cohort evaluations, the PRINCE data suggest that hsCRP detects a component of vascular risk not readily obtained from the Framingham covariates themselves.

Finally, within the Framingham Heart Study, data have also been presented that demonstrate the ability of hsCRP to predict stroke risk independently of the Framingham covariates.[26] After adjustment for age, smoking, blood pressure, diabetes, and total and HDL cholesterol, the risk of future stroke in the Framingham Heart Study increased 25% in men ($P=0.036$) and 29% in women ($P=0.008$) for each increasing quartile of hsCRP. These latter data are consistent with

evidence from several studies showing that hsCRP also predicts first-ever thromboembolic stroke.[10,20,21] With regard to hard coronary heart disease end points within Framingham, power is limited because of a small number of events. However, the age-adjusted relative risks of hard coronary heart disease within Framingham for those with baseline levels of hsCRP <1, 1 to 3, and >3 mg/L are 1.0, 1.47, and 1.63, data fully consistent with those from the other larger studies.

Although a predictor of vascular events, hsCRP levels do not track closely with subclinical atherosclerosis, as measured by cardiac catheterization, intimal-medial thickness, the ankle-brachial index, or coronary calcification.[28–30] This observation likely reflects the fact that inflammation is more tightly associated with plaque vulnerability and rupture than with total plaque burden per se. Clinically, this observation also helps to explain why hsCRP levels not only add to the Framingham Risk Score but also add to coronary risk prediction based on coronary calcification; in the South Bay Heart Study, elevated hsCRP levels resulted in a doubling of risk at low, moderate, and high levels of coronary calcification.[31] Thus, measures of inflammation such as hsCRP seem to provide independent and complementary information on risk beyond that achievable by direct measures of atherosclerotic burden.

## Evidence That hsCRP Correlates With and Adds Prognostic Information to Formal Definitions of Metabolic Syndrome

Part of the clinical interest in adding hsCRP to current risk algorithms derives from the fact that inflammation also plays a major role in the development of diabetes and is intimately related to several difficult-to-measure components of the metabolic syndrome.[32] In cross-sectional studies, hsCRP levels have been found to correlate with elevated triglycerides, low HDL levels, midline obesity, elevated blood pressure, and high fasting glucose levels, the key easily measured components of the ATP III definition of metabolic syndrome.[33,34] However, hsCRP levels also correlate with insulin resistance and impaired fibrinolysis, major components of the metabolic syndrome that are not easily evaluated in an outpatient practice setting. In one study of women, hsCRP and body mass index were the only independent correlates of fasting insulin levels when modeled as a continuous dependent variable.[35]

In other investigations, hsCRP levels have been found to correlate with direct measures of insulin resistance and endothelial dysfunction.[36,37] Among nondiabetic participants in the Insulin Resistance Atherosclerosis Study (IRAS), the correlation coefficients between hsCRP and fasting glucose, fasting insulin, and insulin sensitivity were 0.18, 0.33, and −0.37, respectively (all $P$ values <0.001).[33] The IRAS investigators also found correlations between hsCRP and plasminogen activator inhibitor, indicating interrelationships between inflammation and hypofibrinolysis.[38] Not all of these effects are attributable to obesity, as insulin resistance per se appears responsible for higher production of cytokines.[39] Thus, because of its relation to these additional pathophysiological components of risk, it has been hypothesized that hsCRP evaluation might also add prognostic information as an additional clinical criterion for diagnosis of the metabolic syndrome.[40]

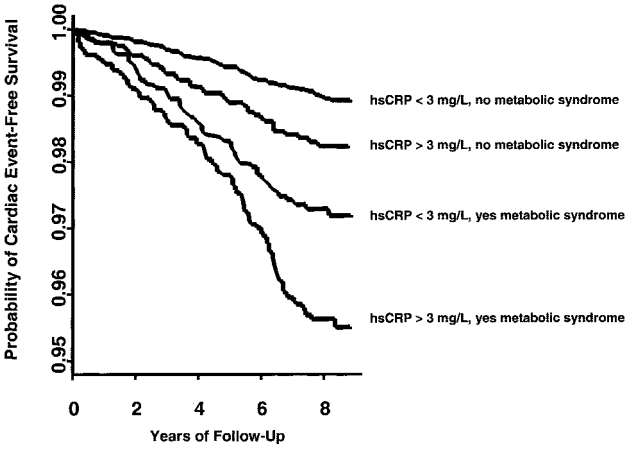


**Figure 5.** Cardiovascular event-free survival according to hsCRP levels above or below 3 mg/L among individuals with and without metabolic syndrome. Reprinted with permission from Reference 34.

Evidence supporting this hypothesis is now available from several major prospective studies, of which the WHS and the West of Scotland Coronary Prevention Study (WOSCOPS) are the largest. In the WHS, levels of hsCRP were shown to correlate with the major components of the metabolic syndrome, and in univariate analyses, the finding of an hsCRP level greater than 3 mg/L had almost identical prognostic value in terms of cardiovascular event-free survival, as did a full assessment of the metabolic syndrome (area under the receiver-operating characteristic curve, 0.77 for hsCRP alone and 0.78 for having at least 3 of 5 ATP III components of the metabolic syndrome).[34] More importantly, in this large-scale prospective evaluation, hsCRP levels were found to add prognostic information to the metabolic syndrome definition. As shown in Figure 5, those who had hsCRP levels <3 mg/L without metabolic syndrome had the best vascular survival, whereas those who had hsCRP levels >3 mg/L with the metabolic syndrome had the worst vascular survival.

An almost identical additive interaction between hsCRP, metabolic syndrome, and subsequent vascular risk was observed in WOSCOPS, a randomized intervention trial of pravastatin that monitored 6447 middle-aged men over a 5-year period. In WOSCOPS, hsCRP levels above and below 3 mg/L at baseline were highly predictive of incident vascular events after stratification by the presence or absence of the metabolic syndrome.[41] Specifically, the observed relative risks of future coronary events in the low CRP/metabolic syndrome–absent, high CRP/metabolic syndrome–absent, low CRP/metabolic syndrome–present, and high CRP/metabolic syndrome–present subgroups within WOSCOPS were 1.0 (referent), 1.6, 1.6, and 2.8, respectively (all $P$ values <0.05).

Additional evidence of the interrelationships between inflammation and metabolic syndrome derive from 6 prospective studies that have reported hsCRP levels to predict the onset of type 2 diabetes, often after controlling for obesity and other diabetes-related risk factors. In the WHS, those

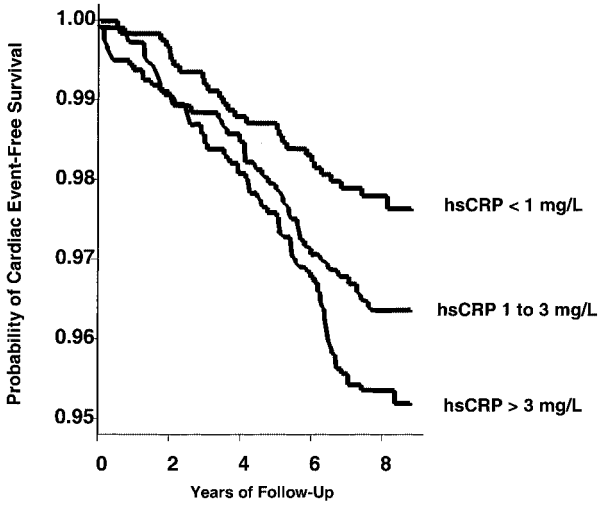


**Figure 6.** Clinical predictive value of hsCRP levels <1, 1 to 3, and >3 g/L among individuals already defined as having metabolic syndrome by ATP III criteria. Reprinted with permission from Reference 34.

with hsCRP levels in the top quartile were more than 4 times as likely to develop diabetes compared with those with hsCRP levels in the lowest quartile (multivariate adjusted relative risk, 4.2; 95% CI, 1.2 to 12.0).[42] Similarly, in WOSCOPS, those with the highest levels of hsCRP at study entry had a 3-fold increase in risk of incident diabetes during the 5-year follow-up period (multivariate adjusted relative risk, 3.1; 95% CI, 1.3 to 7.1).[43] Smaller but consistent effects were observed in the Cardiovascular Health Study, which included 5888 older individuals where the multivariate adjusted relative risk of incident diabetes for those with the highest quartile of baseline hsCRP was 1.8 (95% CI, 1.2 to 2.9).[44] Finally, in the MONICA cohort of 2052 middle aged men, the Insulin Resistance Atherosclerosis Study (IRAS) of 1047 middle-aged men and women, and the Nurses Health Study of middle-aged women, significant age-adjusted associations between baseline hsCRP and incident diabetes were observed.[38,45,46] hsCRP levels have additionally been found to predict cardiovascular risk among those with diabetes; in a prospective cohort of 746 men with type 2 diabetes who were free of cardiovascular disease at study entry, those with hsCRP levels in the top quartile were 3 times as likely to develop cardiovascular events even after control for all available covariates (95% CI, 1.3 to 5.3).[47]

On the basis of the above observations, an argument can be made for including hsCRP as one of the clinical criteria for the diagnosis of the metabolic syndrome. Newly reported clinical data support this contention. Within the WHS population, 3597 women with the ATP III criteria for the metabolic syndrome were prospectively followed up over an 8-year period for first-ever cardiovascular events.[34] In that prospective cohort, cardiovascular event-free survival among patients with metabolic syndrome was markedly different when information on hsCRP was taken into consideration. As shown in Figure 6, baseline hsCRP levels <1, 1 to 3, and >3 mg/L differentiated between low-, moderate-, and higher-risk groups among women already identified as having metabolic syndrome by ATP III criteria. Those with metabolic syndrome and the highest levels of hsCRP had a relative risk of future cardiovascular events twice that of individuals with metabolic syndrome and low levels of hsCRP (95% CI, 1.1 to 4.2; $P=0.001$).[34]

The studies above demonstrate that vascular risk prediction and the prediction of type 2 diabetes can be improved by knowledge of hsCRP levels, even among those with metabolic syndrome. Recent studies relating hsCRP to incident hypertension serve to reinforce the importance of blood pressure in the metabolic syndrome complex.[48,49] Whether to formally incorporate hsCRP as one criterion for diagnosis of metabolic syndrome is presently an area of intense debate. The simplicity of hsCRP evaluation strengthens the argument, particularly because direct measures of insulin resistance and hypofibrinolysis are difficult and formal oral glucose tolerance testing impractical in usual outpatient settings. From a practical standpoint, the measurement of hsCRP at the time of triglyceride, fasting glucose, and HDL assessment has appeal for improving metabolic syndrome diagnosis in daily practice.

## Has the Time Come to Consider hsCRP as a Clinical Criterion for Metabolic Syndrome and as a Formal Addition to Global Risk Prediction?

Inexpensive evaluation of hsCRP in outpatient settings is now possible with the availability of standardized commercial assays capable of detecting the very low levels of CRP needed for coronary risk prediction. No circadian variation exists for hsCRP, nor does food consumption alter plasma levels, so there is no need for a fasting blood sample to be obtained. Despite being an acute-phase reactant, the decade-to-decade variation in hsCRP is similar to that of cholesterol,[25] demonstrating long-term stability for risk prediction. Because therapy with HMG CoA reductase inhibitors lowers hsCRP as well as LDL cholesterol,[50] many clinicians have begun measuring hsCRP at the time of cholesterol evaluation, using information on inflammation both to motivate patients for lifestyle changes and to better target statin therapy.[23,51]

After publication of the CDC/AHA guidelines,[16] outpatient use of hsCRP increased in the United States, a change that reflects the translation of the biology of inflammation into daily clinical practice. Observations that hsCRP also has predictive value in unstable angina and acute myocardial infarction[52] have additionally encouraged some emergency room physicians to obtain hsCRP levels at the time of hospital admission.[53] Multiple clinical trials have specified hsCRP as part of their entry criteria to identify high–cardiovascular risk patients.[54]

In consideration of the consistency of these data, we believe the time has come to examine the possibility of incorporating hsCRP into the criteria for the diagnosis of metabolic syndrome and as a risk factor in calculation of global cardiovascular risk. To begin a dialogue on this issue and to better understand the potential role of hsCRP as an adjunct to the Framingham Risk Score, we reanalyzed data from 27 939 participants in the prospective WHS using 5 clinically defined categorical levels of hsCRP (<0.5, 0.5 to <1, 1 to <3, 3 to <10, and ≥10 mg/L) and after dividing the full WHS population into those with 10-year Framingham risks estimated as being <5%,

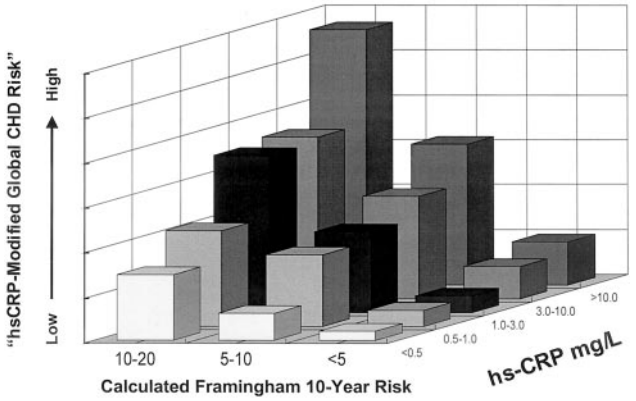


**Figure 7.** Moving toward an hsCRP-modified CHD risk score. Relative risks of future vascular disease using baseline levels of hsCRP in addition to calculated 10-year Framingham risk.

5% to 10%, and 10% to 20%. This new analysis also takes advantage of continued follow-up of the WHS and thus includes 685 incident hard cardiovascular events.

The results of these exploratory analyses are presented in Figure 7. As shown, a risk gradient exists on the basis of hsCRP levels across all levels of the Framingham Risk Score, not only those deemed at intermediate risk as suggested by the CDC/AHA guidelines. Risk levels increase consistently for those at estimated 10-year Framingham Risks of <5% and between 5% and 10% in almost the same manner as for those with estimated 10-year Framingham risks between 10% and 20%. These data suggest that an hsCRP-modified CHD risk score can be calculated that may improve the overall prediction of vascular events (Figure 7).

Thus, given the consistency of data for hsCRP observed in analyses from the PHS, WHS, ARIC, AFCAPS/TexCAPS, MONICA, and Reykjavik studies as well as in the Framingham Heart Study itself, we believe the time has come for a careful consideration of adding hsCRP as a clinical criterion for metabolic syndrome and for the creation of an hsCRP-modified CHD risk score useful for global risk prediction in both men and women. Toward this end, we believe investigators from the major prospective cohort studies as well as experts in the fields of epidemiology, prevention, vascular biology, and clinical cardiology should be convened to begin discussing the merits of this proposal.

## Acknowledgments

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# C-Reactive Protein and the 10-Year Incidence of Coronary Heart Disease in Older Men and Women

## The Cardiovascular Health Study

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***Background***—High C-reactive protein (CRP) is associated with increased coronary heart disease risk. Few long-term data in the elderly are available.

***Methods and Results***—Baseline CRP was measured in 3971 men and women ≥65 years of age without prior vascular diseases; 26% had elevated concentrations (>3 mg/L). With 10 years of follow-up, 547 participants developed coronary heart disease (CHD; defined as myocardial infarction or coronary death). With elevated CRP, the 10-year cumulative CHD incidences were 33% in men and 17% in women. The age-, ethnicity-, and sex-adjusted relative risk of CHD for CRP >3 mg/L compared with <1 mg/L was 1.82 (95% CI, 1.46 to 2.28). Adjusting for conventional risk factors reduced the relative risk to 1.45 (95% CI, 1.14 to 1.86). The population-attributable risk of CHD for elevated CRP was 11%. Risk relationships did not differ in subgroups defined by baseline risk factors. We assessed whether CRP improved prediction by the Framingham Risk Score. Among men with a 10-year Framingham-predicted risk of 10% to 20%, the observed CHD incidence was 32% for elevated CRP. Among women, CRP discriminated best among those with a 10-year predicted risk >20%; the incidences were 31% and 10% for elevated and normal CRP levels, respectively.

***Conclusions***—In older men and women, elevated CRP was associated with increased 10-year risk of CHD, regardless of the presence or absence of cardiac risk factors. A single CRP measurement provided information beyond conventional risk assessment, especially in intermediate-Framingham-risk men and high-Framingham-risk women. **(*Circulation*. 2005;112:25-31.)**

**Key Words:** coronary disease ■ epidemiology ■ inflammation ■ myocardial infarction ■ risk factors

Older adults are the demographic group at highest risk of myocardial infarction (MI). Although cardiovascular risk factor levels in middle-aged individuals are important in MI prediction, utility of some risk factors such as lipid measures has been questioned among older individuals.[1,2] The inflammation marker C-reactive protein (CRP) has been reported as a risk factor for MI in several studies of initially healthy subjects.[3] In 4 studies including middle-aged subjects, CRP measurement added to the predictive value of the Framingham Risk Score or lipid determination.[4–8] A consensus panel reported possible roles of CRP measurement in primary prevention, suggesting that concentrations of 1 to 3 mg/L indicate intermediate risk, levels >3 mg/L indicate increased risk, and levels >10 mg/L might indicate other inflammatory diseases.[3] However, a recent large study reported only a modest association of CRP with future coronary heart disease (CHD), and the investigators questioned these recommendations.[9]

Long-term prospective studies assessing CRP and MI in elderly men and women are not available. In prospective studies of elderly subjects without baseline coronary disease, CRP was associated with short-term risk of MI or angina in the population assessed here[10] and with vascular mortality in 2 other studies[11,12] but not with MI in older adults in the Rotterdam Study[13] or with acute coronary syndrome in the Health, Aging and Body Composition cohort.[14]

In several studies, associations of CRP with MI were substantially reduced by adjustment for conventional cardiovascular risk factors.[9,15–20] Measures of subclinical atherosclerosis such as carotid intima-media thickness and ankle-brachial index predict future MI, possibly because they reflect the lifetime burden of cardiac risk factors or host response to

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risk factors.[21] Adjustment for subclinical disease might provide more complete adjustment for potential confounding, yielding new information on the independence of the association of CRP with MI.

In this report, we analyzed baseline CRP and 10-year incidence of first MI or CHD death in the Cardiovascular Health Study (CHS), a cohort of men and women ≥65 years of age. We assessed the recommended clinical cut points for elevated CRP[3]; independence of CRP associations with CHD from risk factors, including subclinical disease; and associations of CRP with CHD in subgroups defined by baseline risk factors, including subclinical disease and the Framingham Risk Score.

## Methods

The CHS is an observational study of risk factors for cardiovascular disease in 5888 men and women ≥65 years of age who were enrolled as 2 cohorts at 4 centers in either 1989 to 1990 or 1992 to 1993. The first cohort consisted of 5201 primarily white participants; the second consisted of 687 blacks. Invited participants were a random sample of Health Care Financing Administration eligibility lists and their household members.[22] Exclusion criteria included institutionalization, active cancer treatment, or expectation of moving from the area within 2 years. Participants provided informed consent, and institutional review committees approved the study methods.

Interview, lipid determination, and testing for subclinical atherosclerosis were completed at enrollment.[22] Subclinical disease was measured by carotid ultrasound, ECG, and ankle-brachial blood pressure index as previously described.[21] Previous vascular diagnoses were confirmed by medical record review. In 1997, CRP was measured in stored baseline plasma by immunoassay with a coefficient of variation of 6.2%.[23,24]

### Baseline Definitions

Primary analysis of CHD events categorized CRP as low (<1 mg/L), intermediate (1 to 3 mg/L), or elevated (>3 mg/L) to address the utility of recent guidelines.[3] CRP >10 mg/L was assessed in secondary analyses.[3] Diabetes was defined using the American Diabetes Association criteria. Hypertension was defined as blood pressure ≥140/90 mm Hg or self-reported hypertension with the use of antihypertensive drugs. Hyperlipidemia was defined as cholesterol ≥6.22 mmol/L (240 mg/dL), LDL cholesterol ≥4.14 mmol/L (160 mg/dL), or use of medications for hyperlipidemia. Cigarette use was categorized as never, former, or current and by number of pack-years. Presence of subclinical vascular disease was defined as any 1 of the following: ankle-brachial index <0.9, internal or common carotid artery wall thickness >80th percentile, carotid stenosis >25%, major ECG abnormalities, or positive response to the Rose questionnaires for angina and claudication.[21] Regular aspirin use was defined as use during at least 7 of the previous 14 days or by prescription. Framingham Risk Score was calculated[25] and reported as low (<10%), intermediate (10% to 20%), or high (>20%) 10-year predicted risk of CHD.

### Subjects Included in Analysis

We excluded 1536 participants with confirmed prebaseline cardiovascular disease (MI, angina, congestive heart failure, stroke, transient ischemic attack, claudication, coronary artery bypass surgery, angioplasty, carotid endarterectomy; 715 women, 821 men). Of the remaining women, 326 using oral postmenopausal hormones were excluded because users have higher CRP concentrations with uncertain clinical consequence.[26,27] Fifty-five subjects did not have CRP levels available. Exclusions yielded 3971 participants.

### Events Ascertainment

Subjects were followed up every 6 months by alternating field center visits and telephone calls between enrollment and June 30, 2000. Vascular outcomes were ascertained by self-report and review of discharge codes for all hospitalizations. For suspected coronary events, medical records were abstracted and then reviewed and classified by a committee using standardized criteria.[28,29] CHD death was defined as the absence of nonatherosclerotic cause of death and 1 or both of the following: chest pain within 72 hours of death or history of chronic ischemic heart disease in the absence of valvular heart disease or nonischemic cardiomyopathy.

### Statistical Analysis

The SPSS package was used for analysis with a CHS database updated November 18, 2002. Associations of CRP with risk factors were assessed by tests for trend across categories of CRP through the use of $\chi^2$ statistics or ANOVA. When indicated, CRP was log transformed and described by its geometric mean. Incidence rates of CHD by CRP category were calculated separately for men and women and within categories of the Framingham Risk Score. Cox proportional-hazards models were used to compute hazard ratios as estimates of relative risk of CHD with increasing category of CRP with adjustment for age, sex, and race in all participants and in subgroups defined by known risk factors. Censoring occurred at death, last follow-up, or June 30, 2000, whichever occurred first. Differences in findings by sex, race, and presence or absence of vascular risk factors were evaluated formally by adding interaction terms of each for these factors with CRP to the model ($P=0.05$ indicated significance). To determine whether CRP remained predictive after adjustment for known risk factors, 2 additional levels of adjustment were considered. First, models were additionally adjusted for field center and the risk factors related to CRP or MI: hypertension, diabetes, smoking status, pack-years of smoking, body mass index, waist circumference, total and HDL cholesterol, and regular aspirin use. Second, subclinical atherosclerosis measures were added to the models.

## Results

Among 3971 participants, 29% had CRP <1.0 mg/L, 45% had levels of 1 to 3 mg/L, and 26% had elevated values (>3 mg/L). The median CRP was 1.76 mg/L (interquartile range, 0.88 to 3.10 mg/L). Associations of CRP with risk factors and subclinical and clinical cardiovascular disease are shown in Table 1. CRP was higher among women and blacks and with obesity, aspirin use, lower HDL cholesterol, hypertension, diabetes, smoking, and pack-years. CRP was higher with than without subclinical disease, with geometric mean values of 1.64 mg/L without subclinical disease and 1.98 mg/L with subclinical disease ($P<0.001$).

With 10 years of follow-up, there were 547 first MI or CHD deaths (354 nonfatal MI, 41 fatal MI, and 152 CHD deaths). Incidence rates were 22.2 and 12.0 per 1000 person-years in men and women, respectively. Figure 1 shows the cumulative incidence of CHD by gender and baseline CRP. There was little difference between participants with low and intermediate CRP levels, but for those with elevated CRP, the incidence was higher, with a 10-year cumulative incidence of 33% in men and 17% in women. Figure 2 shows the incidence of CHD over the full range of CRP values, demonstrating an increase in risk throughout the range.

Incidence rates and relative risks of CHD by baseline CRP categories are shown in Table 2. Incidence increased with each higher CRP category. Age, sex, and race-adjusted relative risks of CHD were slightly increased for intermediate CRP and were nearly doubled for CRP >3 mg/L. Adjustment for other risk factors attenuated these relative risks, leaving little association with CHD for CRP 1 to 3 mg/L and a 45%

**TABLE 1. Distribution of Cardiovascular Risk Factors by Baseline CRP Concentration**

| | CRP, mg/L | | | | |
|---|---|---|---|---|---|
| | <1 (n=1144) | 1–3 (n=1783) | >3–10 (n=811) | >10 (n=233) | *P* for Trend |
| Continuous variables | | | | | |
| Age, y | 72.9 | 72.4 | 72.4 | 72.6 | 0.16 |
| Body mass index, kg/m$^2$ | 24.7 | 26.8 | 28.6 | 29.0 | <0.001 |
| Waist, cm | 89.5 | 94.7 | 99.1 | 100.2 | <0.001 |
| LDL cholesterol, mmol/L (mg/dL) | 3.29 (127) | 3.47 (134) | 3.44 (133) | 3.23 (125) | 0.21 |
| HDL cholesterol, mmol/L (mg/dL) | 1.50 (58) | 1.40 (54) | 1.35 (52) | 1.32 (51) | <0.001 |
| Total cholesterol, mmol/L (mg/dL) | 5.41 (209) | 5.57 (215) | 5.54 (214) | 5.18 (200) | 0.67 |
| Pack-years (among ever smokers) | 29.0 | 32.6 | 36.8 | 36.7 | <0.001 |
| Categorical variables, % | | | | | |
| Male sex | 43.4 | 42.5 | 37.0 | 41.2 | 0.02 |
| Black race | 10.9 | 13.5 | 20.0 | 29.2 | <0.001 |
| Hypertension | 46.7 | 56.5 | 64.6 | 66.5 | <0.001 |
| Diabetes | | | | | |
| IFG | 11.2 | 14.0 | 16.9 | 19.4 | |
| Diabetes | 8.2 | 13.7 | 21.8 | 25.0 | <0.001 |
| Smoking status | | | | | |
| Former | 40.9 | 38.8 | 40.8 | 41.2 | |
| Current | 8.5 | 11.9 | 16.6 | 20.2 | <0.001 |
| Regular aspirin use | 19.2 | 19.1 | 22.5 | 20.2 | 0.17 |
| Any subclinical cardiovascular disease | 55.8 | 63.3 | 67.8 | 69.1 | <0.001 |
| Ankle-arm index <0.9 | 6.7 | 9.3 | 14.4 | 13.2 | <0.001 |
| Carotid intima-media thickness >80th percentile | 23.1 | 30.3 | 35.1 | 36.1 | <0.001 |
| Major ECG abnormality | 19.7 | 23.9 | 25.0 | 25.6 | 0.004 |
| Carotid stenosis >25% | 39.4 | 43.8 | 47.7 | 50.4 | <0.001 |
| Rose angina positive | 3.1 | 2.4 | 2.6 | 4.3 | 0.78 |
| Rose claudication positive | 0.6 | 1.0 | 1.7 | 2.6 | 0.002 |

IFG indicates impaired fasting glucose. Values for continuous variables are means.

increased risk for CRP >3 mg/L. There was no effect of additional adjustment for baseline statin use. Further adjustment for subclinical disease yielded little attenuation; a 37% increased risk of CHD for elevated CRP remained. When CRP was considered a continuous variable, with adjustment for risk factors, the relative risk associated with a 1-ln-unit-higher baseline CRP was 1.27 (95% CI, 1.12 to 1.44). There were no significant differences in associations by sex or race. The population-attributable risk percentage for elevated CRP was 11%.

Table 3 shows the relative risks of incident CHD for CRP >3 mg/L compared with CRP <1 mg/L in subgroups based on the presence or absence of cardiovascular risk factors. Baseline CRP was associated with CHD in all of these groups, including those without subclinical disease and those at low risk by the Framingham Risk Score (*P* for interaction >0.05 for all). Although the relative risk did not differ by subclinical disease status, among men and women, the presence of elevated CRP together with subclinical disease was associated with a higher incidence of CHD compared with those with lower CRP and no subclinical disease (Figure 3).

Figure 4 shows the 10-year sex-specific incidence of CHD according to CRP concentration in categories of the Framingham Risk Score. In intermediate- and low-risk women, CRP >3 mg/L added little to risk prediction, whereas in high-risk women, CRP provided additional risk information. Among women with a 10-year predicted risk >20%, for intermediate or elevated CRP, the observed incidences were 28%, and 31%, respectively, compared with only 16% for those with low CRP. In men, CRP provided additional risk information in intermediate- and high-Framingham-risk groups. Among men with a 10-year predicted risk 10% to 20%, those with CRP >3 mg/L had an observed risk of 32%. Among high-Framingham-risk men, this observed risk was 41%.

We investigated the utility of CRP >10 mg/L for determining risk of CHD and the impact of hormone replacement therapy among women. Of participants with elevated CRP, 22% were >10 mg/L. Of these 233 participants, 49 (21%) developed CHD during follow-up compared with 498 of 3738 (13.3%) with lower CRP. The age-, sex-, and race-adjusted relative risk of CHD was 2.16 (95% CI, 1.55 to 3.00) for CRP >10 compared with <1 mg/L and 1.78 (95% CI, 1.26 to 2.51) after adjustment for traditional risk factors. Among 326

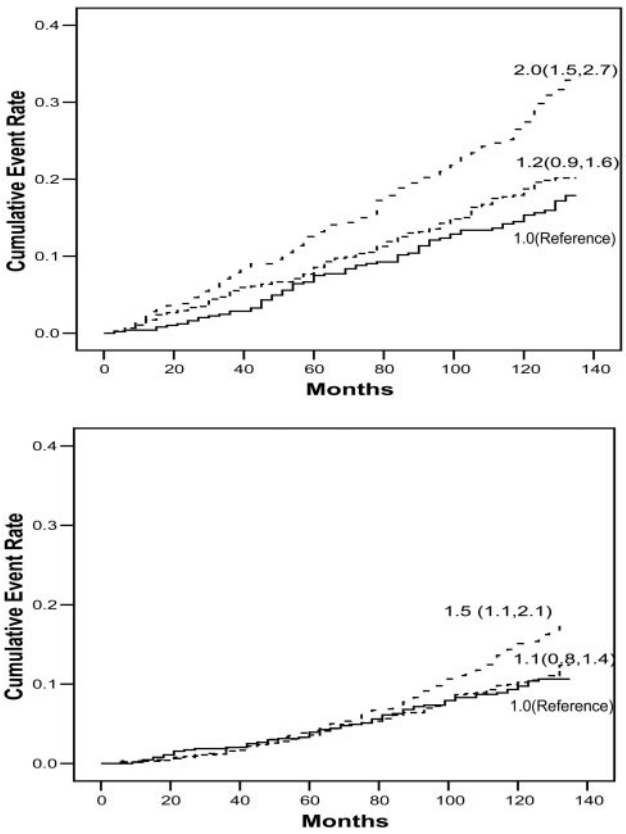


**Figure 1.** Cumulative rate of MI or CHD death. Top, data for men; bottom, data for women. Unadjusted hazard ratios and 95% CIs for each group compared with reference group (CRP <1 mg/L) are shown. Solid line indicates CRP <1 mg/L; dotted line, CRP 1 to 3 mg/L; and dashed line, CRP >3 mg/L.

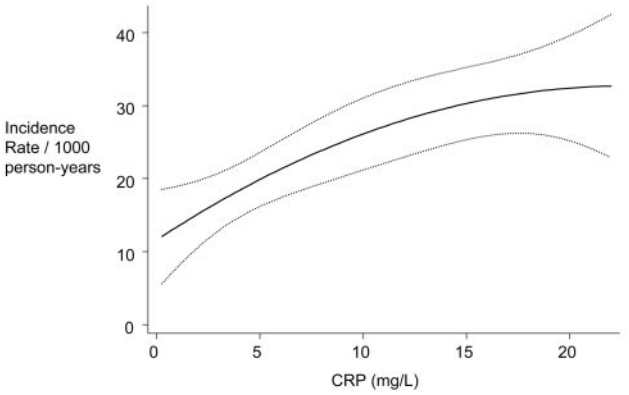


**Figure 2.** Incidence rate per 1000 person-years of MI or CHD death by baseline CRP. Incidence rates were calculated within small intervals of CRP values and plotted with a scatterplot smoother. Association was well fit by a quadratic function of CRP, plotted with 95% confidence bands.

women excluded from analysis for hormone replacement therapy use, the age-adjusted relative risk of CHD for CRP >3 mg/L was 1.35 (95% CI, 0.42 to 4.32).

## Discussion

In this 10-year prospective study in men and women ≥65 years of age, CHD risk increased with increasing CRP. When recent clinical guidelines were applied, intermediate CRP concentrations (1 to 3 mg/L) were weakly related to future CHD, and elevated CRP (>3.0 mg/L) was associated with a 1.45-fold increased risk of CHD, with adjustment for other vascular risk factors. There was little further confounding with adjustment for the presence of noninvasively assessed subclinical atherosclerosis. Elevated CRP was associated with CHD in all subgroups defined by conventional cardiac risk factors or subclinical disease. Among men with intermediate and high Framingham Risk Scores, CRP identified those with higher-than-predicted risk. Among women, CRP discriminated risk best among those at high Framingham-predicted risk.

The relative risk of CHD for elevated CRP observed here was smaller than in most studies of middle-aged subjects and might seem modest at 1.45. However, event rates were high in this age group, so the attributable risk percent for elevated CRP was high at 11%, even given a modest relative risk.[30] Thus, a much higher percentage of subjects with elevated CRP subsequently had events in this study compared with studies of younger subjects.[7,8] In a recent report by Danesh et

**TABLE 2. Association of Baseline CRP With Incident MI or CHD Death Over 10 Years***

| | Relative Risk (95% CI) in CRP Categories | | | |
|---|---|---|---|---|
| | <1.0 mg/L (N=1144, n=135) | 1.0–3.0 mg/L (N=1783, n=230) | >3.0 mg/L (N=1044, n=182) | *P* |
| Incidence rate, % (n of events) | | | | |
| Men | 17.1 (74) | 20.6 (127) | 33.3 (96) | |
| Women | 10.4 (61) | 11.0 (103) | 15.5 (86) | |
| Model 1 | 1.0 (ref) | 1.18 (0.96–1.46) | 1.82 (1.46–2.28) | <0.001 |
| Model 2 | 1.0 (ref) | 1.08 (0.86–1.35) | 1.45 (1.14–1.86) | <0.004 |
| Model 3 | 1.0 (ref) | 1.04 (0.82–1.31) | 1.37 (1.06–1.78) | 0.01 |

N is number at risk in given group; n, number of cases in given group; and ref, reference.

*Model 1 is adjusted for age, race, and sex. Model 2 is adjusted for age, sex, race, field center, hypertension, diabetes, smoking status, log pack-years, body mass index, waist circumference, total cholesterol, HDL cholesterol, and regular aspirin use. Model 3 is adjusted for model 2 variables plus ankle-arm index <0.9, internal or common carotid intima-media thickness >80th percentile, positive responses to the Rose angina or claudication questionnaires, major ECG abnormalities, and maximum stenosis of the carotid artery >25%.

**TABLE 3. Relative Risk of CHD for CRP >3 mg/L Compared With <1 mg/L by Categories of Baseline Risk Factors**

| | Risk Factor Present | | Risk Factor Absent | |
|---|---|---|---|---|
| Risk Factor | n/N | RR* (95% CI) | n/N | RR* (95% CI) |
| Smoking (former+current) | 307/2076 | 1.84 (1.37–2.47) | 240/1890 | 1.68 (1.18–2.40) |
| Pack-years (>median; ever smokers only) | 171/967 | 2.13 (1.40–3.25) | 120/1000 | 1.61 (1.02–2.53) |
| Hypertension | 365/2219 | 1.74 (1.32–2.29) | 182/1748 | 1.55 (1.04–2.32) |
| Diabetes or impaired fasting glucose | 213/1130 | 1.49 (1.02–2.18) | 334/2833 | 1.74 (1.30–2.32) |
| Hyperlipidemia | 139/966 | 1.99 (1.25–3.19) | 404/2971 | 1.74 (1.34–2.26) |
| Regular aspirin use | 123/788 | 1.89 (1.17–3.05) | 423/3176 | 1.79 (1.39–2.31) |
| Estimated 10-year Framingham Risk Score >20% | 181/772 | 2.00 (1.28–3.13) | 357/3127 | 1.51 (1.15–1.98) |
| Subclinical disease | 417/2477 | 1.85 (1.42–2.42) | 130/1494 | 1.42 (0.91–2.22) |
| Carotid wall thickness >80th percentile | 250/1167 | 1.56 (1.11–2.20) | 293/2784 | 1.71 (1.26–2.32) |
| Ankle-arm index <0.9 | 95/381 | 1.45 (0.82–2.59) | 443/3514 | 1.78 (1.39–2.28) |
| Carotid stenosis ≥25% | 315/1726 | 1.68 (1.25–2.25) | 230/2223 | 1.74 (1.22–2.47) |
| Major ECG abnormality | 177/886 | 1.73 (1.15–2.61) | 355/2963 | 1.81 (1.38–2.38) |

n/N indicates number of events/number at risk in all 3 levels of CRP in specified category of each risk factor.
*Adjusted for age, race, and sex.

al,[9] a similar adjusted relative risk was observed in a large population, but in that case-control study, attributable risk was not estimated. If elevated CRP represents a causal risk factor as suggested by several experimental studies,[31] our estimate of attributable risk indicates a hypothesis that correction of elevated CRP could eliminate up to 11% of incident CHD in this age group.

It has been suggested that novel risk factors or atherosclerosis imaging may identify those at intermediate CHD risk who might benefit from aggressive risk factor interventions.[32] Along with findings in middle-aged populations,[7,8] our data provide evidence that CRP assessment can identify older patients at higher or lower than their predicted risk of coronary events. Our findings with regard to women at low and intermediate risk differ from findings in middle-aged women in which CRP predicted cardiovascular events across the entire range of Framingham Risk Scores.[33] Further work is needed to validate our findings in this age group and to determine appropriate values defining elevated CRP in various age and sex groups.

Other studies reported weak or no associations of CRP with subclinical disease measures.[34–38] Here, in the absence of clinical disease, CRP was higher among those with any single type of subclinical disease. Moreover, CHD incidence was higher among those with elevated CRP and subclinical disease compared with groups with only 1 or neither of these risk factors. In this cohort, the 10-year stroke risk associated with elevated CRP was larger among those with higher compared with lower carotid intima-media thickness.[39] In a short-term study, the risk of MI was higher among those with higher coronary artery calcium scores if CRP was also elevated.[40] Taken together, findings from these few studies suggest possible roles for the assessment of both inflammation and subclinical disease. It is also possible that CRP is a marker of subclinical disease, and if better measures of subclinical disease were available, adjustment for subclinical disease would further lessen the association of CRP with CHD.

The CDC/AHA guideline for CRP testing suggest that values >10 mg/L indicate acute inflammation and have uncertain implications for vascular risk prediction.[3] In this older population, 6% of subjects had CRP >10 mg/L; when traditional risk factors were accounted for, these subjects had a 1.8-fold increased risk of CHD, a higher risk estimate than

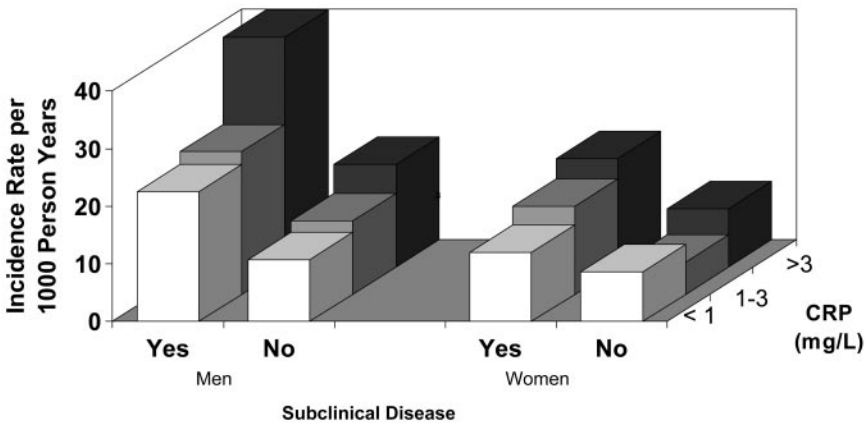


**Figure 3.** Incidence rates per 1000 person-years of first MI or CHD death by baseline CRP, stratified by sex and presence of subclinical atherosclerosis.

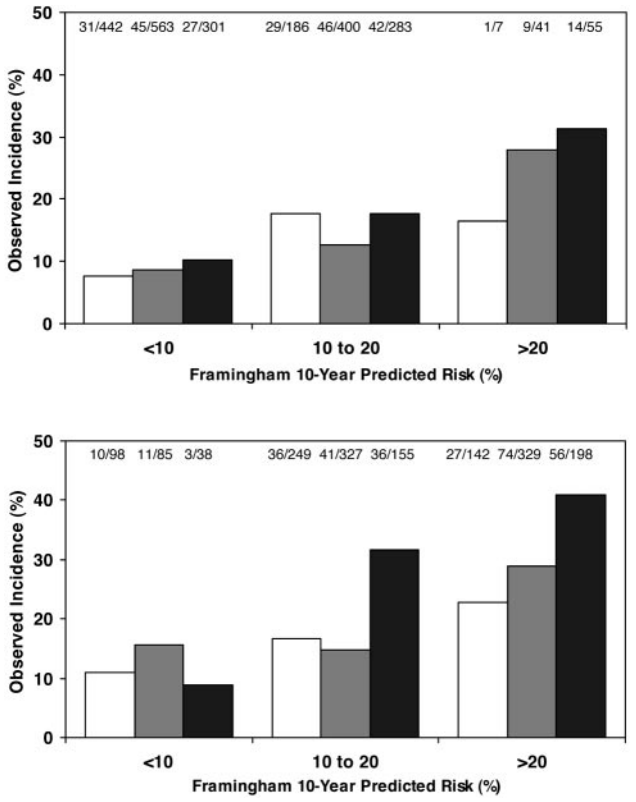


**Figure 4.** Ten-year rate of CHD according to baseline CRP and 10-year predicted risk from the Framingham Risk Score. Top, data for women; bottom, data for men. Observed incidence based on categories of CRP was determined within each category of Framingham-predicted risk. For each category, numbers across top represent number of events per number at risk in that group.

for CRP >3.0 mg/L. Our finding agrees with recently reported results in middle-aged women.[33] Thus, CRP values >10 mg/L appear to be important in CHD risk prediction.

Limitations of this study merit consideration. The cohort, free-living elderly who were willing to enroll in the study, may not represent the general older population. The observational study design, even with extensive multivariate analysis, cannot prove causal relationships. Competing risks may have diluted associations of CRP with CHD because CRP may be associated with other disease outcomes. In some cases, analysis of subgroups was limited by small sample sizes. Finally, CRP was measured only once at baseline, and it has been suggested that repeated testing for confirmation be considered in those with high values.[3]

Strengths of this study include its large size, extensive baseline data collection, and long-term event follow-up. Several new findings were observed on the basis of unique aspects of the study. First, we confirmed an association of elevated CRP with CHD incidence in an older age group; most previous studies included younger subjects or clinical trial participants. Second, independence of associations from noninvasively measured subclinical atherosclerosis was documented. Third, more complete adjustment for smoking status was made by assessing pack-years, a major determinant of CRP concentration in smokers.[34] Fourth, because CRP was measured in the whole cohort, incidence rates of CHD by baseline CRP were calculated, and subgroup analyses could be done.

In conclusion, we extend previous reports on the association of CRP with CHD to men and women ≥65 years of age. CRP appears to be useful for risk assessment in this age group. Because event rates are high overall in older age, further study is required to determine optimal clinical roles of CRP measurement, especially as related to interventions for elevated CRP.

## Acknowledgments

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# COMPARISON OF C-REACTIVE PROTEIN AND LOW-DENSITY LIPOPROTEIN CHOLESTEROL LEVELS IN THE PREDICTION OF FIRST CARDIOVASCULAR EVENTS

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## ABSTRACT

***Background*** Both C-reactive protein and low-density lipoprotein (LDL) cholesterol levels are elevated in persons at risk for cardiovascular events. However, population-based data directly comparing these two biologic markers are not available.

***Methods*** C-reactive protein and LDL cholesterol were measured at base line in 27,939 apparently healthy American women, who were then followed for a mean of eight years for the occurrence of myocardial infarction, ischemic stroke, coronary revascularization, or death from cardiovascular causes. We assessed the value of these two measurements in predicting the risk of cardiovascular events in the study population.

***Results*** Although C-reactive protein and LDL cholesterol were minimally correlated ($r=0.08$), base-line levels of each had a strong linear relation with the incidence of cardiovascular events. After adjustment for age, smoking status, the presence or absence of diabetes mellitus, categorical levels of blood pressure, and use or nonuse of hormone-replacement therapy, the relative risks of first cardiovascular events according to increasing quintiles of C-reactive protein, as compared with the women in the lowest quintile, were 1.4, 1.6, 2.0, and 2.3 ($P<0.001$), whereas the corresponding relative risks in increasing quintiles of LDL cholesterol, as compared with the lowest, were 0.9, 1.1, 1.3, and 1.5 ($P<0.001$). Similar effects were observed in separate analyses of each component of the composite end point and among users and nonusers of hormone-replacement therapy. Overall, 77 percent of all events occurred among women with LDL cholesterol levels below 160 mg per deciliter (4.14 mmol per liter), and 46 percent occurred among those with LDL cholesterol levels below 130 mg per deciliter (3.36 mmol per liter). By contrast, because C-reactive protein and LDL cholesterol measurements tended to identify different high-risk groups, screening for both biologic markers provided better prognostic information than screening for either alone. Independent effects were also observed for C-reactive protein in analyses adjusted for all components of the Framingham risk score.

***Conclusions*** These data suggest that the C-reactive protein level is a stronger predictor of cardiovascular events than the LDL cholesterol level and that it adds prognostic information to that conveyed by the Framingham risk score. (N Engl J Med 2002;347:1557-65.)



BECAUSE of its critical importance in atherogenesis, low-density lipoprotein (LDL) cholesterol is the focus of current guidelines for the determination of the risk of cardiovascular disease.[1] However, atherothrombosis often occurs in the absence of hyperlipidemia, and recent consensus panels assembled by the National Heart, Lung, and Blood Institute and the Centers for Disease Control and Prevention have concluded that population-based data on other risk factors are urgently needed.[2,3]

Among the biologic markers considered by those panels, there was particular interest in C-reactive protein, a marker of inflammation that has been shown in several prospective, nested case–control studies to be associated with an increased risk of myocardial infarction,[4-9] stroke,[4,6,10,11] sudden death from cardiac causes,[12] and peripheral arterial disease.[13] Although the results of these studies are highly consistent, limitations inherent in the design of nested case–control studies make it difficult to assess the relative merit of C-reactive protein. In particular, population-based cutoff points for C-reactive protein remain uncertain, and reliable data describing receiver-operating-characteristic curves for C-reactive protein have not been available. Moreover, there are insufficient data from prospective cohort studies directly comparing the predictive value of C-reactive protein with that of LDL cholesterol.

In a previous hypothesis-generating report limited to 122 women in whom cardiovascular disease developed (case patients) and 244 controls who were participants in the Women's Health Study, we observed that several markers of inflammation, including C-reactive protein, had prognostic value for the detection of first vascular events over a three-year period.[6] However, the relatively small number of events and the short follow-up limit the reliability of those data. Furthermore, because of the matched-pairs case–control study design, we were unable to define general population-

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based cutoff points or to evaluate directly characteristics of C-reactive protein used as a diagnostic test.

To overcome these limitations, we measured C-reactive protein and LDL cholesterol in all 27,939 participants in the Women's Health Study who provided usable base-line blood samples; these women had been followed for a mean of eight years. Using these data, we were able to calculate survival curves associated with C-reactive protein levels, to compare the predictive value of C-reactive protein and LDL cholesterol directly in a large, representative population sample, and to define the population distribution of C-reactive protein levels. We also determined the predictive value of each biologic marker among users and nonusers of hormone-replacement therapy; this is a clinically relevant issue, since hormone-replacement therapy affects levels of both C-reactive protein and LDL cholesterol.[14-16] Finally, we evaluated whether C-reactive protein provided prognostic information on risk after adjustment for all components of the Framingham risk score.

## METHODS

### Study Design

The Women's Health Study is an ongoing evaluation of aspirin and vitamin E for the primary prevention of cardiovascular events among women 45 years of age or older. Participants were enrolled between November 1992 and July 1995, at which time they provided information regarding demographic, behavioral, and lifestyle factors. All participants were followed for the occurrence of first cardiovascular events, including nonfatal myocardial infarction, nonfatal ischemic stroke, coronary revascularization procedures, and death from cardiovascular causes. The occurrence of myocardial infarction was considered confirmed if symptoms met the criteria of the World Health Organization and if the event was associated with abnormal levels of cardiac enzymes or diagnostic electrocardiographic criteria. Stroke was confirmed if the participant had new neurologic deficits that persisted for more than 24 hours. Computed tomographic scans or magnetic resonance images were available for the great majority of events and were used to distinguish hemorrhagic from ischemic events. The performance of either percutaneous coronary revascularization or coronary-artery bypass surgery was confirmed by a review of hospital records. Deaths from cardiovascular causes were confirmed by review of autopsy reports, death certificates, medical records, and information obtained from family members.

Before randomization, blood samples were collected in tubes containing EDTA from 28,345 study participants and stored in liquid nitrogen until the time of analysis. Samples were then transferred to a core laboratory facility, where they were assayed for C-reactive protein with a validated, high-sensitivity assay (Denka Seiken) and for LDL cholesterol with a direct-measurement assay (Roche Diagnostics). This laboratory is certified for the measurement of lipids and is a core facility for ongoing standardization programs regarding the measurement of C-reactive protein. Of the samples received, 27,939 could be evaluated and were assayed for C-reactive protein and LDL cholesterol.

### Statistical Analysis

Because hormone-replacement therapy affects levels of C-reactive protein and LDL cholesterol, we first established population-based distributions for each analyte among the 15,745 women who were not taking hormone-replacement therapy at study entry — a method consistent with the guidelines of the Department of Health and Human Services for lipid standardization.[17] We then divided these population data into increasing quintiles with respect to C-reactive protein and LDL cholesterol and constructed Kaplan–Meier curves for event-free survival. The relative risks of new cardiovascular events were computed for quintiles 2 through 5, as compared with the lowest quintile, in both crude Cox proportional-hazards models and models adjusted for risk factors. Stratified analyses were used to address the predictive value of LDL cholesterol and C-reactive protein among users and nonusers of hormone-replacement therapy at base line. To evaluate whether different cutoff points might affect the risk estimates for users of hormone-replacement therapy, we repeated the analysis among users with cutoff points for C-reactive protein and LDL cholesterol defined by the values in the 12,139 women who were using hormone-replacement therapy at base line. The 55 women for whom hormone-replacement status was unknown were excluded from the stratified analyses.

To estimate the discriminative value of predictive models, we calculated the C statistic on the basis of the minimal follow-up time of six years for both C-reactive protein and LDL cholesterol in crude and risk-factor–adjusted models. This statistic is analogous to the area under the receiver-operating-characteristic curve.[18] To compute the C statistic, we compared each woman's status with respect to cardiovascular disease (present or absent) at six years with the predicted six-year probability of event-free survival, estimated from the Cox proportional-hazards model. Subjects whose data were censored before six years of follow-up (less than 1 percent) were excluded from this calculation.

We tested for trend across the quintiles of C-reactive protein or LDL cholesterol by entering a single ordinal term for the quintile in the Cox regression model. In addition, we tested for deviation from linearity by comparing models containing quintile indicators with those containing a linear term in a likelihood-ratio test with 3 degrees of freedom. We also tested the additional prognostic

**TABLE 1.** DISTRIBUTION OF C-REACTIVE PROTEIN AND LDL CHOLESTEROL LEVELS AMONG 15,745 STUDY PARTICIPANTS WHO WERE NOT TAKING HORMONE-REPLACEMENT THERAPY AT THE TIME OF THE BASE-LINE BLOOD COLLECTION.

| AGE GROUP | NO. OF WOMEN | PERCENTILE | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 5TH | 10TH | 25TH | 50TH | 75TH | 90TH | 95TH |
| | | milligrams per liter | | | | | | |
| **C-reactive protein** | | | | | | | | |
| 45–54 yr | 10,075 | 0.17 | 0.25 | 0.52 | 1.31 | 3.18 | 6.15 | 8.80 |
| 55–64 yr | 3,604 | 0.25 | 0.39 | 0.82 | 1.89 | 4.12 | 7.47 | 9.76 |
| 65–74 yr | 1,862 | 0.33 | 0.46 | 0.91 | 1.99 | 3.92 | 6.79 | 8.77 |
| ≥75 yr | 204 | 0.29 | 0.43 | 0.80 | 1.52 | 3.55 | 7.56 | 13.33 |
| Total | 15,745 | 0.19 | 0.29 | 0.61 | 1.52 | 3.48 | 6.61 | 9.14 |
| | | milligrams per deciliter* | | | | | | |
| **LDL cholesterol** | | | | | | | | |
| 45–54 yr | 10,075 | 72.7 | 82.1 | 97.6 | 117.3 | 139.6 | 162.5 | 178.2 |
| 55–64 yr | 3,604 | 83.4 | 94.9 | 113.4 | 134.4 | 158.8 | 181.9 | 198.3 |
| 65–74 yr | 1,862 | 86.4 | 97.0 | 115.1 | 137.0 | 157.9 | 183.5 | 199.3 |
| ≥75 yr | 204 | 91.2 | 100.4 | 117.3 | 139.3 | 159.6 | 178.4 | 189.4 |
| Total | 15,745 | 75.8 | 85.3 | 102.4 | 123.7 | 147.4 | 170.5 | 187.2 |

*To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

contribution of quintiles of C-reactive protein or LDL cholesterol to models containing the other variable with a likelihood-ratio test with 4 degrees of freedom.

To evaluate joint effects, we repeated the analyses after classifying all study participants in one of four groups on the basis of whether their C-reactive protein and LDL cholesterol levels were above or below the respective study medians. Finally, using these data, we assessed whether C-reactive protein had independent predictive value after simultaneous adjustment for all components of the Framingham risk score[19] (including age, smoking status, categorical levels of blood pressure, presence or absence of diabetes mellitus, and high-density lipoprotein and LDL cholesterol levels) and whether C-reactive protein contributed information on risk beyond that conveyed by the 10-year risk calculated with the Framingham risk score and beyond the risk associated with LDL cholesterol, as defined by current guidelines.[1] All P values are two-tailed, and 95 percent confidence intervals were calculated.

## RESULTS

### Base-Line Characteristics

The mean age of the 27,939 women at base line was 54.7 years. Forty-four percent were current users of hormone-replacement therapy, 25 percent had hypertension, 12 percent were current smokers, and 2.5 percent had diabetes mellitus. The mean body-mass index (the weight in kilograms divided by the square of the height in meters) was 25.9.

### Distribution of C-Reactive Protein and LDL Cholesterol Levels

Table 1 presents data on the distribution of C-reactive protein and LDL cholesterol values among the

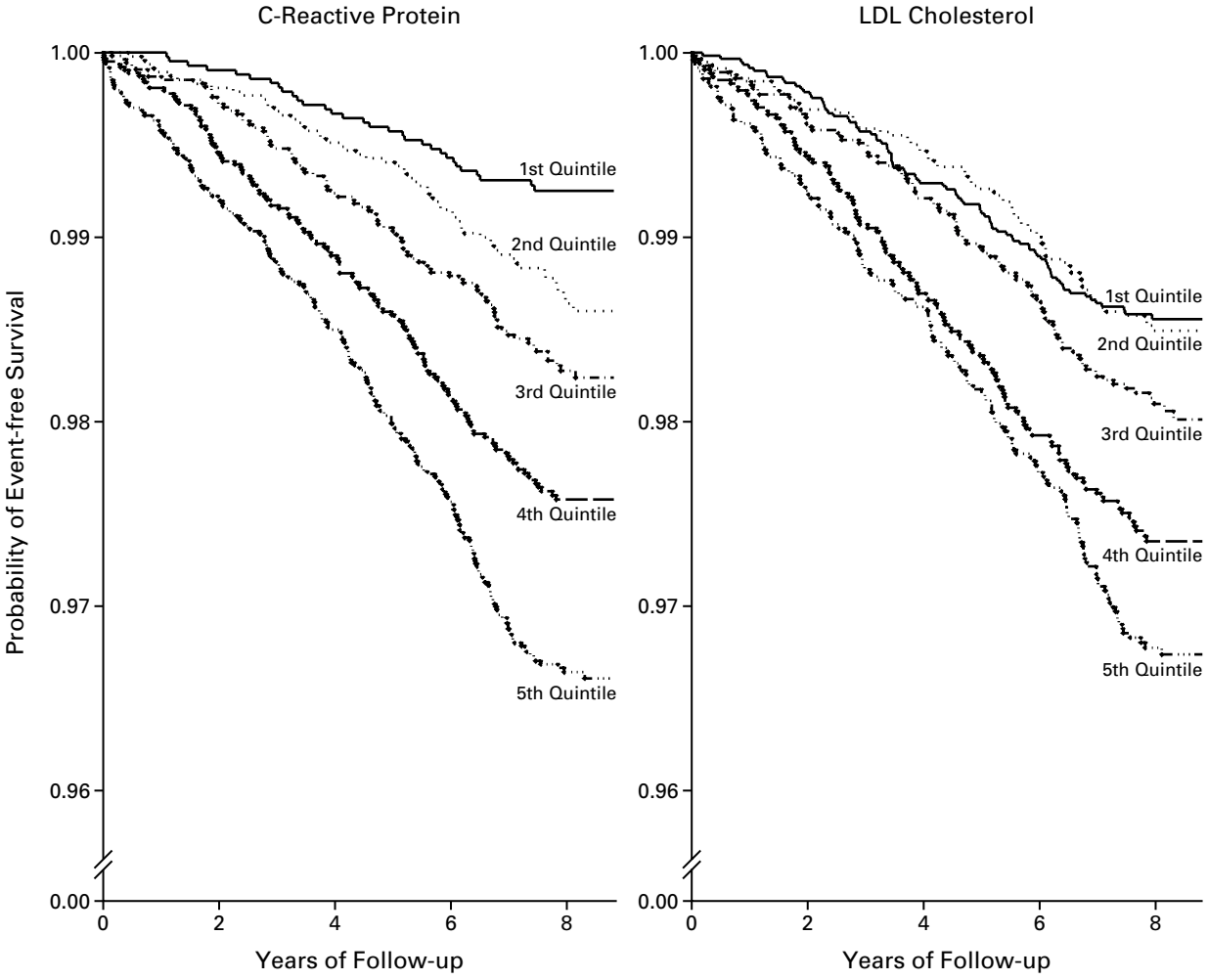


**Figure 1.** Event-free Survival According to Base-Line Quintiles of C-Reactive Protein and LDL Cholesterol.

The range of values for C-reactive protein was as follows: first quintile, ≤0.49 mg per liter; second quintile, >0.49 to 1.08 mg per liter; third quintile, >1.08 to 2.09 mg per liter; fourth quintile, >2.09 to 4.19 mg per liter; fifth quintile, >4.19 mg per liter. For LDL cholesterol, the values were as follows: first quintile, ≤97.6 mg per deciliter; second quintile, >97.6 to 115.4 mg per deciliter; third quintile, >115.4 to 132.2 mg per deciliter; fourth quintile, >132.2 to 153.9 mg per deciliter; fifth quintile, >153.9 mg per deciliter. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586. Note the expanded scale on the ordinate.

15,745 women who were not using hormone-replacement therapy at the time of blood collection. These distributions are very similar to those reported for men and women in previous U.S. and European studies. On the basis of this sample, the cutoff points for quintiles of C-reactive protein were less than or equal to 0.49, more than 0.49 to 1.08, more than 1.08 to 2.09, more than 2.09 to 4.19, and more than 4.19 mg per liter.

### Event-free Survival

The probability of event-free survival for all study participants is presented in Figure 1 according to base-line quintiles of C-reactive protein and LDL cholesterol. Table 2 presents crude relative risks of a first cardiovascular event according to increasing quintiles of base-line C-reactive protein and LDL cholesterol, along with relative risks adjusted for age and other risk factors. For both C-reactive protein and LDL cholesterol, strong linear risk gradients were observed. After adjustment for age, smoking status, the presence or absence of diabetes, blood pressure, and use or nonuse of hormone-replacement therapy, the multivariable relative risks of a first cardiovascular event for women in increasing quintiles of C-reactive protein were 1.0 (the first quintile was the reference category), 1.4, 1.6, 2.0, and 2.3 ($P<0.001$), whereas the relative risks associated with increasing quintiles of LDL cholesterol were 1.0 (the first quintile was the reference category), 0.9, 1.1, 1.3, and 1.5 ($P<0.001$). No significant deviations from linearity in the log relative risks were detected in either model. The apparent superiority of C-reactive protein over LDL cholesterol in terms of the prediction of risk was observed in similar analyses of the individual components of the composite end point (coronary heart disease, stroke, and death from cardiovascular causes) (Fig. 2).

### Predictive Models

Table 2 also presents results of the C statistic analyses (area under the receiver-operating-characteristic curve). In models of crude rates including the entire cohort (27,939 women), the calculated area under the receiver-operating-characteristic curve was 0.64 for C-reactive protein and 0.60 for LDL cholesterol. In prediction models including age, smoking status, presence or absence of diabetes, blood pressure, use or nonuse of hormone-replacement therapy, and treatment assignment, the ability of the model based on C-reactive protein to discriminate events from nonevents was virtually identical to that of the model based on LDL cholesterol (C statistic for both models, 0.81). However, the likelihood-ratio chi-square statistic was higher for the model based on C-reactive protein than for that based on LDL cholesterol (716.4 vs. 706.0, both with 16 df). This statistic, a more sensitive measure of model fit than the rank-based C statistic, suggests that the model based on C-reactive protein has better discrimination than the model based on LDL cholesterol. In addition, in likelihood-ratio tests of the contribution of each variable, the addition of C-reactive protein to the model based on LDL cholesterol was stronger (chi-square=25.4, 4 df;

**TABLE 2.** CRUDE, AGE-ADJUSTED, AND RISK-FACTOR–ADJUSTED RELATIVE RISK OF A FIRST CARDIOVASCULAR EVENT ACCORDING TO THE QUINTILE OF C-REACTIVE PROTEIN AND LDL CHOLESTEROL AT BASE LINE.*

| VARIABLE | QUINTILE OF C-REACTIVE PROTEIN | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 (≤0.49 mg/liter) | 2 (>0.49–1.08 mg/liter) | 3 (>1.08–2.09 mg/liter) | 4 (>2.09–4.19 mg/liter) | 5 (>4.19 mg/liter) | P VALUE | AREA UNDER ROC CURVE |
| Crude relative risk (95% CI) | 1.0 | 1.8 (1.1–2.7) | 2.3 (1.5–3.4) | 3.2 (2.2–4.8) | 4.5 (3.1–6.6) | <0.001 | 0.64 |
| Age-adjusted relative risk (95% CI) | 1.0 | 1.5 (1.0–2.4) | 1.8 (1.2–2.8) | 2.5 (1.7–3.7) | 3.6 (2.5–5.2) | <0.001 | 0.74 |
| Risk-factor–adjusted relative risk (95% CI) | 1.0 | 1.4 (0.9–2.2) | 1.6 (1.1–2.4) | 2.0 (1.3–3.0) | 2.3 (1.6–3.4) | <0.001 | 0.81 |
| | QUINTILE OF LDL CHOLESTEROL | | | | | | |
| | 1 (≤97.6 mg/dl) | 2 (>97.6–115.4 mg/dl) | 3 (>115.4–132.2 mg/dl) | 4 (>132.2–153.9 mg/dl) | 5 (>153.9 mg/dl) | P VALUE | AREA UNDER ROC CURVE |
| Crude relative risk (95% CI) | 1.0 | 1.0 (0.8–1.4) | 1.3 (1.0–1.8) | 1.8 (1.4–2.4) | 2.2 (1.7–2.9) | <0.001 | 0.60 |
| Age-adjusted relative risk (95% CI) | 1.0 | 0.9 (0.7–1.3) | 1.1 (0.9–1.5) | 1.5 (1.1–1.9) | 1.7 (1.3–2.2) | <0.001 | 0.73 |
| Risk-factor–adjusted relative risk (95% CI) | 1.0 | 0.9 (0.7–1.2) | 1.1 (0.8–1.4) | 1.3 (1.0–1.7) | 1.5 (1.1–2.0) | <0.001 | 0.81 |

*P values are for tests of trend across quintiles. ROC denotes receiver operating characteristic, and CI confidence interval. Risk-factor–adjusted relative risks have been adjusted for age, smoking status, the presence or absence of diabetes mellitus, blood pressure, and use or nonuse of hormone-replacement therapy. All models have been adjusted for treatment assignment. For all relative risks, the reference category is the first quintile. To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

P< 0.001) than the addition of LDL cholesterol to the model based on C-reactive protein (chi-square= 15.0, 4 df; P=0.005).

#### Effects of Hormone-Replacement Therapy

Table 3 presents stratified analyses according to the use or nonuse of hormone-replacement therapy at base line. Among women who did not use hormone-replacement therapy, the multivariable-adjusted relative risks of a first cardiovascular event in increasing quintiles of C-reactive protein were 1.0, 1.8, 1.8, 2.4, and 3.0 (P<0.001), whereas the multivariable-adjusted relative risks in increasing quintiles of LDL cholesterol were 1.0, 0.8, 0.9, 1.1, and 1.4 (P=0.002). Among users of hormone-replacement therapy, risk estimates were lower for both C-reactive protein and LDL cholesterol but remained significant in crude and age-adjusted models. Risk estimates based on C-reactive protein among users of hormone-replacement therapy were similar regardless of whether the quintiles were defined by measurements in nonusers or users of hormone-replacement therapy.

#### Interactions between C-Reactive Protein and LDL Cholesterol

Of all events in the study participants, 77 percent occurred among those with LDL cholesterol levels below 160 mg per deciliter (4.14 mmol per liter), and 46 percent occurred among those with LDL cholesterol levels below 130 mg per deciliter (3.36 mmol per liter). However, C-reactive protein and LDL cholesterol levels were minimally correlated (r=0.08), suggesting that each biologic marker was detecting a different high-risk group. We therefore constructed survival curves after dividing the study participants into four groups on the basis of whether they were

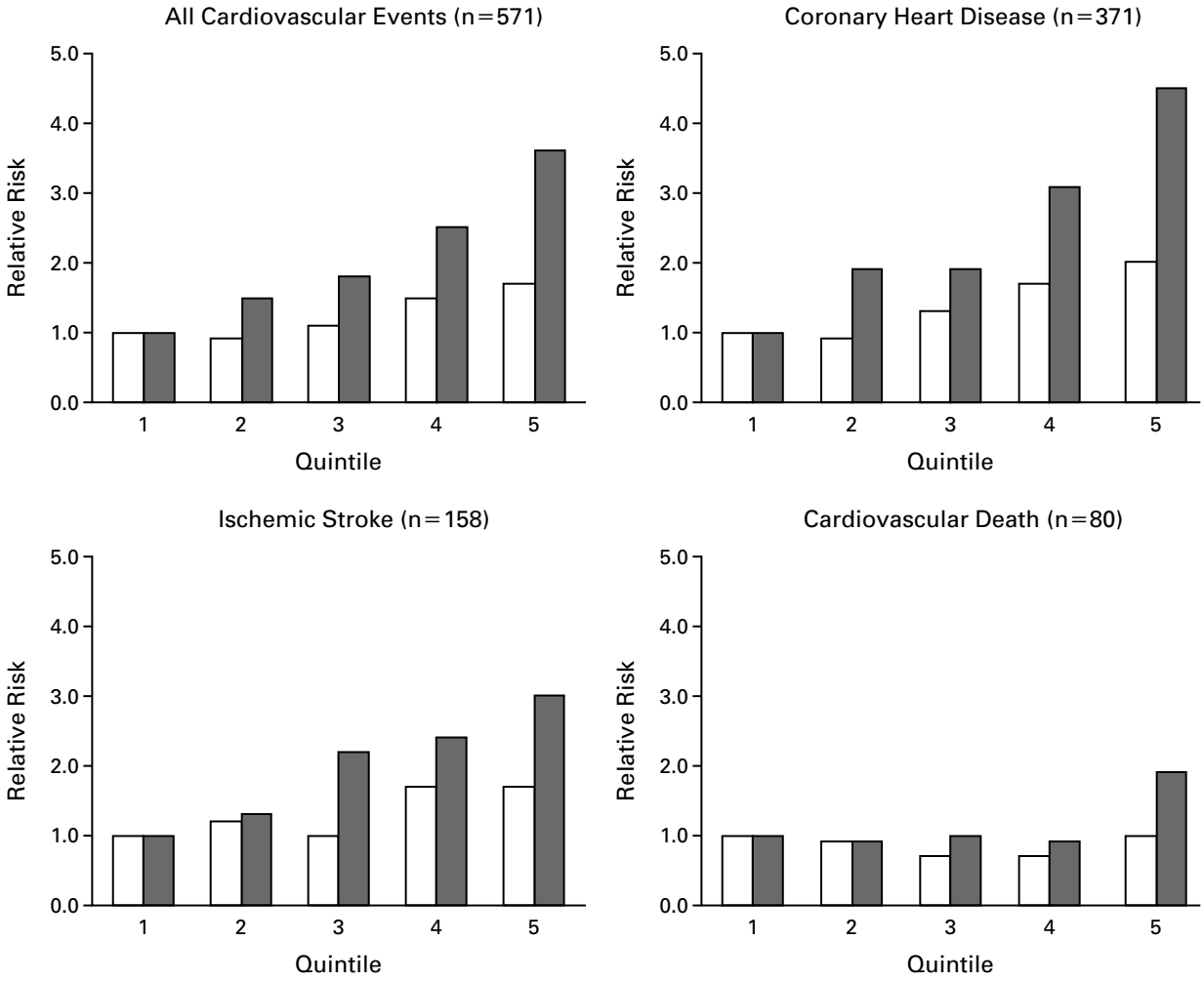


**Figure 2.** Age-Adjusted Relative Risk of Future Cardiovascular Events, According to Base-Line C-Reactive Protein Levels (Solid Bars) and LDL Cholesterol Levels (Open Bars).

**TABLE 3.** CRUDE, AGE-ADJUSTED, AND RISK-FACTOR–ADJUSTED RELATIVE RISK OF A FIRST CARDIOVASCULAR EVENT, ACCORDING TO THE QUINTILE OF C-REACTIVE PROTEIN AND LDL CHOLESTEROL AT BASE LINE, AMONG 12,139 WOMEN WHO USED POSTMENOPAUSAL HORMONE-REPLACEMENT THERAPY AND 15,745 WOMEN WHO DID NOT USE SUCH THERAPY.*

| VARIABLE | QUINTILE OF C-REACTIVE PROTEIN | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | P VALUE | AREA UNDER ROC CURVE |
| **Nonusers of HRT** | | | | | | | |
| Crude relative risk | 1.0 | 2.3 | 2.8 | 4.3 | 6.9 | <0.001 | 0.67 |
| Age-adjusted relative risk | 1.0 | 1.9 | 2.2 | 3.2 | 5.4 | <0.001 | 0.78 |
| Risk-factor–adjusted relative risk | 1.0 | 1.8 | 1.8 | 2.4 | 3.0 | <0.001 | 0.84 |
| **Users of HRT** | | | | | | | |
| Crude relative risk | 1.0 | 1.0 | 1.5 | 1.9 | 2.4 | <0.001 | 0.60 |
| Age-adjusted relative risk | 1.0 | 0.9 | 1.3 | 1.6 | 2.1 | <0.001 | 0.69 |
| Risk-factor–adjusted relative risk | 1.0 | 0.9 | 1.1 | 1.3 | 1.3 | 0.08 | 0.77 |
| | **QUINTILE OF LDL CHOLESTEROL** | | | | | | |
| | 1 | 2 | 3 | 4 | 5 | P VALUE | AREA UNDER ROC CURVE |
| **Nonusers of HRT** | | | | | | | |
| Crude relative risk | 1.0 | 1.0 | 1.2 | 1.8 | 2.6 | <0.001 | 0.61 |
| Age-adjusted relative risk | 1.0 | 0.8 | 1.0 | 1.3 | 1.6 | <0.001 | 0.75 |
| Risk-factor–adjusted relative risk | 1.0 | 0.8 | 0.9 | 1.1 | 1.4 | 0.002 | 0.84 |
| **Users of HRT** | | | | | | | |
| Crude relative risk | 1.0 | 1.1 | 1.5 | 1.8 | 1.7 | 0.001 | 0.58 |
| Age-adjusted relative risk | 1.0 | 1.1 | 1.3 | 1.7 | 1.5 | 0.005 | 0.68 |
| Risk-factor–adjusted relative risk | 1.0 | 1.0 | 1.3 | 1.5 | 1.5 | 0.02 | 0.77 |

*ROC denotes receiver operating characteristic, and HRT hormone-replacement therapy. P values are for tests of trend across quintiles. Risk-factor–adjusted relative risks have been adjusted for age, smoking status, presence or absence of diabetes mellitus, and blood pressure. All models have been adjusted for treatment assignment. For all relative risks, the reference category is the first quintile. For 55 women in the study, status with regard to hormone-replacement therapy was unknown.

above or below the median C-reactive protein value (1.52 mg per liter) and the median LDL cholesterol value (123.7 mg per deciliter [3.20 mmol per liter]). For the entire cohort (Fig. 3), the multivariable-adjusted relative risks were as follows: low C-reactive protein–low LDL cholesterol, 1.0 (this was the reference category); low C-reactive protein–high LDL cholesterol, 1.5 (95 percent confidence interval, 1.0 to 2.1); high C-reactive protein–low LDL cholesterol, 1.5 (95 percent confidence interval, 1.1 to 2.1); and high C-reactive protein–high LDL cholesterol, 2.1 (95 percent confidence interval, 1.5 to 2.8). The corresponding age-adjusted rates of events per 1000 person-years of follow-up were 1.3, 2.0, 2.6, and 3.9, respectively.

On the assumption that recent evidence from clinical trials will lead to a marked reduction in the use of hormone-replacement therapy among American women,[20] we sought to increase the generalizability of our findings by repeating these analyses including only the 15,745 women who were not using hormone-replacement therapy at base line. In this analysis, the multivariable-adjusted relative risks were as follows: low C-reactive protein–low LDL cholesterol, 1.0 (the reference category); low C-reactive protein–high LDL cholesterol, 1.5 (95 percent confidence interval, 1.0 to 2.4); high C-reactive protein–low LDL cholesterol, 1.7 (95 percent confidence interval, 1.1 to 2.6); and high C-reactive protein–high LDL cholesterol, 2.4 (95 percent confidence interval, 1.6 to 3.6). The corresponding age-adjusted rates of events per 1000 person-years were 1.2, 1.9, 3.1, and 4.5, respectively. As in the total cohort, event-free survival among nonusers of hormone-replacement therapy was worse in the high C-reactive protein–low LDL cholesterol group than in the low C-reactive protein–high LDL cholesterol group (Fig. 3).

### C-Reactive Protein, LDL Cholesterol Categories, and the Framingham Risk Score

We performed several further analyses to evaluate the addition of measurements of C-reactive protein to the Framingham risk score and to the LDL cholesterol categories of less than 130, 130 to 160, and more than 160 mg per deciliter, which are defined in current guidelines for risk detection.[1] After adjustment for all components of the Framingham risk score,[19] quintiles of C-reactive protein remained a strong, independent predictor of risk in the cohort as a whole (relative risks of future cardiovascular events in increasing quintiles, 1.0, 1.3, 1.4, 1.7, and 1.9; $P<0.001$) and among nonusers of hormone-replacement therapy (relative risks, 1.0, 1.6, 1.5, 1.8, and 2.2; $P=0.001$). As shown in Figure 4, increasing levels of C-reactive protein were associated with increased risk of cardiovascular events at all levels of estimated 10-year risk based on the Framingham risk score.[19] Similarly, increasing C-reactive protein levels were associated with increased risk of cardiovascular events at LDL cholesterol levels below 130, 130 to 160, and above 160 mg per deciliter (Fig. 4).

## DISCUSSION

The current study suggests that C-reactive protein, a marker of systemic inflammation, is a stronger predictor of future cardiovascular events than LDL cholesterol. In this study, C-reactive protein was superior to LDL cholesterol in predicting the risk of all study end points; this advantage persisted in multivariable analyses in which we adjusted for all traditional cardiovascular risk factors and was clear among users as well as nonusers of hormone-replacement therapy at base line. However, C-reactive protein and LDL cholesterol levels were minimally correlated. Thus, the combined evaluation of both C-reactive protein and LDL cholesterol proved to be superior as a method of

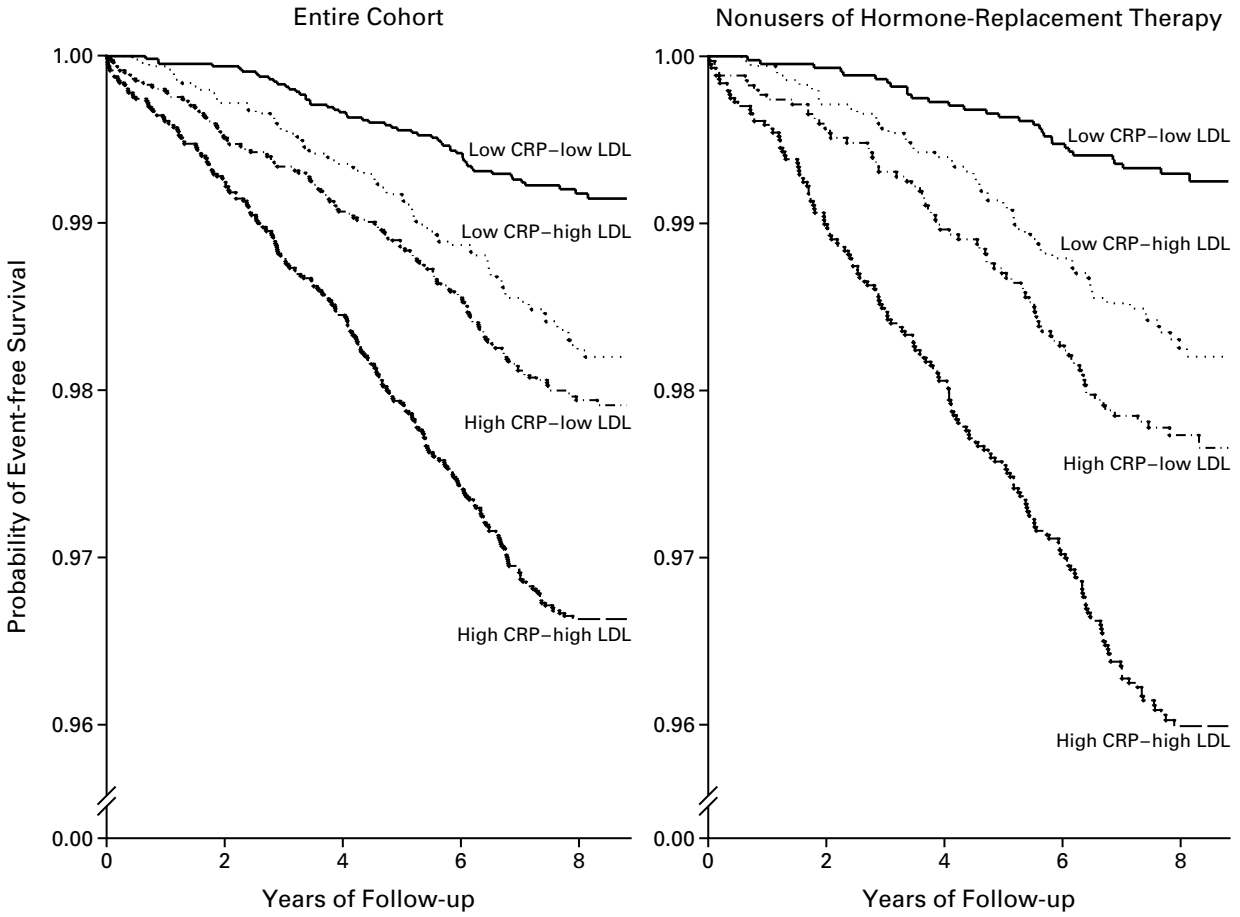


**Figure 3.** Event-free Survival among Women with C-Reactive Protein (CRP) and LDL Cholesterol Levels above or below the Median for the Study Population.

Data are shown for the entire cohort (27,939 women) and for women who were not taking hormone-replacement therapy at base line (15,745 women). The median values were as follows: for C-reactive protein, 1.52 mg per liter; for LDL cholesterol, 123.7 mg per deciliter (3.20 mmol per liter). Note the expanded scale on the ordinate.

risk detection to measurement of either biologic marker alone. Finally, at all levels of estimated 10-year risk for events according to the Framingham risk score and at all levels of LDL cholesterol, C-reactive protein remained a strong predictor of future cardiovascular risk.

In addition to their pathophysiological implications with regard to inflammation and atherothrombosis,[21-23] we believe these data have implications for the detection and prevention of cardiovascular disease. Seventy-seven percent of first cardiovascular events among the 27,939 women in this study occurred in those with LDL cholesterol levels below 160 mg per deciliter, and 46 percent occurred in those with levels below 130 mg per deciliter. Thus, large proportions of first cardiovascular events in women occur at LDL cholesterol levels below the threshold values for intervention and treatment in the current guidelines of the National Cholesterol Education Program.[1]

Our data also help establish the population distribution of C-reactive protein. That the cutoff points for the quintiles in the current population are very close to those previously described in smaller studies from the United States and Europe is reassuring and consistent with evidence describing the stability and reproducibility of values obtained for C-reactive protein with new, high-sensitivity assays.[24] These data also demonstrate that a single set of cutoff points for C-reactive protein in women can be used regardless of their status with regard to hormone-replacement therapy — an issue that has been of concern in previous work.[14-16]

The current data also have implications for the targeting of preventive therapies. We previously demonstrated in a randomized trial that statin therapy may have clinical value for primary prevention among persons with elevated C-reactive protein but low LDL cholesterol levels.[25] According to the survival analy-

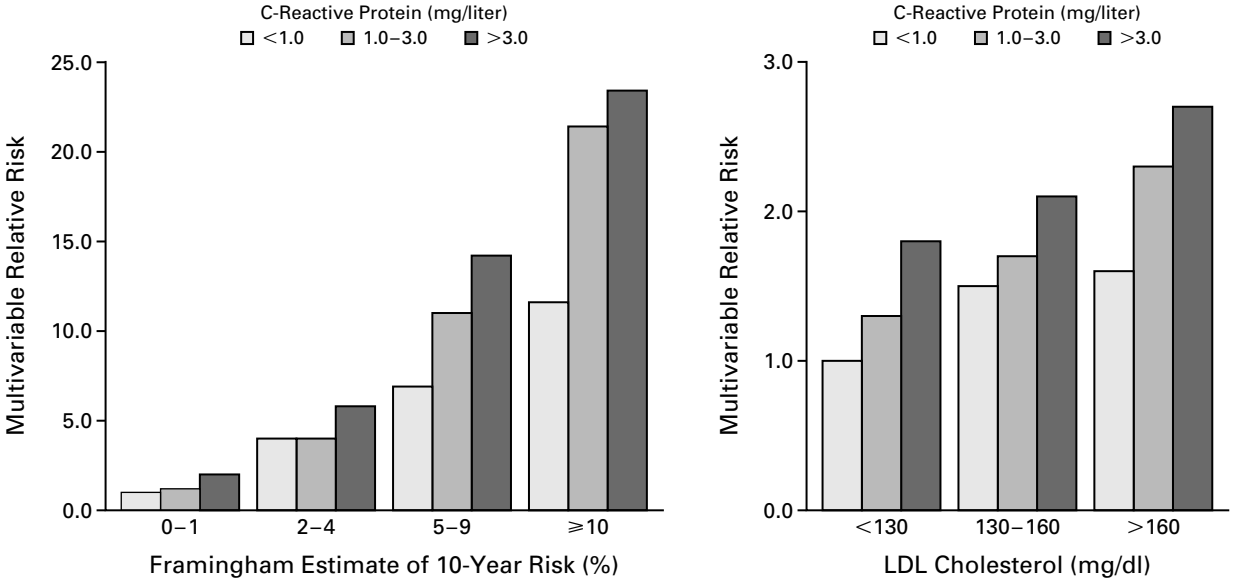


**Figure 4.** Multivariable-Adjusted Relative Risks of Cardiovascular Disease According to Levels of C-Reactive Protein and the Estimated 10-Year Risk Based on the Framingham Risk Score as Currently Defined by the National Cholesterol Education Program and According to Levels of C-Reactive Protein and Categories of LDL Cholesterol.

To convert values for LDL cholesterol to millimoles per liter, multiply by 0.02586.

ses in the current study (Fig. 3), women in the high C-reactive protein–low LDL cholesterol subgroup were at higher absolute risk than those in the low C-reactive protein–high LDL cholesterol subgroup, yet it is only the latter group for whom aggressive prevention is likely to be considered by most physicians. These observations suggest that continued reliance on LDL cholesterol to predict the risk of cardiovascular events will not lead to optimal targeting of statin therapy for primary prevention; this suggestion is consistent with data from the Heart Protection Study, in which LDL cholesterol levels did not predict the efficacy of statins for secondary prevention.[26] Our data thus strongly support the need for a large-scale trial of statin therapy among persons with low levels of LDL cholesterol but high levels of C-reactive protein.[27]

Unlike other markers of inflammation, C-reactive protein levels are stable over long periods, have no diurnal variation, can be measured inexpensively with available high-sensitivity assays, and have shown specificity in terms of predicting the risk of cardiovascular disease.[24,28-30] However, despite the consistency of prospective data in diverse cohorts,[4-13,16,25,31] decisions regarding the clinical use of C-reactive protein remain complex. To evaluate fully the clinical usefulness of any new biologic marker requires more than a direct comparison with LDL cholesterol or the Framingham risk score; other factors, such as lipid subfractions, triglycerides, Lp(a) lipoprotein, homocysteine, insulin resistance, and hypofibrinolysis, either in combination with or in place of other traditional markers, must also be taken into account. Furthermore, it is increasingly clear that no single common pathway is likely to account for all cardiovascular events and that interactions between novel biologic markers and more traditional risk factors, such as high blood pressure, smoking, obesity, diabetes, low levels of physical activity, and use of hormone-replacement therapy, may be more or less important for individual patients. Thus, as our findings indicate, new biologic and statistical approaches will be needed as information from basic vascular biology begins the transition into clinical practice.

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Dr. Ridker is named as a coinventor on patents filed by Brigham and Women's Hospital that relate to the use of inflammatory biologic markers in cardiovascular disease.

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