Inflammation and Atherosclerosis: Role of C-Reactive Protein in Risk Assessment

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Inflammation participates critically in atherosclerosis. Circulating levels of several inflammatory markers rise in individuals at risk for atherosclerotic events. In particular, elevation of plasma C-reactive protein (CRP), a nonspecific acute-phase reactant that is easily and reliably measured, has strong predictive power for cardiovascular events. Indeed, measurements of high-sensitivity CRP (hs-CRP) plasma levels add to both the prognostic information gleaned from assay of plasma lipid risk factors and the risk levels estimated by means of Framingham study–based criteria. Retrospective data suggest the hypothesis that hs-CRP plasma levels may be useful for guiding use of lipid-lowering therapy in individuals who appear to be at low risk according to traditional risk assessment. A large-scale, randomized clinical trial—Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER)—will test whether rosuvastatin therapy will reduce incident cardiovascular disease in subjects with elevated plasma hs-CRP concentrations who do not meet current criteria for initiation of lipid-lowering drug therapy. Such clinical trial data may provide an evidence base for the use of plasma CRP assay as an adjuvant guide to therapy to complement the established traditional risk factors such as plasma lipid levels. Thus, medical practitioners are ushering in an era in which the biology of inflammation in atherosclerosis will find its way into clinical application. *Am J Med.* 2004;116(6A):9S–16S.

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Investigation of the mechanisms of atherosclerosis has determined that inflammation plays a central role in the development, progression, and outcomes of this prevalent disease. While improving the understanding of atherosclerotic disease, current insights hold promise for meaningful clinical applications in risk assessment and guidance of targeted therapy.

OVERVIEW OF ATHEROSCLEROSIS: AN INFLAMMATORY DISEASE

Atherosclerotic plaque development begins with endothelial cell activation, including overexpression of leukocyte adhesion proteins (**Figure 1**).[1,2] Triggers of this inflammatory response may include oxidized lipoproteins, hypertension, diabetes mellitus, and conditions such as obesity. The healthy endothelium resists prolonged leukocyte attachment. Expression of adhesion molecules, such as vascular cell adhesion molecule–1 (VCAM-1), enhances the recruitment of inflammatory cells from the blood. VCAM-1 binds leukocytes (e.g., monocytes) found in nascent atheromas; in models of experimental atherosclerosis, expression of VCAM-1 has been shown to increase before leukocytes adhere to the arterial endothelium. Chemoattractant stimuli—e.g., monocyte chemoattractant protein–1—promote migration of leukocytes into the intima, where macrophage colony-stimulating factor promotes the differentiation of monocytes into macrophages. The macrophages express scavenger receptors that allow them to engulf and modify lipoproteins and become foam cells. These lipid-laden phagocytes secrete a number of inflammatory mediators that amplify inflammation in the vessel wall and can contribute to additional leukocyte accumulation, smooth muscle cell proliferation, and extracellular matrix remodeling. By elaboration of matrix metalloproteinases that degrade the protective collagen structure of the plaque's fibrous cap, macrophages can contribute to plaque vulnerability, to rupture, and to formation of thrombi that can precipitate acute coronary events. Simultaneously, the inflammatory response inhibits collagen production and stimulates macrophage expression of the potent procoagulant tissue factor, contributing to the prothrombotic milieu. Thus, inflammation plays a central role in the inception and progression of atherosclerosis as well as in the dreaded thrombotic complications of this disease.

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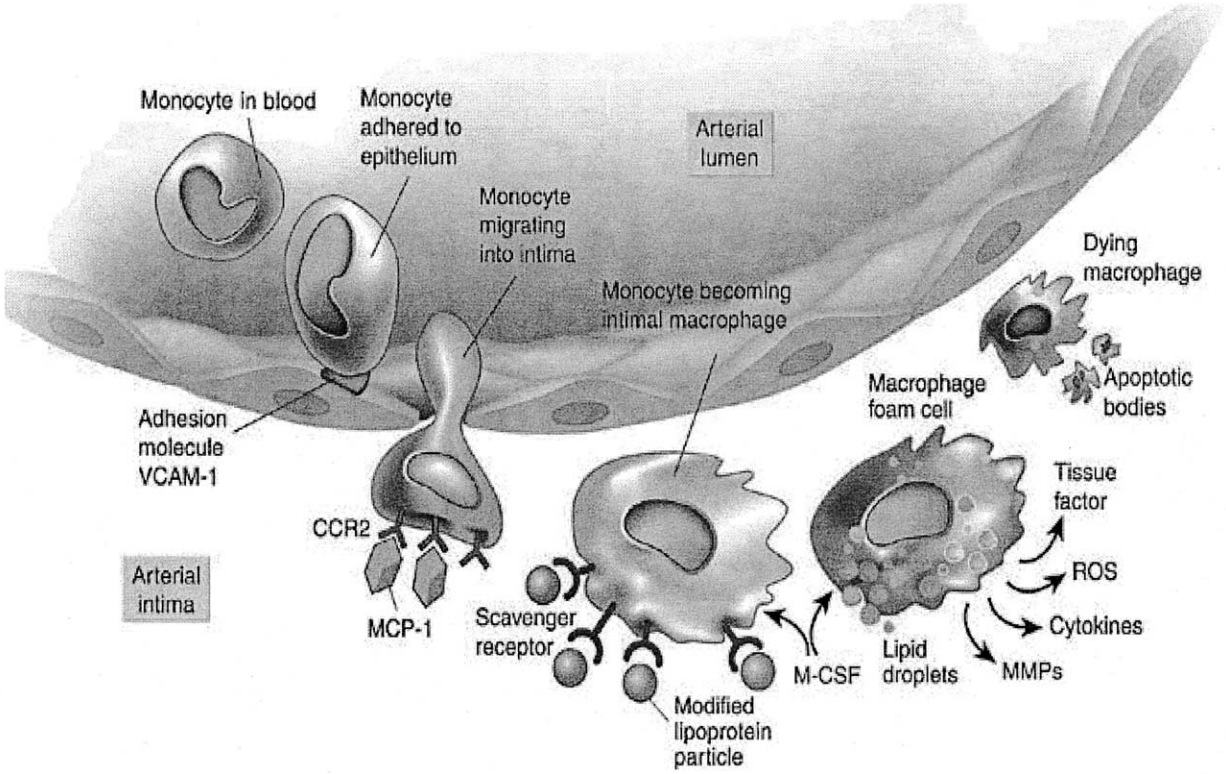

Figure 1. Role of monocytes in atherogenesis after inflammatory activation of endothelial cells, including (*left to right*) adhesion to the vascular endothelium; migration into the intima; maturation to macrophage phenotype; formation of the foam cell, the hallmark of arterial atherosclerotic lesions; and elaboration of inflammatory mediators and matrix metalloproteinases (MMPs). CCR2 = chemokine receptor-2; MCP-1 = monocyte chemoattractant protein–1; M-CSF = macrophage colony-stimulating factor; ROS = reactive oxygen species; VCAM-1 = vascular cell adhesion molecule–1. (Reprinted with permission from *Nature.*[2])

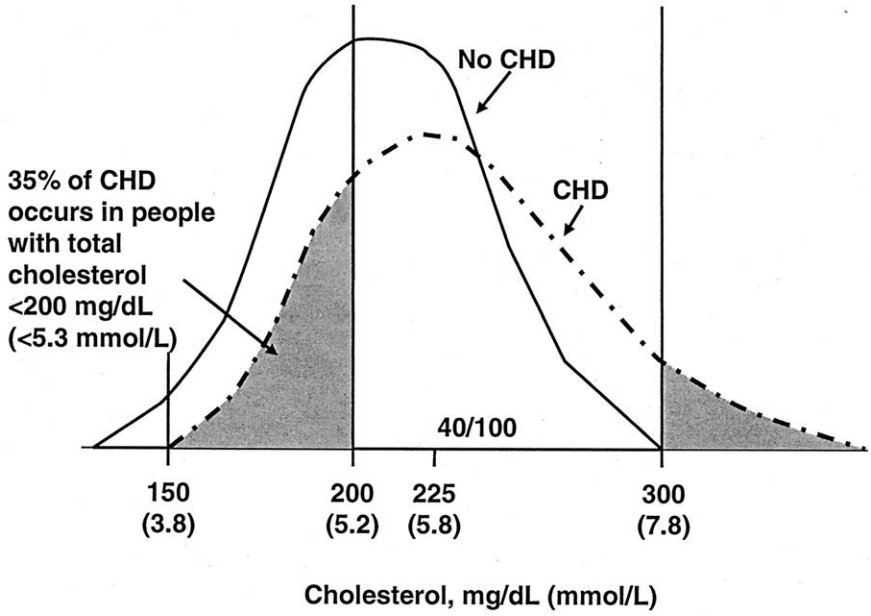

Figure 2. Distribution of plasma total cholesterol levels in individuals with and without coronary heart disease (CHD), i.e., myocardial infarction, in a 26-year follow-up of the Framingham study. (Reprinted with permission from *Atherosclerosis.*[3])

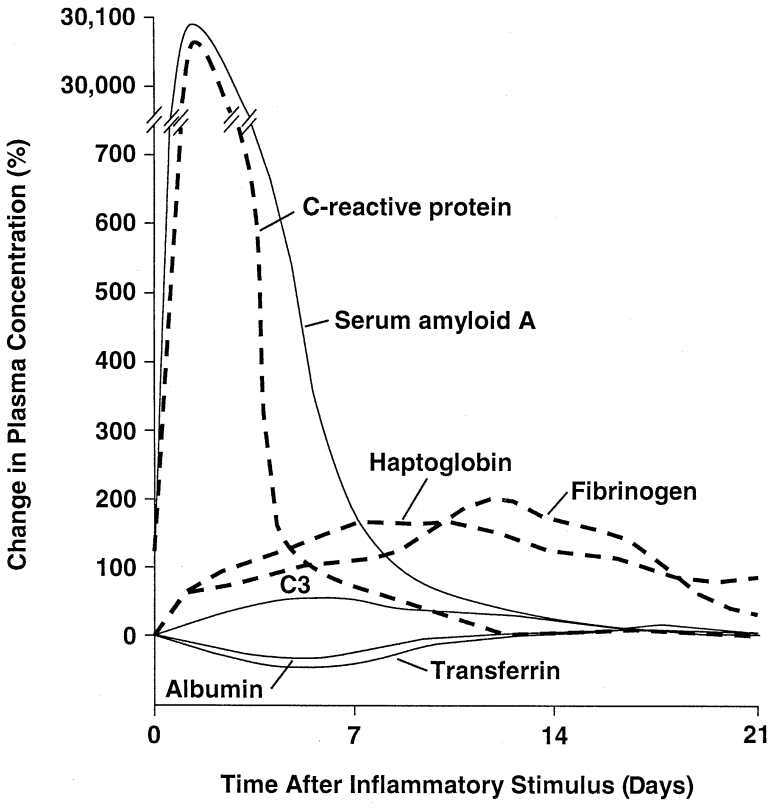

Figure 3. Changes in acute-phase reactants after a moderate inflammatory stimulus such as exercise. Severe stimuli include infection and cancer; minor stimuli include stress and some psychiatric illnesses. Note the break in scale between 700% and 30,000%. C3 = complement. (Reprinted with permission from *N Engl J Med.*[5])

ROLE OF INFLAMMATORY MARKERS IN RISK ASSESSMENT: C-REACTIVE PROTEIN AND CARDIOVASCULAR DISEASE

One of the great success stories of modern medicine is the ability to harness the basic knowledge of cholesterol and its metabolism, with clinical use of this knowledge allowing the modification of plasma cholesterol levels in a way that uniformly improves clinical outcomes. Although determination of plasma lipid profiles constitutes an important component of risk assessment for atherosclerosis, the picture provided by lipid profiles alone is incomplete. Castelli[3] demonstrated that plasma total cholesterol levels poorly discriminate risk for coronary heart disease (CHD) on a population level: much of the total incidence of CHD occurs in individuals with below-average plasma levels of total cholesterol (**Figure 2**). Although global risk assessment offers improved prediction of coronary events,[4] emerging data suggest that measurement of inflammatory markers may enhance risk evaluation.

Circulating levels of certain proteins involved in host defenses change markedly during the acute response to inflammatory stimuli (e.g., infectious agents). Although the levels of some acute-phase reactants change minimally, plasma concentrations of C-reactive protein (CRP) and serum amyloid A levels show dramatic increases (**Figure 3**).[5] Current data suggest a pathway of inflammation that culminates in higher concentrations of various markers in peripheral blood.[6] Systemic or local inflammation, in either blood vessels or tissue, likely results in production of multipotent, primary proinflammatory cytokines capable of inducing endothelial and other cells to produce adhesion molecules, procoagulant factors, and other mediators released into the circulation in soluble form. The cytokines also induce production of the "messenger" cytokine interleukin-6, which stimulates the liver to produce acute-phase proteins, including CRP and serum amyloid A. The large increases in circulating levels of plasma CRP and serum amyloid A indicate major changes in the regulation of these genes as they respond to inflammation.

Although the circulating levels of several inflammatory mediators correlate with increased coronary risk, CRP has attracted particular attention.[1,7] Plasma CRP has a long half-life, exhibits stable levels in individuals, and has negligible circadian variation. It is easily measured, and inexpensive standardized high-sensitivity assays provide similar results in fresh, stored, or frozen plasma, reflecting the high stability of the protein. As a downstream

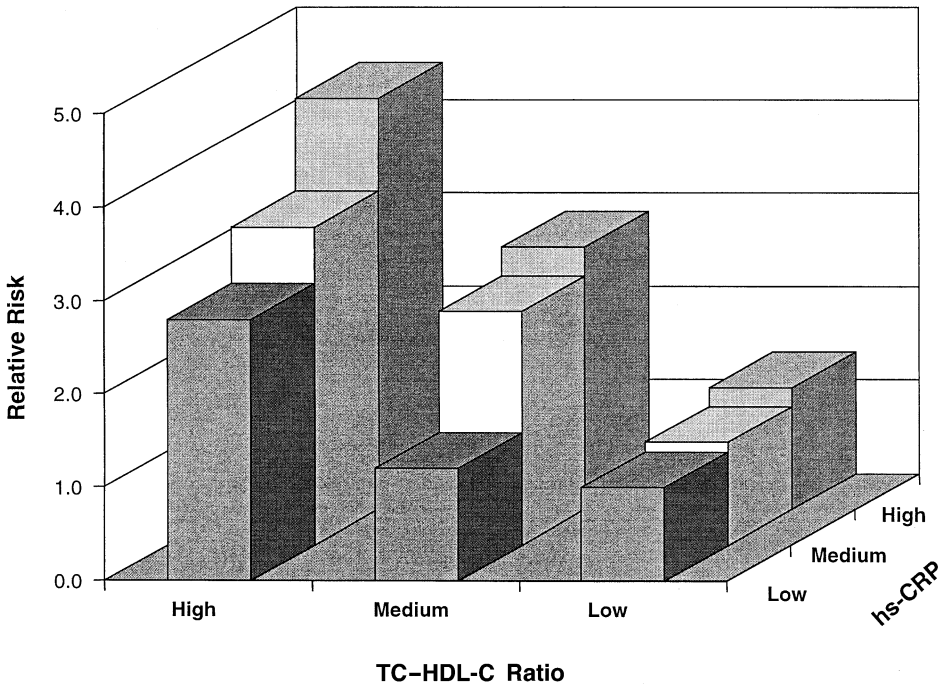

Figure 4. Risk for myocardial infarction according to tertiles of baseline measurements for plasma total cholesterol (TC)–high-density lipoprotein cholesterol (HDL-C) ratio and high-sensitivity C-reactive protein (hs-CRP) in apparently healthy men in the Physicians' Health Study. (Reprinted with permission from *Circulation*.[8])

biomarker, CRP provides functional integration of overall upstream cytokine activation; it also exhibits activities that may exert direct effects on vascular disease, including the binding and activation of complement. Some experiments indicate that high concentrations of plasma CRP elevate levels of cell adhesion molecules and tissue factor, mediate low-density lipoprotein cholesterol (LDL-C) uptake by endothelial macrophages, induce recruitment of monocytes into blood vessel walls, and augment levels of monocyte chemoattractant protein–1. CRP classically emanates from the liver; however, it may also be produced by vascular sources, including cells residing in atherosclerotic plaques. Mice transgenic for human CRP expression have been shown to be both prothrombotic and proatherogenic.

Data from many prospective studies demonstrate that baseline plasma CRP levels predict the likelihood of cardiovascular events in apparently well people.[1,7] These data also indicate that high-sensitivity CRP (hs-CRP) measurements add to the predictive ability of plasma lipid risk factors. Indeed, elevated plasma CRP levels were associated with increased relative risk of initial myocardial infarction (MI) at every level of the plasma total cholesterol–high-density lipoprotein cholesterol (HDL-C) ratio in apparently healthy men in the Physicians' Health Study (**Figure 4**).[8] Recently, Ridker et al.[9] assessed the ability of a single, nonfasting baseline plasma hs-CRP measurement to predict first cardiovascular events over a mean of 8 years in nearly 28,000 apparently healthy women in the Women's Health Study.[9] The plasma hs-CRP level at baseline predicted risk for first cardiovascular events better than did baseline plasma levels of LDL-C (**Figure 5**).[9] Further, plasma hs-CRP assay added prognostic information to the Framingham scoring estimates of 10-year CHD risk and LDL-C categories used in the Adult Treatment Panel III (ATP III) guidelines of the National Cholesterol Education Program (NCEP) (**Figure 6**).[9] Notably, plasma hs-CRP measurement aided risk prediction in individuals with the metabolic syndrome. Accumulation of components of the metabolic syndrome, as currently defined by ATP III guidelines (i.e., central obesity, elevated plasma triglyceride concentrations, low plasma levels of HDL-C, hypertension, and elevated levels of blood glucose), were associated with increased plasma hs-CRP levels in 14,719 women in the Women's Health Study (**Figure 7**).[10]

POTENTIAL CLINICAL UTILITY OF C-REACTIVE PROTEIN MEASUREMENT

The suitability of plasma CRP assay for clinical application compares favorably with determination of other inflammatory markers.[11] For example, there is no standard assay for measuring lipoprotein(a), and circadian variations in fibrinogen make its measurement difficult. Although measurement of homocysteine is reliable, pro-

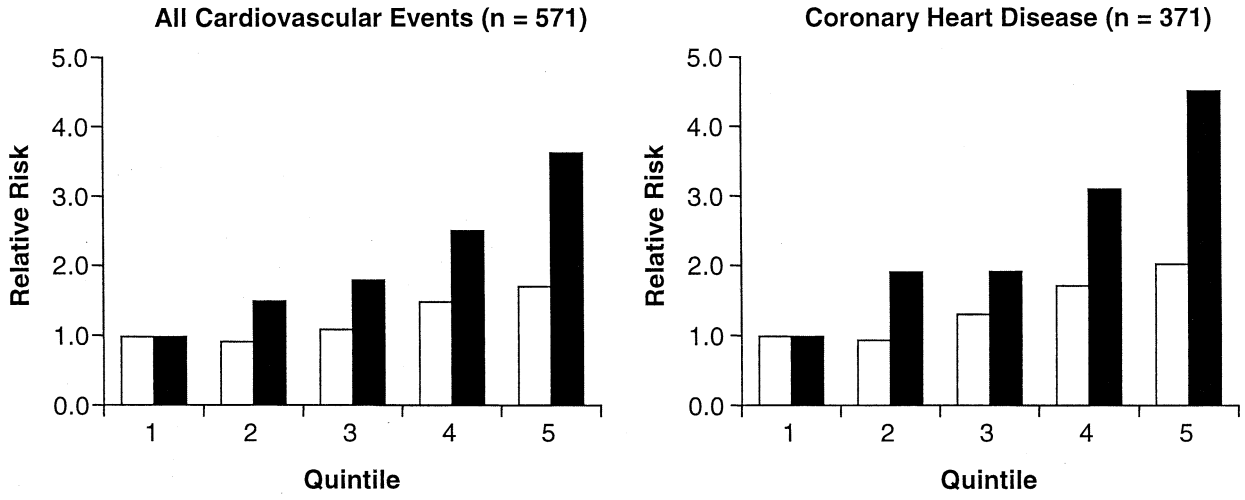

Figure 5. Age-adjusted relative risk of first cardiovascular events and coronary heart disease according to baseline measurements of plasma low-density lipoprotein cholesterol (*open bars*) and high-sensitivity C-reactive protein (*solid bars*) quintiles in the Women's Health Study population. (Reprinted with permission from *N Engl J Med*.[9])

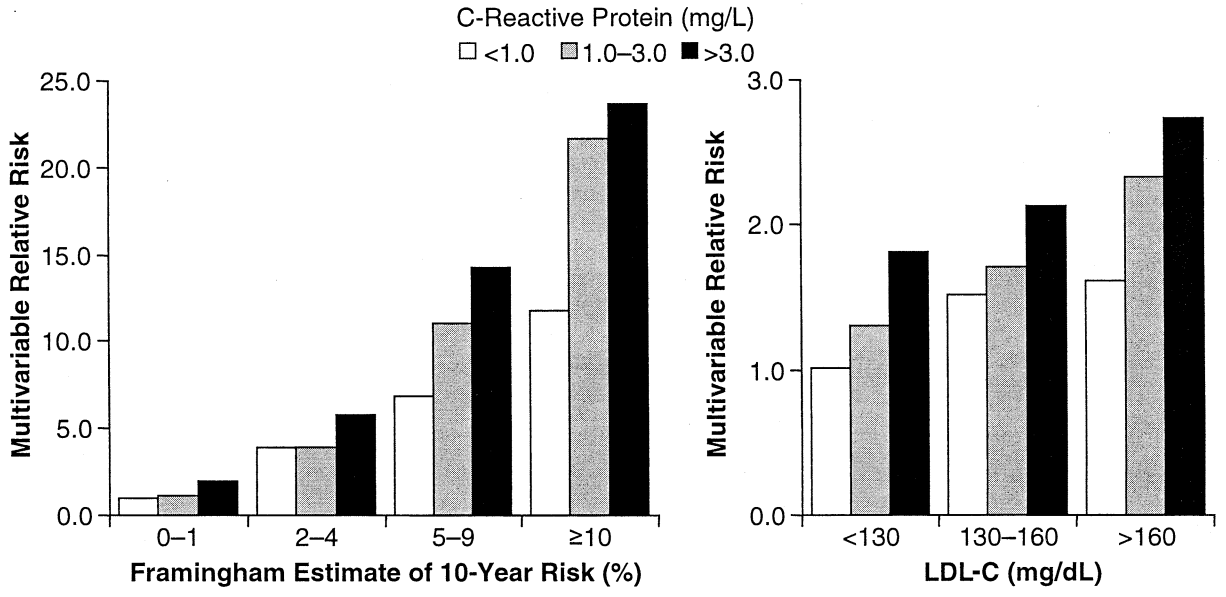

Figure 6. Multivariable-adjusted risk for cardiovascular disease by C-reactive protein cut points according to Adult Treatment Panel III risk score and low-density lipoprotein cholesterol (LDL-C) category in the Women's Health Study population. (Reprinted with permission from *N Engl J Med*.[9])

spective data on its predictive utility are inconsistent, as are prospective data on utility of the lipoprotein(a).[4] The predictive abilities of both plasma hs-CRP and fibrinogen levels add to that of plasma lipid measures (e.g., total cholesterol–HDL-C ratio); however, it remains unclear whether risk assessment can be improved by the addition of assays for lipoprotein(a), homocysteine, or such fibrinolytic markers as tissue plasminogen activator and plasminogen activator inhibitor–1. Indeed, performance assessment of markers for cardiovascular risk prediction in the Women's Health Study[12] showed that an elevated plasma hs-CRP value was the single best marker and, when combined with the plasma total cholesterol–HDL-C ratio, provided an even more potent prediction of prospective risk (**Figure 8**[7]).

Accumulating data on the ability of plasma hs-CRP levels to predict cardiovascular risk, add prognostic information to measurement of plasma lipid levels, and discriminate levels of risk within current risk categories prompted a joint scientific statement from the American Heart Association (AHA) and the Centers for Disease Control and Prevention (CDC).[13] Although this state-

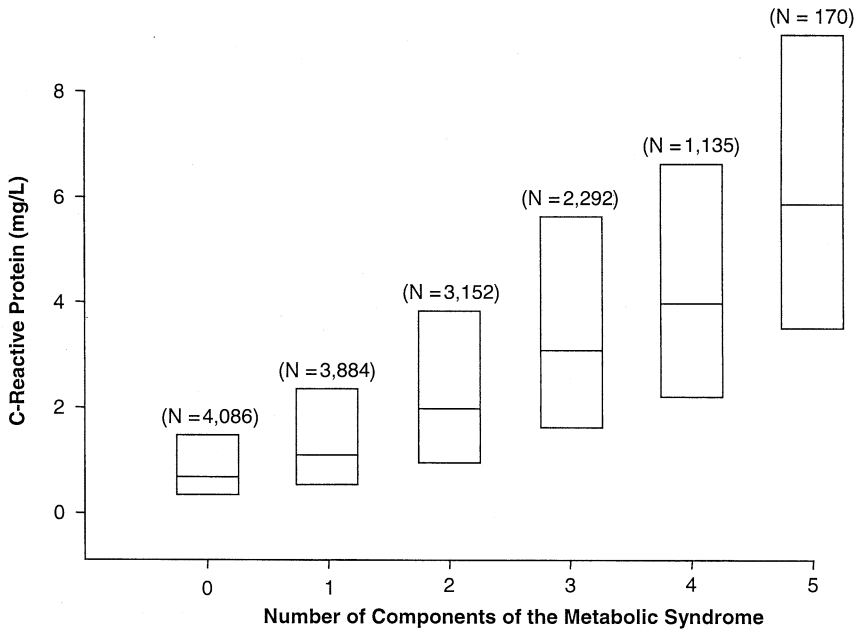

Figure 7. C-reactive protein (CRP) plasma levels according to number of components of the metabolic syndrome present in subjects in the Women's Health Study. The box plots show median and 25th and 75th percentile values for hs-CRP. (Reprinted with permission from *Circulation*.[10])

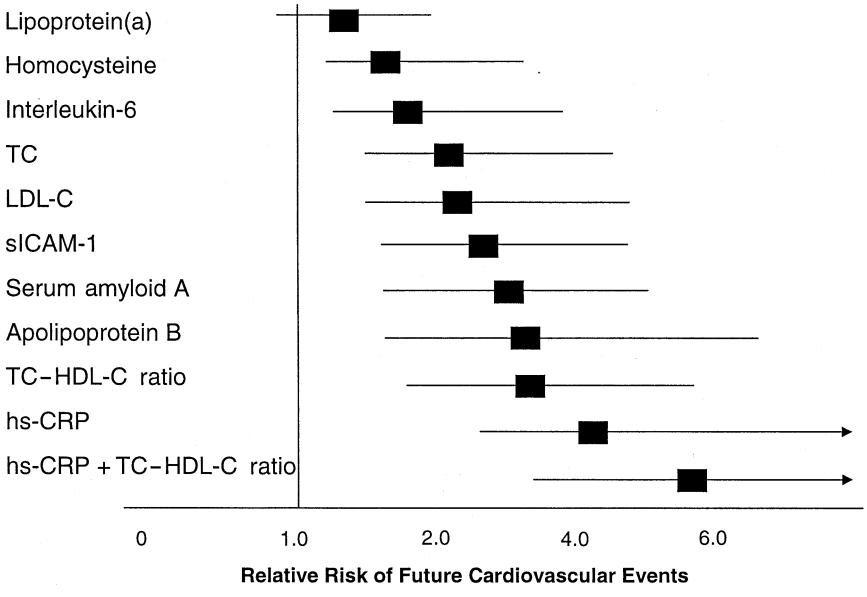

Figure 8. Direct comparison of C-reactive protein (CRP) to several other lipid and nonlipid risk factors for cardiovascular disease. HDL-C = high-density lipoprotein cholesterol; hsCRP = high sensitivity C-reactive protein; LDL-C = low-density lipoprotein cholesterol; sICAM-1 = soluble intercellular adhesion molecule–1; TC = total cholesterol. (Reprinted with permission from *Circulation*.[7])

ment recommends against plasma hs-CRP screening of the entire adult population, it concludes that measurement of plasma hs-CRP is reasonable for assessing absolute risk for coronary disease primary prevention, particularly in intermediate-risk individuals. The currently recommended plasma hs-CRP cut points are <1.0 mg/L for low risk, 1.0 to 3.0 mg/L for average risk, and >3.0 mg/L for high risk. The AHA/CDC position paper suggests averaging 2 fasting or nonfasting plasma hs-CRP measurements taken 2 weeks apart from metabolically

Table 1. Acute Coronary Event Rates and Number of Patients Needed to Treat to Prevent 1 Event According to Baseline Cholesterol Ratio and High-Sensitivity C-Reactive Protein (hs-CRP) Plasma Value in the Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS)

Relation to Median Value at Baseline*	Event Rate†		Number Needed to Treat†
	Statin Group	Placebo Group	
↑ TC–HDL-C ratio/ ↑ hs-CRP	0.041	0.057	62
↑ TC–HDL-C ratio/ ↓ hs-CRP	0.021	0.050	35
↓ TC–HDL-C ratio/ ↓ hs-CRP	0.024	0.025	983
↓ TC–HDL-C ratio/ ↑ hs-CRP	0.025	0.050	42

HDL-C = high-density lipoprotein cholesterol; statin = 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor; TC = total cholesterol.

* ↑ signifies above and ↓ signifies below the baseline median value of 5.9 for TC–HDL-C ratio or 1.6 mg/L for hs-CRP.

† The rates of events and the numbers needed to treat to prevent 1 event were calculated on the basis of 5 patient-years at risk.

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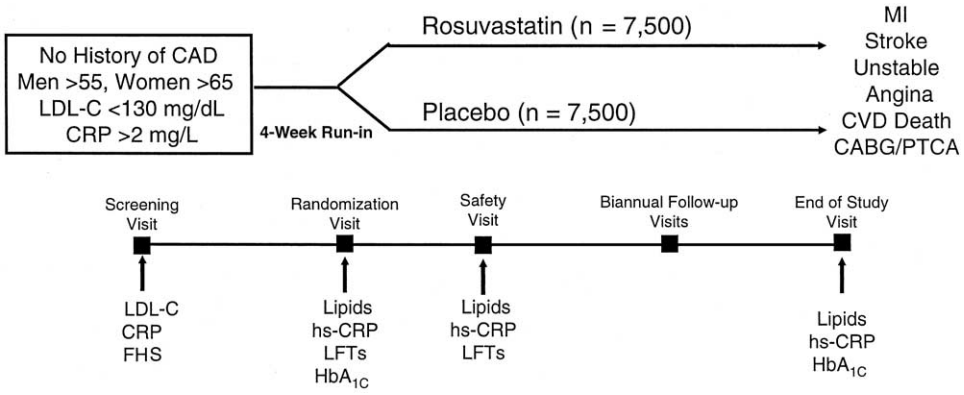

Figure 9. Design of the Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER) that assesses lipid-lowering therapy in patients who are at low risk and have higher plasma levels of C-reactive protein (CRP). CABG/PTCA = coronary artery bypass grafting/percutaneous transluminal coronary angioplasty; CAD = coronary artery disease; CVD = cardiovascular disease; FHS = familial hypercholesterolemia syndrome; HbA_{1c} = glycated hemoglobin; hs-CRP = high-sensitivity CRP; LDL-C = low-density lipoprotein cholesterol; LFTs = liver function tests; MI = myocardial infarction. (Reprinted with permission from *Circulation.*[17])

stable patients. An averaged level of >10.0 mg/L indicates the need for repeat testing and consideration of an ongoing infectious or inflammatory disease. New data indicates, however, that these chronically high levels also represent quite high vascular risk, and are not false-positive results.

Future investigations will determine whether plasma hs-CRP measurement can identify individuals who are apparently at low risk and may benefit from lipid-lowering therapy. Retrospective evidence supports this hypothesis. The Air Force/Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS) included men and women without CHD who had average total and LDL-C plasma levels and below-average HDL-C plasma levels (patients with uncontrolled hypertension, insulin-dependent diabetes, and body mass ≥50% of desirable value were excluded). Treatment with lovastatin (a 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor [statin]) in AFCAPS/TexCAPS showed a risk reduction of 37% for first acute coronary event over 5 years. An outcomes analysis was performed with data from the participants (aged 35 to 62 years) stratified by high or low (above or below median baseline) plasma total cholesterol–HDL-C ratio and plasma hs-CRP values. Compared with results of the placebo arm, the statin arm demonstrated marked event reduction in the high ratio/high CRP, high ratio/low CRP, and low ratio/high CRP groups. In contrast, statin therapy had little effect on the rate of events in individuals with low ratio/low CRP values. Similar results occurred in subjects stratified by plasma LDL-C and hs-CRP levels above and below baseline median values (**Table 1**).[14] These findings suggest

that plasma CRP assay can indeed identify individuals at seemingly low risk who will nonetheless benefit from statin treatment.

Although such data suggest that elevated CRP plasma levels define risk that warrants therapy among individuals who do not meet current criteria, definitive prospective evidence for a broader application in event reduction remains undetermined. A large-scale, randomized clinical trial—Justification for the Use of Statins in Primary Prevention: an Intervention Trial Evaluating Rosuvastatin (JUPITER)—will evaluate the effects of statin therapy in subjects who have both plasma LDL-C levels below those currently used to target therapy and plasma CRP levels that indicate heightened risk of a CHD event. This trial is randomizing 15,000 men (>55 years) and women (>65 years) with no history of coronary disease, plasma LDL-C levels of <130 mg/dL, and plasma hs-CRP levels of >2.0 mg/dL to placebo or rosuvastatin, a new high-potency statin.[15,16] Participants will be followed up for a composite end point of MI, stroke, unstable angina, cardiovascular death, or coronary intervention (coronary artery bypass surgery or percutaneous transluminal coronary angioplasty) (**Figure 9**).[17] The results of JUPITER should provide important information regarding the use of plasma CRP values to guide initiation of lipid-lowering therapy in a primary prevention population deemed to be at low cardiovascular risk by means of current criteria. This trial heralds a new era—the clinical application of the biology of inflammation in atherosclerosis—in the progress of combating atherosclerotic cardiovascular disease.

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