

# Emerging Noninvasive Biochemical Measures to Predict Cardiovascular Risk

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**N**ew predictors of cardiovascular events are needed to improve the accuracy of risk stratification. Such predictors should be easily measurable in the population and potentially modifiable. This review reports on new biomarkers that are closely linked to the pathogenic mechanisms underlying the progression of the atherosclerotic plaque leading to rupture and thrombosis that ultimately precipitate acute clinical events, such as stroke and myocardial infarction. These risk factors have been associated with subclinical or clinical cardiovascular disease in large populations and include markers of lipoprotein and lipid metabolism, vitamin B<sub>12</sub> metabolism, fibrinolysis, coagulation, inflammation, infection, endothelial dysfunction, the angiotensin system, and oxidative stress. For other key processes of atherosclerosis and cardiac disease, such as apoptosis or programmed cell death, there are currently no markers that can be measured noninvasively. Atherosclerosis is a multifactorial condition and possibly only a subset of factors are the main determinants of disease in a given patient. A better definition of the cardiovascular risk profile will help to better target primary and secondary prevention. Further epidemiological studies are needed to characterize the actual predictive and clinical value of these new emerging cardiovascular biomarkers.

*Arch Intern Med.* 1999;159:237-245

In the United States the age-adjusted rates of cardiovascular mortality have declined by more than 50% in the last quarter century among whites and blacks of both sexes. This achievement is in part a shared success of both improved treatment and effective primary prevention. Current disease prevention strategies focus on reducing blood lipids (particularly low-density lipoprotein [LDL]), blood pressure control, serum glucose control, weight control, increasing physical activity, and smoking cessation. As currently implemented, these strategies may have reached the limits of their effectiveness. During the past few years the decline from coronary disease death has tended to level and the death rates from stroke have slightly increased.<sup>1</sup> As important as the established risk factors are for predicting disease, only a fraction of those who have 1 or more of these risk factors will actually develop a cardiovascular event, and some

with no known risk factors will experience an event. There is, however, cause for optimism. Important advances in our understanding of the pathogenesis of disease and in the genetics that mediate the relationship between risk factors and disease have opened large new areas for epidemiological exploration. This article provides a brief overview of emerging biomarkers of atherosclerosis that are suitable for evaluation in prospective epidemiological studies. As they are evaluated, we should be better able to identify individuals at risk of clinical cardiovascular events and new targets for intervention.

The transition from subclinical cardiovascular disease to overt clinical disease is usually precipitated by acute coronary events such as unstable angina or myocardial infarction. Pathologic evidence indicates that rupture, erosion, and fissure of lipid-rich vulnerable atherosclerotic plaques are probably the most frequent underlying mechanisms that trigger the cascade of effects leading to acute

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coronary occlusion.<sup>2</sup> Current hypotheses concerning the pathogenesis of this cascade focus on endothelial injury, the oxidation of LDPs and their effects on the endothelium, the interaction of growth factors and cytokines leading to increased oxidative stress, increased free radical formation, destruction of nitric oxide, endothelial dysfunction, increased platelet aggregation, inflammation, proteolysis, impaired thrombolysis, and thrombosis. Although there are no sensitive and practical means of detecting vulnerable plaques in the coronary arteries in vivo, several serologic markers associated with each of the pathophysiological processes leading to plaque rupture and fissure, and thrombosis might help to identify those individuals with subclinical disease who will most likely experience a new coronary event. Recently identified biomarkers including markers of lipoprotein metabolism, endothelial dysfunction, fibrinolysis, and inflammation have been associated with an excess risk of subclinical or clinical cardiovascular disease, and have been linked with key atherogenic or prothrombotic mechanisms. These biomarkers may better predict clinical events either alone or in combination with traditional established cardiovascular risk factors. However, the independent relation of these new risk factors with atherosclerotic disease has not yet been conclusively proven.

### LIPID METABOLISM

Specific lipoprotein and fatty acid metabolism markers are linked with the formation of lipid-rich plaques and thrombosis. Lipoprotein(a) [Lp(a)] can promote atherosclerotic disease by increasing deposition of cholesterol into the arterial wall, enhancing oxidation of LDL cholesterol, and interfering with fibrinolysis. Lipoprotein(a) is emerging as one promising target for intervention such as niacin.<sup>3</sup> In the Framingham studies and in another large cohort Lp(a) was found to be a powerful independent predictor of coronary disease,<sup>4,6</sup> and in Atherosclerosis Risk in Communities study Lp(a) was independently

associated with cerebrovascular disease and asymptomatic atherosclerosis.<sup>7,8</sup> Genetic factors affect Lp(a) levels that vary substantially according to sex and race.<sup>9</sup> For example, the distribution of Lp(a) in white Americans and Asian Indians are more skewed to the left, while this distribution is nearly normal in African Americans.<sup>10</sup>

Cholesteryl ester transfer protein (CETP), lipoprotein lipase (LPL), and hepatic lipase are major determinants of the plasma high-density lipoprotein (HDL) cholesterol and triglyceride levels, and play an important role in the reverse cholesterol transport system. Although the relationship between the actions of these proteins and atherosclerosis is complex and not fully documented, a body of evidence suggests that polymorphisms of the CETP, LPL, and hepatic lipase genes influence the levels of these lipoproteins and may be independent predictors of atherosclerotic risk.<sup>11-15</sup> Furthermore, these polymorphisms are relatively frequent in the population and may be more suitable measures in epidemiological studies.<sup>14,16,17</sup> These genetic polymorphisms are particularly relevant because of racial differences in their distribution—for example, CETP mutations are frequent among individuals of Japanese ancestry—and their interaction with environmental factors such as diet, smoking, alcohol intake, and medication use.<sup>11-13,16-20</sup> Emerging evidence suggests that HDL cholesterol subfractions may be independent risk factors of atherosclerotic disease. In the Cardiovascular Health Study (CHS) HDL2-C and HDL3-C were decreased in individuals with prevalent cardiovascular disease, and HDL3-C was correlated with carotid atherosclerosis.<sup>21</sup> In another study,<sup>22</sup> there was a strong association of HDL2 (inverse) and CETP (direct) with carotid intimal media thickness. The strongest predictor of intimal media thickness was the CETP content of the dense HDL3 subfraction expressed as the ratio of CETP/HDL3.<sup>22</sup>

Triglyceride-rich lipoproteins such as intermediate-density lipoproteins and very low-density lipoprotein or chylomicron remnants are

emerging as predictors of atherosclerosis. They are involved in the pathogenesis of atherosclerosis either directly as atherogenic particles,<sup>23</sup> or indirectly by altering the composition of other lipoproteins such as LDL and HDL. There is a strong and consistent association of hypertriglyceridemia with dense LDL.<sup>24,25</sup> Decreased LDL particle size has in turn been associated with premature coronary artery disease,<sup>24,25</sup> and intermediate-density lipoproteins have been independently associated with the progression of carotid<sup>26</sup> and coronary atherosclerosis.<sup>27</sup> Additional relevant lipid markers include fasting and peak postprandial triglyceride and remnant lipoprotein,<sup>28,29</sup> plasma fatty acid composition (phospholipid and cholesterol ester),<sup>30</sup> total plasma apoA<sub>1</sub> and apoB, and common apo(a) (kringle type 2), apoB, apoE gene polymorphisms (E2/E4).<sup>7,31-35</sup> The recent development of a rapid method for separating very low-density lipoprotein and chylomicron remnant particles from whole plasma offers a new tool for studying the relationship of remnant particles with atherosclerotic disease.<sup>36</sup> This method isolates apoB48 and apoB100 particles that are enriched in apoE and cholesteryl ester and/or depleted in C apoproteins, characteristics of remnant particles of chylomicron and very low-density lipoprotein, respectively. In limited studies to date, elevated levels of these remnant lipoproteins have been observed in subjects with coronary artery disease and in diabetes mellitus.<sup>37</sup>

Studies of the red blood cell membrane fatty acid and lipid composition can gather important information on the antioxidant defenses.<sup>38,39</sup> Changes in red blood cell membrane fatty acid and lipid composition reflect dietary fat intake,<sup>40</sup> and are associated with modifications in sodium-lithium countertransport,<sup>41</sup> an independent predictor of onset of hypertension in normotensive individuals.<sup>42</sup>

### HOMOCYSTEINE

Homocysteine can promote vascular disease by means of direct cytotoxic effects on the endothelium, increased adhesiveness of the platelets,

and effects on clotting factors.<sup>43</sup> Elevated levels of homocysteine have been linked with increased risk of carotid stenosis,<sup>44</sup> vascular disease,<sup>45</sup> myocardial infarction in young women,<sup>46</sup> venous thromboembolism,<sup>47</sup> and mortality among patients with coronary disease.<sup>48</sup> Levels of homocysteine vary according to age, sex, race, and genotype and environmental factors such as diet and medication use.<sup>43</sup> Polymorphisms of the thermolabile methylenetetrahydrofolate reductase, the key enzymes of homocysteine metabolism, are associated with increased levels of homocysteine and excess cardiovascular risk.<sup>46,49</sup>

Fasting plasma levels of plasma homocysteine in a normal population generally range from 5 to 15  $\mu\text{mol/L}$ .<sup>50</sup> Even mild elevations ( $>10\text{-}15\ \mu\text{mol/L}$ ) are associated with increased risk of atherosclerotic vascular disease, and a homocysteine level in excess of 16  $\mu\text{mol/L}$  is associated with a 3-fold increase in risk of coronary heart disease.<sup>51</sup> Elevated plasma homocysteine may arise from both nutritional and genetic factors. Plasma levels of homocysteine are higher in individuals whose plasma levels of vitamins B<sub>12</sub>, B<sub>6</sub> (pyridoxine), B<sub>9</sub> (folate), and pyridoxal phosphate are in the bottom quartile and substantial health benefits are expected from an increase in dietary folate.<sup>51</sup> Plasma homocysteine increases with age, postmenopausal state, renal disease, and with administration of diphenylhydantoin and carbamazepine.<sup>43</sup>

### THROMBOSIS AND HEMOSTASIS

Alterations in thrombosis and hemostasis are causally linked to atherosclerotic disease and can precipitate acute events. Markers, such as increased levels of fibrinogen, fibrinogen  $\beta$  mutation,<sup>31,52</sup> plasmin- $\alpha$ -antiplasmin complex, plasminogen activator inhibitor 1 (PAI-1), tissue-type plasminogen activator antigen (TPA), and D-dimer can identify high-risk individuals.<sup>53-60</sup> Some of these markers, such as PAI-1 activity or antigen, are indicators of inhibited fibrinolysis, others, such as TPA antigen, D-dimer, and plasmin- $\alpha$ -antiplasmin complex, are in-

dicators of the activation of the fibrinolytic system. In CHS increased levels of plasmin- $\alpha$ -antiplasmin complex independently predicted myocardial infarction and coronary death.<sup>61</sup> In the Atherosclerosis Risk in Communities Study, participants with subclinical atherosclerosis had significantly increased levels of PAI-1, TPA, and D-dimer, compared with those with no atherosclerotic disease.<sup>54</sup> Increased PAI-1 is likely one mechanistic link between glucose intolerance or diabetes with increased risk of atherothrombotic events.<sup>55</sup> In prospective studies<sup>56-58</sup> PAI-1 and TPA independently predicted cardiovascular events or mortality. The PAI-1 activity or antigen seem to be better predictors of events than the known polymorphisms that regulate PAI-1 (4G/5G mutation).<sup>31,62</sup> Gene polymorphism of the TPA seems to be a better predictor of myocardial infarction than TPA activity or antigen.<sup>63</sup> Fibrinolytic markers are affected by medication use and diet.<sup>64-66</sup> While some studies<sup>57,67</sup> found that the significant associations of TPA with cardiovascular disease were independent of other risk factors, others<sup>55,60,63,68,69</sup> have found that fibrinolytic markers were correlated with insulin resistance, obesity, and serum lipids such as total cholesterol and Lp(a), suggesting that the prothrombotic effect of altered lipids and diabetes are mediated by an impairment of fibrinolysis.

Angiotensin and cellular calcium play an important role in regulating the production of PAI-1,<sup>70-72</sup> and antihypertensive agents, such as angiotensin-converting enzyme (ACE) inhibitors and calcium channel blockers, may have beneficial effects on fibrinolysis. In 1 study<sup>65</sup> 120 patients were randomized to ramipril or placebo 24 hours after an acute myocardial infarction to assess the effects on fibrinolytic markers. After 2 weeks PAI-1 antigen and activity were significantly decreased in the ramipril group compared with the placebo group by 44% and 22%, respectively. In a smaller trial<sup>73</sup> of 81 patients enrolled after an acute myocardial infarction, those randomized to enalapril had significantly lower levels of TPA antigen compared with placebo, but no differ-

ence was found in PAI-1 after 12 weeks of treatment, although baseline levels of PAI-1 and TPA were unknown and blood samples were not optimally stored. Another small study<sup>74</sup> in 12 patients with coronary heart disease, obesity, and hypertension failed to find any significant effect of lisinopril on TPA or PAI-1 antigen after 12 weeks of treatment. The use of captopril significantly reduced the levels of PAI-1 and TPA in 14 patients with postmyocardial infarction but not in placebo-treated controls.<sup>75</sup> In a trial<sup>56</sup> that compared verapamil with metoprolol in 809 patients with stable angina, 631 patients were evaluated for fibrinolytic markers. The TPA antigen and PAI-1 activity, but not the type of treatment, predicted coronary events. Compared with baseline values, the use of verapamil and metoprolol were associated with a 10% decrease and a 2% increase in TPA, respectively ( $P < .001$  for treatment difference,  $P$  was not significant for the variation vs baseline).

Other relevant hemostatic factors that predict coronary events or that are associated with atherosclerosis include increased levels of factor VII,<sup>76</sup> factor VII polymorphisms vary with race and are associated with an increased risk of myocardial infarction,<sup>77,78</sup> factor VIII, thromboplastin, von Willebrand factor,<sup>59</sup> thromboglobulin,<sup>79</sup> and markers of platelet activation, such as prothrombin fragment 1+2,<sup>80,81</sup> and in young women factor Leiden V mutation.<sup>82</sup> The frequency of factor V Leiden varies depending on geographic locations and ethnic diversity. In CHS mutations of the factor V gene that were particularly frequent among blacks, were significantly associated with intimal media thickness and prevalent cardiovascular disease.<sup>83</sup>

### INFLAMMATION AND INFECTIOUS AGENTS

Inflammation may be both important in the pathogenesis of atherothrombosis and a distal marker of an advancing disease process. Increases in the serum C-reactive protein prospectively predict coronary events.<sup>84-87</sup> The protective effect of aspirin on coronary events

may be in part mediated by the anti-inflammatory properties of the drug,<sup>84</sup> suggesting that the association between inflammation and coronary disease is likely causal. In experimental studies<sup>88</sup> increases in interleukin 6 (IL-6) and tumor necrosis factor  $\alpha$  cause coronary arteriosclerosislike changes in the smooth muscle. Increased serum levels of IL-6 and tumor necrosis factor  $\alpha$  are associated with severity of left ventricular dysfunction<sup>89</sup> and are candidates for predicting atherosclerotic disease. It is well established that IL-6 levels are increased in heart failure and in specific inflammatory diseases. Increased levels of circulating transforming growth factor  $\beta$ 1 are associated with both diabetes and coronary disease.<sup>90,91</sup> Emerging evidence is showing that in the general population IL-6 levels are correlated with several chronic conditions. In a community sample of men from the general population in the United Kingdom plasma IL-6 levels were significantly correlated with smoking, chronic pulmonary disease, high triglyceride levels, high fibrinogen, and coronary disease.<sup>92</sup> In a small sample of postmenopausal women plasma IL-6 was negatively correlated with serum estradiol and bone density.<sup>93</sup> In a large population sample of the Established Populations for Epidemiologic Studies of the Elderly, IL-6 levels were correlated with functional disability, cancer, heart disease, and hypertension.<sup>94</sup> Polymorphisms of the transforming growth factor  $\beta$ 1 and tumor necrosis factor  $\alpha$  genes have been associated with severity of atherosclerosis and hypertension or diabetes.<sup>95,96</sup> Frequent IL-6 polymorphisms modulate IL-6 levels.<sup>97</sup>

Infectious agents, such as *Chlamydia*, cytomegalovirus, *Helicobacter pylori*, and herpes simplex virus, have been associated with an excess risk of coronary disease.<sup>98-102</sup> These pathogens have been implicated in the pathogenesis of atherosclerosis by in vitro studies in which infection of smooth muscle cells with these agents (herpes simplex virus I and II) produces changes similar to those observed in the atherosclerotic plaque, by

detection of viral and bacterial antigens and genetic material in human atherosclerotic lesions (*Chlamydia*, *H pylori*, cytomegalovirus, and herpes simplex virus 2)<sup>102</sup> and by seroepidemiological studies.<sup>103</sup> The presence of seropositivity for cytomegalovirus and the titer of anticytomegalovirus IgG antibodies were powerful and independent predictors of coronary restenosis after directional atherectomy.<sup>101</sup>

The evidence of a role of *H pylori* in the pathogenesis of atherosclerosis is less clear.<sup>99,104</sup> Although 1 cross-sectional study<sup>99</sup> reported a higher prevalence of seropositivity for *H pylori* in patients with vs those without coronary heart disease (76.6% vs 45.5%, respectively), another prospective cross-sectional study<sup>104</sup> found no evidence of increased titers to *H pylori* among 342 patients admitted with myocardial infarction. In 1 cross-sectional study,<sup>105</sup> 22% of patients with coronary heart disease had high (>1:64) antibody titers against *Chlamydia pneumoniae* vs 4.7% of healthy controls. It remains debated whether these infectious agents have a causal effect on atherothrombosis or are merely an epiphenomenon of the inflammatory process.

#### ENDOTHELIAL FUNCTION

Increased levels of biochemical markers of endothelial function and cell adhesion—P-selectin, E-selectin, soluble intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and thrombomodulin—have been found in patients with atherosclerosis and dyslipidemia.<sup>106-110</sup> Increased circulating levels of E-selectin, soluble intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and thrombomodulin have been found in patients with atherosclerosis and dyslipidemia.<sup>106-110</sup> In the Atherosclerosis Risk in Communities Study, increased plasma levels of intercellular adhesion molecule 1 and E-selectin prospectively predicted coronary events.<sup>111</sup> In the Physician's Health Study, intercellular adhesion molecule 1 was significantly and independently associated with incident myocardial

infarction.<sup>112</sup> DNA polymorphisms of adhesion molecule genes have been identified in young subjects with angiographically documented coronary atherosclerosis.<sup>113,114</sup> Endothelin is an endothelial-derived vasoconstrictor peptide with mitogenic properties that has been associated with severity of coronary disease.<sup>115</sup> Apoptosis, or programmed cell death, is an important mechanism underlying the atherothrombotic process, myocardial ischemia, and heart failure, but, currently there are no noninvasive measures of apoptosis.<sup>116-118</sup>

#### ANGIOTENSIN

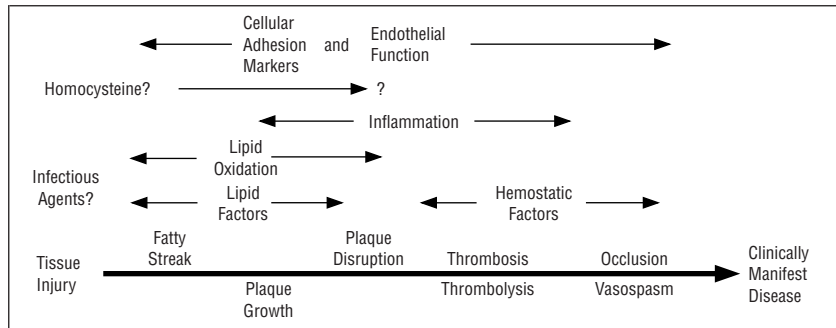
Angiotensin plays a pivotal role in vascular remodeling, and regulation of vasoconstriction and thrombolysis. The insertion and deletion polymorphisms of the ACE gene account for up to 50% of interindividual variability in ACE levels.<sup>31</sup> Although genotyping is a more reliable marker of tissue levels of ACE than ACE serum levels, the findings on the associations of ACE polymorphisms with coronary disease have been contradictory.<sup>119,120</sup> Racial differences may have accounted for the contradictory findings. For example, the I allele and the risk associated with ACE polymorphism are almost twice as high in Japanese than in whites.<sup>31,119</sup>

#### ANTIOXIDANTS

Antioxidants inhibit monocyte adhesion, protect against cytotoxic effects of oxidized LDL, inhibit platelet activation, preserve endothelium-derived nitric oxide activity, and contribute to the production of LDL that is resistant to oxidation. These effects may stabilize the plaque, maintain the vasomotor function, and inhibit platelet activation. Randomized trials and prospective observational studies<sup>121-123</sup> indicate that vitamin E and vitamin C levels predict cardiovascular events. New findings document the health effects of antioxidant agents. In the Cambridge Heart Antioxidant Study, 2002 patients with coronary heart disease were randomized to receive 400 to 800 IU of vitamin E per day or placebo and followed up for a me-

dian of 1.4 years.<sup>121</sup> In the active treatment group the combined end point of cardiovascular death or nonfatal myocardial infarction was significantly reduced (relative risk, 0.53; 95% confidence interval, 0.34-0.83). These favorable findings were not confirmed in a trial conducted in Finland in 1862 men who had a previous myocardial infarction and were smokers. The participants were randomized to 50 mg/d of vitamin E, 20 mg/d of beta carotene, both vitamins, or placebo.<sup>124</sup> There were no significant differences in the risk of coronary events between the 4 groups. Use of low doses or different sources of vitamin E (synthetic vs natural) might have accounted for the differences in results between this study and the Cambridge Heart Antioxidant Study. The benefits of vitamin E are not limited to pharmacological supplementation. In observational studies,<sup>122,125,126</sup> high dietary intake of vitamin E was associated with a significantly decreased risk of coronary heart disease.

The results of supplementation with beta carotene are less favorable. A randomized trial in 22 071 male physicians found no effect of beta carotene supplementation on incidence of cancer and cardiovascular disease after 14 years of follow-up.<sup>127</sup> In a trial involving a total of 18 314 smokers, the combination of beta carotene and vitamin A increased the risk of lung cancer (relative risk, 1.28; 95% confidence interval, 1.04-1.57) and all-cause mortality (relative risk, 1.17; 95% confidence interval, 1.03-1.33), and tended to increase the risk of death from cardiovascular disease (relative risk, 1.26; 95% confidence interval, 0.99-1.61). A recent cohort study<sup>128</sup> in Finnish men younger than 60 years has shown that vitamin C deficiency, as assessed by low plasma ascorbate concentration, is a risk factor for coronary heart disease. It is not known whether vitamin C supplementation decreases the risk. The findings from trials using antioxidants suggest that vitamin E supplementation seems to provide the greatest benefits. Among the nonantioxidant vitamins, vitamin D levels have been inversely associated with coronary calcifications.<sup>129</sup>



Proatherogenic mechanisms and progression pattern from initial artery injury through clinically manifest disease.

### Summary of the Emerging Cardiovascular Risk Biomarkers

Factors	Measures
Lipid	High-density lipoprotein cholesterol (HDL-C) subfractions (HDL2-C and HDL3-C), low-density lipoprotein (LDL) cholesterol, lipoprotein(a), apoA <sub>1</sub> and apoB, LDL particle size, intermediate density lipoproteins, plasma fatty acid composition (phospholipid and cholesterol ester), red blood cell membrane fatty acid, and lipid composition Peak postprandial triglyceride and remnant lipoprotein Gene polymorphisms: cholesteryl ester transfer protein, lipoprotein lipase, and hepatic lipase, apoA, apoB, apoE gene polymorphisms (E2/E4)
Homocysteine	Homocysteine, folate, vitamin B <sub>12</sub> metabolites Gene polymorphisms: methylenetetrahydrofolate reductase
Hemostasis	Fibrinogen, plasmin- $\alpha$ -antiplasmin complex, plasminogen activator inhibitor 1 (PAI-1), tissue-type plasminogen activator antigen, D-dimer, factor VII, factor VIII, thromboplastin, von Willebrand factor, thromboglobulin, prothrombin fragment 1+2 Gene mutations: fibrinogen $\beta$ , plasminogen activator inhibitor 1 (4G/5G) mutation, tissue-type plasminogen activator antigen, factor Leiden V, factor VII, postprandial-activated factor VII
Inflammation	C-reactive protein, interleukin 6, transforming growth factor $\beta$ 1, tumor necrosis factor $\alpha$ Gene polymorphisms: interleukin 6, tumor necrosis factor $\alpha$ , transforming growth factor $\beta$ 1
Infection	Serologic markers of <i>Chlamydia</i> , cytomegalovirus, <i>Helicobacter pylori</i> , and herpes simplex virus
Endothelial function	Endothelin, P-selectin, E-selectin, soluble intercellular adhesion molecule 1, vascular cell adhesion molecule 1 and thrombomodulin Gene polymorphisms: E-selectin
Oxidative stress	Vitamin E, vitamin C, F <sub>2</sub> -isoprostanes, 7 $\beta$ -hydroxycholesterol, thiobarbituric acid-reactive substances, oxidation susceptibility of LDL and very low-density lipoprotein, flavonoids Gene polymorphisms: nitric oxide synthase (Asp298 variant)
Vitamins	Vitamin D

### MARKERS OF OXIDATIVE STRESS

In a prospective study,<sup>130</sup> serum 7  $\beta$ -hydroxycholesterol, a major oxidation product of cholesterol in membranes, lipid hydroperoxides in LDL measured as thiobarbituric acid-reactive substances and oxidation susceptibility of LDL and very low-density lipoprotein were the strongest predictors of a 3-year increase in carotid wall thickness. F<sub>2</sub>-isoprostanes are prostaglandin isomers formed by peroxidation of arachidonic acid. The 8-epi prosta-

glandin F<sub>2 $\alpha$</sub>  and isoprostane F<sub>2 $\alpha$</sub>  are F<sub>2</sub>-isoprostanes produced in human atherosclerotic lesions.<sup>131</sup> Foam cells adjacent to the lipid necrotic core of the plaque are markedly positive for 8-epi IPF<sub>2 $\alpha$</sub> .<sup>132</sup> F<sub>2</sub>-isoprostanes circulate in plasma and are excreted in urine. Measurement of F<sub>2</sub>-isoprostanes may be a sensitive, specific, and noninvasive method for measuring oxidative stress.<sup>131</sup> Frequent nitric oxide synthase polymorphisms (10% among controls and 30% among cases) have been associated with an excess risk of coronary disease.<sup>132</sup>

## ROLE OF RACE AND SEX ON RISK FACTORS FOR ATHEROSCLEROTIC DISEASE

As these markers are evaluated it will be important to determine their predictive power in various ethnic groups and in women as well as men. There is evidence that the relative contribution of various risk factors may differ according to race and sex. Compared with white Americans, black and Native Americans tend to have a higher risk of stroke and coronary disease, Japanese have higher rates of stroke but lower rates of coronary disease, and Hispanics tend to have lower rates of both conditions.<sup>133-141</sup> Such differences in cardiovascular risk are associated with different distributions in the prevalence of established risk factors. For example, Japanese have low serum cholesterol levels, high intake of alcohol, and low intake of fat and animal proteins.<sup>133</sup> In a combined analysis from the Atherosclerosis Risk in Communities Study and CHS, for whites, there was a significantly greater impact on atherosclerosis of smoking and HDL cholesterol among older age groups but a smaller impact of diabetes. For black women, the impact of HDL cholesterol decreased among the older age strata.<sup>142</sup> In the Atherosclerosis Risk in Communities Study, black women were more obese than white women, diabetes and hypertension were more frequent among blacks than whites, and black men had higher levels of HDL cholesterol compared with white men.<sup>143</sup> Similar patterns were found in CHS.<sup>144</sup> In CHS after adjustment for known risk factors, compared with whites, blacks had thicker common carotid walls and lower ankle brachial blood pressure index ratios in both sexes, while internal carotid walls were significantly thinner in black women.<sup>144</sup> These findings in CHS suggest that the established risk factors do not explain all the ethnic and sex-related differences in cardiovascular disease rates, and that additional information on new risk factors is needed to improve the risk stratification algorithms.

## CONCLUSIONS

About 300 cardiovascular risk factors have been reported in the literature. Those described herein address diverse pathogenic mechanisms (**Figure** and **Table**), and are particularly promising for identifying the most parsimonious combination of markers to accurately predict cardiovascular events in individual age, sex, and ethnic origin groups. Although many of these risk factors have been evaluated in epidemiological studies, most of them are not at present approved for patient screening and there are no uniform reference data for normal values of these measures in the population.

It is expected that the assessment of these new biomarkers will help to identify specific mechanistic patterns that lead to cardiovascular events and will be useful for better targeting preventive interventions. For example, an individual with elevated homocysteine levels will mostly benefit from vitamin B<sub>12</sub> and folate supplementation, while a person with increased endothelin levels will benefit from pharmacological interventions that decrease endothelin or antagonize the endothelin receptor. At present, only limited prospective data are available for these new risk factors, and it is unclear which of these new risk factors may have the greatest predictive potential for cardiovascular events. New studies are ongoing or being planned to address this question.

Accepted for publication June 10, 1998.

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