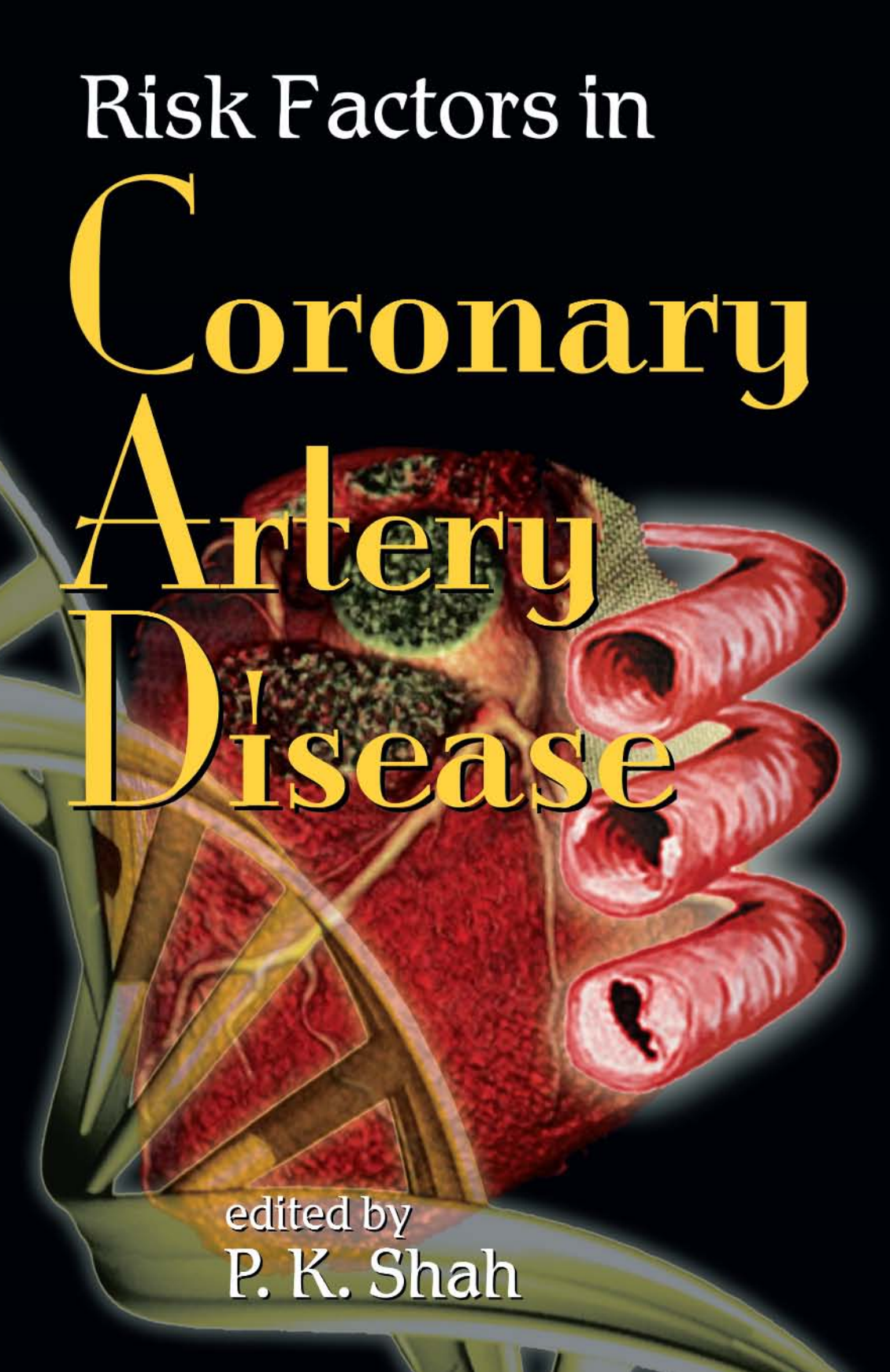
Risk Factors in Coronary Artery Disease



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Risk Factors in Coronary Artery Disease

Fundamental and Clinical Cardiology

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Risk Factors in Coronary Artery Disease

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Series Introduction

Taylor & Francis Group has developed various series of beautifully produced books in different branches of medicine. These series have facilitated the integration of rapidly advancing information for both the clinical specialist and the researcher.

My goal as Editor-in-Chief of the Fundamental and Clinical Cardiology Series is to assemble the talents of world-renowned authorities to discuss virtually every area of cardiovascular medicine. In the current monograph, Dr. Prediman K. Shah has written and edited a much-needed and timely book which addresses those risk factors for coronary artery disease that have until now received insufficient emphasis. Dr. Shah has selected several risk factors for special emphasis, including, hemostatic risk factors, chronic infections, psychosocial factors, and clinical application of genotyping to risk stratification.

Future contributions to this series will include books on molecular biology, interventional cardiology, and clinical management of such problems as coronary artery disease and ventricular arrhythmias.

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Preface

Coronary artery disease (CAD), resulting from atherosclerosis and thrombosis (atherothrombosis), is the leading cause of death and morbidity in much of the industrialized world and is rapidly achieving the same dubious distinction in developing nations as well. In the cardiovascular field, prevention of CAD and its effective treatment remain paramount objectives of clinical practice as well as targets of ongoing research. The precise etiology and mechanism(s) leading to the development of CAD remain incompletely understood although a number of risk factors have been identified over the past several decades. These include abnormal levels of circulating cholesterol with elevated levels of LDL and reduced levels of HDL cholesterol, hypertension, cigarette smoking, diabetes, male gender, post-menopausal state, advancing age, sedentary lifestyle, obesity, and a positive family history of premature vascular disease. Increasing recognition that many patients (as many as 30–50%) with established CAD lack these traditional risk factors has led to a search for additional new risk factors that may predispose individuals to CAD. Over the past several years, observational and epidemiologic studies have identified a host of new and potential risk factors for atherothrombotic vascular disease. Of this growing list of new and emerging risk factors, elevated blood levels of homocysteine, fibrinogen, inflammation and infection, atherogenic lipoprotein phenotype associated with small LDL cholesterol particles and elevated triglycerides, elevated levels of lipoprotein(a) (Lpa), insulin resistance syndrome (syndrome X or Reaven's syndrome or deadly quartet), psychosocial factors and a number of genetic polymorphisms are of particular interest. The goal of this monograph is to bring to our readers the latest update on these new and emerging risk factors that could open up new opportunities for diagnosis, risk prediction, prevention, and treatment of atherothrombotic vascular disease.

P. K. Shah

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Metabolic Syndrome and Cardiovascular Disease: Epidemiology, Pathophysiology, and Therapeutic Considerations

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INTRODUCTION

Metabolic syndrome and diabetes mellitus are as much vascular conditions as they are metabolic disorders. It is now well established that a majority (as high as 80%) of diabetic patients die of cardiovascular complications. Current evidence also suggest that, on average, diabetic patients have evidence of insulin resistance for approximately 5 to 6 years before the onset of clinical hyperglycemia. This prediabetic state can manifest itself as metabolic syndrome, which is a constellation of several cardiovascular risk factors including obesity (especially truncal), hypertension, dyslipidemia, glycemic abnormalities, and other metabolic perturbations, which are caused primarily by insulin resistance (1,2). The final products of this syndrome, affecting the cardiovascular system, are endothelial dysfunction, atherosclerosis, and cardiovascular disease (Fig. 1) (3).

Recent studies suggest that changes leading to metabolic syndrome may originate in utero and continue to progress during childhood and adolescence, reaching almost 50% in prevalence in severely obese youngsters (4–8). It is estimated that about 47 million U.S. residents have metabolic syndrome (including those with diabetes), corresponding to 22% of men and 24% of women age 20 years and above, and it rises to more than 40% in patients older than 60 years

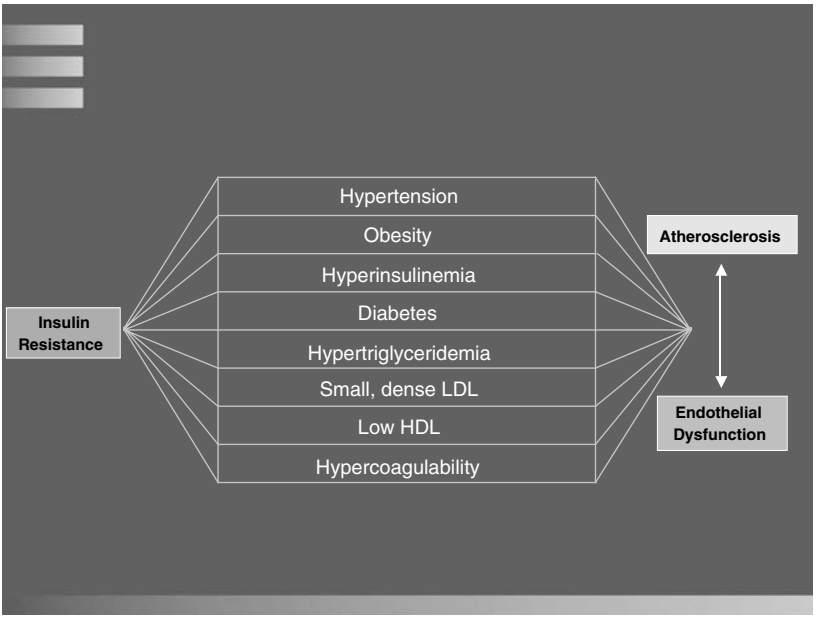

Figure 1 The role of insulin resistance as a key event in metabolic syndrome. *Source*: From Ref. 3.

of age (9). The prevalence of metabolic syndrome in the Hypertension Genetic Epidemiology Network study was found to be 34% in blacks and 39% in whites with compound phenotype with separate domains for obesity, blood pressure, and lipids (10). In this genetic study the dominant factor for metabolic syndrome was obesity and its relationship to lipids and insulin (10).

The growth in prevalence of metabolic syndrome parallels the dramatic rise in prevalence of obesity (11–14).

Individuals with metabolic syndrome are at a three-fold greater risk of coronary heart disease and stroke and more than a four-fold greater risk of cardiovascular mortality (11,15).

The contribution of metabolic syndrome to atherosclerotic disease can be best illustrated by the relationship of diabetes to atherosclerotic disease. Cardiovascular events account for approximately 80% of all diabetic mortality and cardiovascular disorders account for more than 75% of all hospitalizations for diabetic patients (16).

This chapter will review current evidence linking metabolic syndrome with cardiovascular morbidity and mortality and discuss the various available management strategies.

METABOLIC SYNDROME AND CARDIOVASCULAR DISEASE MORBIDITY AND MORTALITY

A number of studies have shown a strong association between metabolic syndrome and CVD morbidity and mortality. One of the first studies, which demonstrated the relationship between cardiovascular diseases and metabolic syndrome, was based on the large population in Italy, which included 22,256 men and 18,495 women. These individuals were participants in a series of epidemiologic investigations of cardiovascular disease conducted in Italy between 1978 and 1987. They were followed for an average of 7 years, during which time a total of 1218 deaths occurred (1003 in men and 215 in women). The risk of death from all causes and cardiovascular disease increased with increased numbers of metabolic abnormalities in both men and women. The majority of individuals who died from cardiovascular disease in this study presented with one or more of the metabolic abnormalities (high blood glucose level, high blood pressure, low HDL-cholesterol level, and high triglyceride level) (17). The Framingham Offspring Study also provided data regarding evaluation of common metabolic coronary disease risk factors in a community sample of 2406 men and 2569 women aged 18 to 74 years. After adjustment for age and weight, a 2.25-kg (5-lb) weight increase over 16 years was associated with an increased prevalence and clustering of metabolic risk factors in both men and women, and a 2.25 kg weight loss was associated with a lower prevalence of the risk factors. Clusters of three or more risk factors were associated with a 2.39 and 5.90 times greater risk of coronary heart disease in men and women, respectively (Fig. 2) (18).

The prevalence of the cardiovascular risks associated with the metabolic syndrome using the definition proposed by the World Health Organization (WHO) was evaluated in 4483 subjects aged 35–70 years participating in a large family study of type 2 diabetes in Finland and Sweden (the Botnia study). Cardiovascular

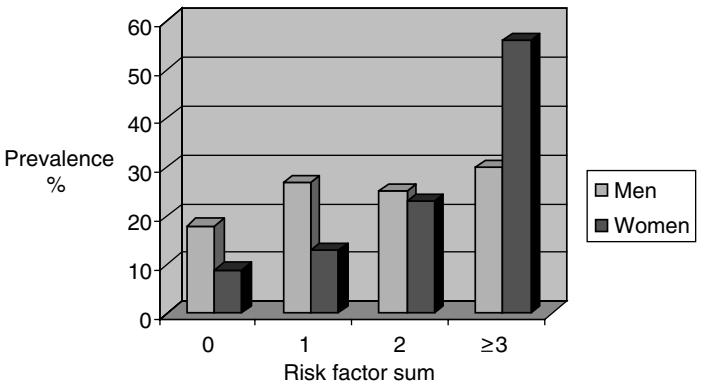

Figure 2 Age-adjusted risk factor sum in correlation with 16 year coronary heart disease risk. *Source*: From Ref. 18.

mortality was assessed in 3606 subjects with a median follow-up of 6.9 years. In women and men, respectively, the metabolic syndrome was seen in 10% and 15% of subjects with normal glucose tolerance, 42% and 64% of those with impaired glucose tolerance, and 78% and 84% of those with type 2 diabetes. The risk for coronary heart disease and stroke was increased three-fold and there was also increased cardiovascular mortality in subjects with the metabolic syndrome (12.0 vs. 2.2%). Of the individual components of the metabolic syndrome, microalbuminuria conferred the strongest risk of cardiovascular death (11). Kuopio Ischemic Heart Disease Study is a population-based, prospective cohort study of 1209 Finnish men aged 42 to 60 years at baseline (1984–1989) who were initially without cardiovascular disease, cancer, or diabetes and were followed for 11.4 years. Using both ATP III (Third Report of the Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults-Adult Treatment Panel III) and WHO definitions of metabolic syndrome, this study demonstrated that even in the absence of diabetes or prior cardiovascular disease, the presence of metabolic syndrome was associated with a significant increase in the risk of cardiovascular disease and overall mortality (Fig. 3) (15). A recently published

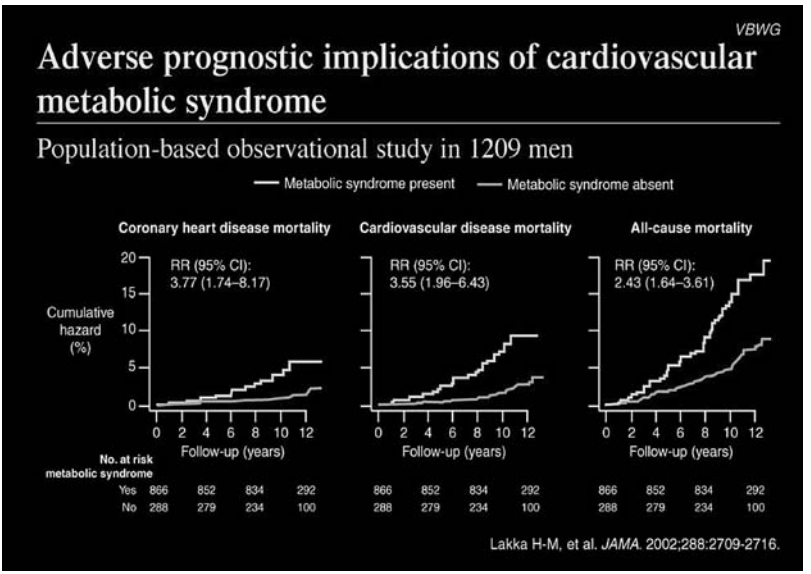

Figure 3 Kuopio Ischemic Heart Disease Study Unadjusted Kaplan-Meier Hazard Curves. Curves for men with versus without the metabolic syndrome based on factor analysis (men in the highest quarter of the distribution of the metabolic syndrome factor were considered to have the metabolic syndrome). Median follow-up (range) for survivors was 11.6 (9.1–13.7) years. Relative risks were determined by age-adjusted Cox proportional hazards regression analysis. *Abbreviations*: RR, relative risk; CI, confidence interval. *Source*: From Ref. 15.

study based on 11 prospective European cohort studies included 6156 men and 5356 women without diabetes, aged 30 to 89 years with 8.8 years follow-up. Based on modified WHO definition, the prevalence of metabolic syndrome was slightly higher in men (15.7%), than in women (14.2%). This study also demonstrated that non-diabetic patients with metabolic syndrome had an increased risk of cardiovascular mortality (19). The Strong Heart Study examined 2283 non-diabetic American Indians who were free of cardiovascular disease at the baseline examination. Based on ATP III definition, metabolic syndrome was present in 798 individuals (35%), and 181 participants (7.9%) developed cardiovascular disease over 7.6+/−1.8 years of follow-up (20).

The Third National Health and Nutrition Examination Survey (NHANES III) was used to categorize adults over 50 years of age by presence of metabolic syndrome (National Cholesterol Education Program definition) with or without diabetes. Demographic and risk factor information was determined for each group, as well as the proportion of each group meeting specific criteria for metabolic syndrome. A subset of adults $\geq$50 years of age representing 76.1 million Americans was used for this adult in-home questionnaire analysis and 3510 patients underwent physical examination. Older Americans over 50 years of age without metabolic syndrome regardless of diabetes status had the lowest cardiovascular disease prevalence (8.7% without diabetes, 7.5% with diabetes). Compared with those with metabolic syndrome, patients with diabetes without metabolic syndrome did not have an increase in prevalence of cardiovascular disease. Those with metabolic syndrome without diabetes had relatively higher cardiovascular disease prevalence (13.9%), and those with both metabolic syndrome and diabetes had the highest prevalence of cardiovascular disease (19.2%) compared to those with neither. Based on these data, metabolic syndrome was considered to be the factor associated with increased prevalence of cardiovascular disease (Fig. 4) (21). Another analysis from the NHANES III database of 10,357 patients revealed that the prevalence of metabolic syndrome was significantly higher in the group of patients with myocardial infarction, stroke, or myocardial infarction and stroke together, compared with subjects with no history of myocardial infarction or stroke (22). Metabolic syndrome was found to be a major predictor of cardiovascular risk in women in the Women's Ischemia Syndrome Evaluations (WISE) study, which evaluated 755 women who were referred for coronary angiography to evaluate for suspected myocardial ischemia (23). A cross-sectional study of 3770 women aged 60–79 years randomly selected from 23 British towns demonstrated that the prevalence of metabolic syndrome is high in older British women and is associated with cardiovascular disease. This study also demonstrated that association was similar when patients were stratified using WHO definition versus ATP III definition of metabolic syndrome (24). The strong association between metabolic syndrome and cardiovascular disease emphasizes the need for better understanding of the pathophysiological mechanisms responsible for this relationship. In the following

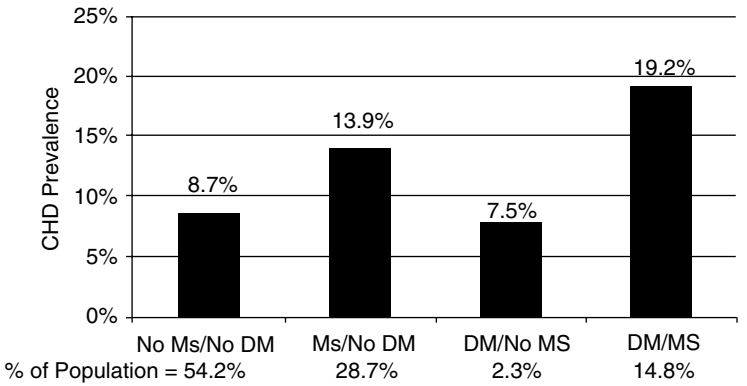

Figure 4 Age-adjusted prevalence of CHD in the U.S. population over 50 years of age categorized by presence of metabolic syndrome and diabetes. Combinations of metabolic syndrome (MS) and diabetes mellitus (DM) status are shown. *Source*: From Ref. 21.

section we will discuss the current concepts regarding the significance of each component of the metabolic syndrome.

VISCERAL OBESITY

Visceral or abdominal obesity is the form of obesity most strongly associated with metabolic syndrome and cardiovascular risk factors. It presents clinically as increased waist circumference (Table 1). However, the absolute waist circumference criteria may not be applicable for certain populations, such as South Asians and other immigrant groups. In these individuals, WHO criteria, which utilize the waist-to-hip ratio might be more suitable in identifying patients with metabolic syndrome (Table 2). Waist circumference was shown to independently predict obesity-related cardiovascular disease even in patients with normal weight (25). ATP III considered the "obesity epidemic" as mainly responsible for the rising prevalence of metabolic syndrome (26).

Increased visceral adipose tissue is considered to be a major factor responsible for many of the abnormalities associated with metabolic syndrome including insulin resistance. Adipocyte is now recognized as an important secretory organ (Fig. 5) (27). Adipocyte-secreted molecules are called "adipokines." Adiponectin is one of the adipokines, which is considered to be an important mediator of insulin sensitivity (28). Adiponectin works via activation of the adenosine monophosphate-activated protein kinase in skeletal muscle and liver, leading to phosphorylation of acetyl coenzyme A carboxylase, increased fatty acid oxidation and glucose uptake, reduced fatty acid synthesis, and reduction of molecules involved in gluconeogenesis (29–36). In the absence

Table 1 Modified Clinical Identification of Metabolic Syndrome

Risk factor	Characteristic
Waist circumference	Men > 102 cm (> 40 inches) Women > 88 cm (> 35 inches)
Triglycerides	≥ 150 mg/dL (≥ 1.69 mmol/L)
HDL-cholesterol	Men < 40 mg/dL (< 1.03 mmol/L) Women < 50 mg/dL (< 1.29 mmol/L)
Blood pressure	≥ 130/≥ 85 mm Hg
Fasting glucose	≥ 100 mg/dl (≥ 5.55 mmol/L)

The diagnosis of metabolic syndrome is made when three or more of these risk factors are present. Information from the Executive summary of the third report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).

of metabolic syndrome, these effects result in reduction in triglyceride content in the liver and skeletal muscle and suppression of hepatic glucose production and increase in high density lipoprotein levels (28,36–39).

Adiponectin may also lower C-reactive protein and other inflammatory cytokines (40). An injection of an adiponectin-producing adenovirus was shown to reverse the significantly increased adipose tissue tumor necrosis factor (TNF-α) messenger RNA and plasma TNF-α levels in adiponectin knockout mice (41). Human studies found negative correlation between adiponectin and the inflammatory markers TNF-α, interleukin 6, and C-reactive protein (42–46). Patients with coronary artery disease were found to have lower adiponectin levels compared to those without coronary artery disease (30). Another recent study found that high plasma adiponectin levels were associated with lower risk of myocardial infarction independent of hypertension, diabetes, glycohemoglobin levels, and C-reactive protein. The relationship between adiponectin level and

Table 2 The WHO Metabolic Syndrome

Risk factor	Characteristic
Central obesity	Waist-to-hip ratio (men > 0.90; women > 0.85) and/or Body mass index > 30 kg/m^2
Triglycerides	> 150 mg/dL (≥ 1.69 mmol/L)
HDL-cholesterol	Men < 35 mg/dL (< 0.9 mmol/L) Women < 39 mg/dL (< 1.0 mmol/L)
Blood pressure	≥ 140/≥ 90 mm Hg
Fasting glucose	≥ 110 mg/dl (≥ 6.1 mmol/L)
Microalbuminuria	Urinary albumin excretion rate ≥ 20 mg/min or Albumin/creatinine ratio ≥ 30 mg/g

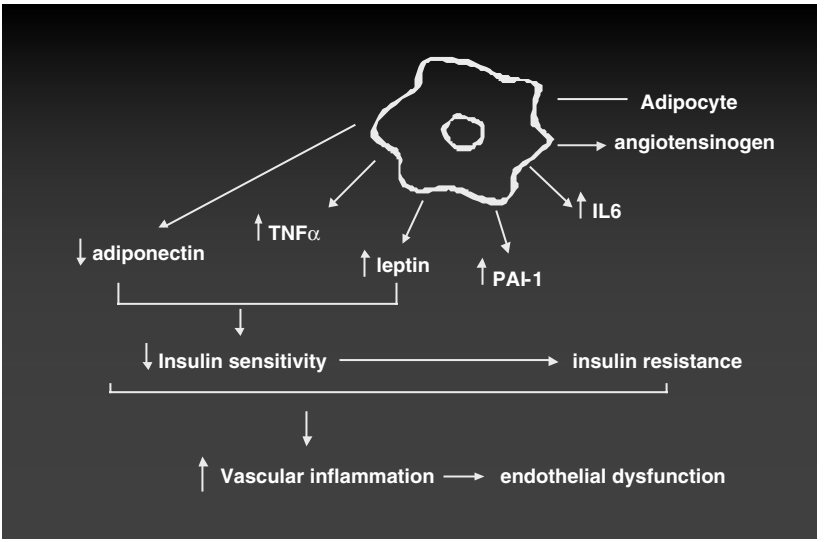

Figure 5 Adipocyte role in visceral obesity and metabolic syndrome.

myocardial infarction was only partly explained by differences in blood lipid levels (36).

Current evidence suggests that adiponectin is not only a marker of cardiovascular risk but also a significant contributor to the pathophysiological process involving atherosclerosis. Previously published animal and human data suggest that adiponectin can lower the risk of cardiovascular disease by improving insulin sensitivity and blood lipid levels (28,34,36,37–46). Adiponectin suppresses lipid accumulation and class A scavenger receptor expression in macrophages and, consequently, the transformation of macrophages to foam cells, which plays an important role in the atherogenic process (36,47,48).

It was also demonstrated that adiponectin binds to subendothelial collagens and suppresses proliferation and migration of human aortic smooth muscle cells (36,48,49).

In apolipoprotien E-deficient mice, adiponectin significantly reduced the development of atherosclerosis that usually occurs in these animals. These findings might help explain the observation in humans showing low adiponectin levels in patients with coronary artery disease (30,50–54).

Modest weight reduction of 10% can lead to significant increase in serum adiponectin levels in overweight diabetic and nondiabetic patients (30). Similar results were observed in another study evaluating obese patients undergoing gastric bypass surgery (30,35). However, the removal of subcutaneous fat by

liposuction did not significantly improve obesity-related metabolic abnormalities and could not achieve the metabolic benefits of weight loss (55). This emphasizes the pivotal role of visceral adipose tissue in the pathophysiology of insulin resistance and related cytokines abnormalities.

Adiponectin is not the only cytokine released by the adipose tissue. Adipose tissue also produces other cytokines, such as resistin, interleukin-6, TNF-α, PAI-1, and angiotensin II. These cytokines seem to have opposite effects compared to adiponectin. Therefore, the overall effects of adipokines on insulin sensitivity and other cardiovascular risk factors depend on the net balance of their production by adipose tissue (Fig. 5) (54).

Based on the above discussion, it is apparent that reduction in visceral adiposity would be beneficial. It has been shown that with a 10% decrease in total body weight there can be as much as 40% loss of visceral adipose tissue. Therapeutic lifestyle changes (TLC) are a crucial part of the obesity treatment. Current evidence suggests that the longer the behavior therapy program, the better the long-term weight loss outcome compared with standard treatment. One of the recently suggested models of TLC, which could be utilized in metabolic syndrome, describes visit intervals and goals for follow up (Fig. 6) (56). Structured meal plans, which provide adequate nutrition with portion size restriction for at least two meals a day, may improve the risk factors for metabolic syndrome and assist with healthy meal choices. Sustained dietary changes will require continued physician counseling and nutritionist support to maintain the weight loss without compromising electrolyte balance and adequate vitamin intake.

A number of studies have shown the benefit of regular physical activity in patients with metabolic syndrome (57–59). A structured exercise program is necessary for successful long-term maintenance of weight loss, although the effect of exercise alone on weight loss is not as powerful as caloric restriction. Although

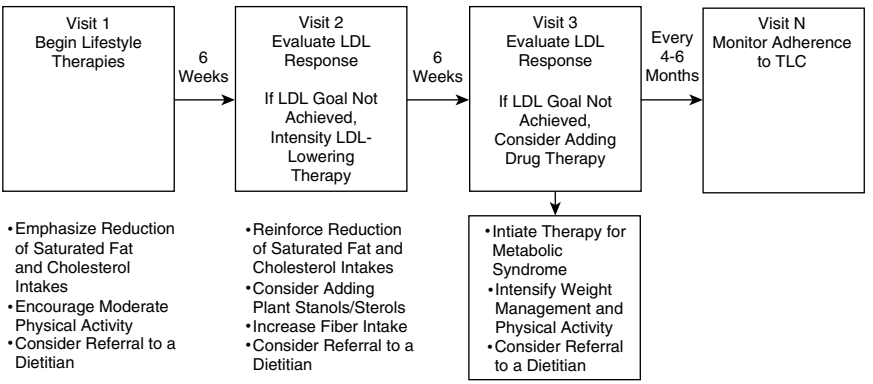

Figure 6 Steps in therapeutic lifestyle changes (TLC). *Abbreviation*: LDL, low-density lipoprotein. *Source*: From Ref. 56.

30 minutes of physical activity a day five times a week was previously considered adequate, the recent multidisciplinary expert panel, after reviewing the data and the results of epidemiologic studies, recommends for adults and children 60 minutes of daily physical activity (59). Every patient needs individual readiness evaluation by his/her primary care physician and obstacles to daily exercise should be discussed and eliminated. Patients need to be advised regarding the importance of the warm-up and cool-down periods and the incorporation of the exercise into daily life. Exercise recommendations should include setting certain goals such, as improving cardiovascular fitness, increasing strength with resistance training, and enhancing flexibility through a wide range of motion.

Medications for treatment of obesity are currently recommended for patients who have a body mass index of 27 kg/m^2 or higher with complications of obesity, or patients with a body mass index of 30 kg/m^2 without complications. The U.S. Food and Drug Administration approved two medications for long-term weight loss—sibutramine (Meredia®) and orlistat (Xenical®). Patients who were treated with sibutramine demonstrated three times more likely to achieve between 5 to 10% weight loss and also maintain their initial weight loss better 90 than placebo patients with the same diet and exercise. Orlistat is an inhibitor of pancreatic lipase, which leads to decreased fat absorption in the intestine. With this medication the weight loss of approximately 10% can be achieved. Steatorrhea is a common side effect, as well as reduction in fat-soluble vitamins (vitamins D and E), leading to the need to supplement these vitamins while on orlistat. A recently published study done in Greece confirmed in 6 months follow-up that orlistat and a hypocaloric diet had modifying effects on cardiovascular risk factors in patients with metabolic syndrome and type 2 diabetes (60).

In addition, there is now considerable interest and emerging evidence regarding the role of drugs that modify the endocannabinoid system in the treatment of obesity and metabolic syndrome. These drugs not only help in achieving sustained weight reduction, but they are also associated with favorable changes in dyslipidemia. The recent data from RIO-EUROPE and RIO-USA presented at the American Hearth Association and American College of Cardiology meetings have provided good evidence in support of the beneficial effects of rimonabant, selective CB1 endocannabinoid receptor antagonist, in the treatment of obesity. It was particularly interesting to note in these studies that there was substantially greater reduction in visceral adiposity in patients receiving rimonabant.

In morbidly obese patients who are unable to achieve appropriate weight reduction, a surgical approach can achieve the most profound and long-lasting weight loss, leading to improvement in the comorbidities related to obesity including hypertension, hyperlipidemia, and insulin resistance. Gastric bypass surgery is considered the best bariatric operation and may help to achieve permanent weight loss of more than 50% of excess body weight in the majority of patients. After bariatric surgery, patients need to be managed with the help of a nutritionist as well as physical and occupational therapists. Although frequently

used for cosmetic reasons liposuction, which predominantly removes subcutaneous fat, is not associated with significant improvement in various hormonal and cytokine abnormalities that have been linked with adverse cardiovascular consequences of obesity (55).

INSULIN RESISTANCE

The primary abnormality responsible for the most features of metabolic syndrome-related cardiovascular complications is insulin resistance. Insulin resistance can be defined as inability to respond appropriately to the various actions of insulin. Insulin resistance and metabolic syndrome are not synonymous. In the insulin resistance state, cells are deprived from glucose, which is critical for metabolic activity. The fasting plasma glucose concentration is the variable with the greatest positive predictive value for insulin resistance and hyperinsulinemia, especially when between 110 and 126 mg/dL. Despite the above, fasting plasma glucose concentration cannot be used as a sensitive indicator due to the fact that the majority of insulin resistant individuals will have glucose concentrations less than 110 mg/dL (61). Due to these limitations, a recent American Diabetic Association statement defines plasma glucose concentration higher than 100 mg/dL as the new criteria for metabolic syndrome (Table 1).

The Collaborative Analysis of Diagnostic Criteria in Europe (DECODE) study, which summarized the results of 22 large, European population-based studies, revealed that increasing fasting plasma glucose and 2-hour post-challenge glucose were associated with increasing risk of cardiovascular death. In this study insulin resistance, which was indicated by increased 2-hour post-challenge glucose, was a better predictor of cardiovascular mortality in subjects without diabetes, than elevated fasting plasma glucose (62). The importance of postprandial hyperglycemia was supported by another study, which revealed a reduction in the risk of cardiovascular disease in insulin resistant patients who were taking an α-glucosidase inhibitor (acarbose), decreasing postprandial hyperglycemia (63).

A recently published study on mice exhibiting a type 2 diabetes phenotype revealed that lipid accumulation in the liver leads to subacute hepatic "inflammation" through NF-kappaB activation and downstream cytokine production. This causes insulin resistance both locally in the liver and systemically (64). Also, IKK-beta acts locally in the liver and systemically in myeloid cells, where NF-kappaB activation induces inflammatory mediators that cause insulin resistance. These findings demonstrate the importance of liver cell IKK-beta in hepatic insulin resistance and the central role of myeloid cells in development of systemic insulin resistance. It was proposed that inhibition of IKK-beta, especially in myeloid cells, may be used to treat insulin resistance (65).

Insulin has multiple other roles besides glucose metabolism. It promotes protein and amino acid metabolism and the storage and utilization of fatty acids; therefore, in cases of insulin resistance, fatty acids and amino acids are not being

metabolized normally. Insulin also plays a role in vascular disorders, especially endothelial dysfunction. The abnormalities of fatty acid metabolism leads to accelerated atherosclerosis, increased risk of myocardial infarction, peripheral artery disease, and stroke (66).

The association between insulin resistance, hyperinsulinemia, and coronary disease is supported by a number of observational studies (67–69). Despite the fact that the strength of insulin resistance as a predictor of cardiovascular disease is substantially attenuated after adjustment for risk factors, which was reflected in review of studies examining both fasting and stimulated insulin concentrations, most recent trials revealed that it could be an independent risk factor for coronary artery disease and stroke (Fig. 7) (69–74).

Although exercise and weight loss ameliorate insulin resistance and may in some cases prevent or delay onset of the metabolic syndrome or diabetes, pharmacologic intervention that improves insulin resistance is often needed in those who fail to respond to TLC. Presently there is no single pharmacologic approach that is effective in improving all of the consequences of insulin resistance, which include hyperglycemia, dyslipidemia, abnormal coagulation and fibrinolysis, and hypertension. Thus, currently, treatment of individual

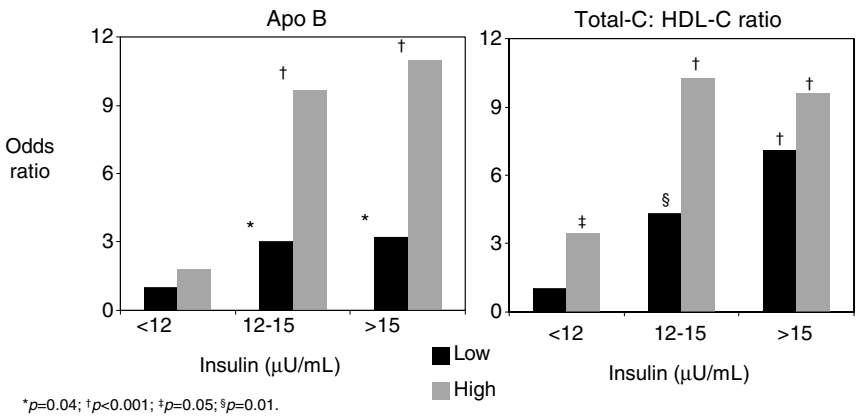

Figure 7 Odds ratios for ischemic heart disease according to plasma insulin and triglyceride concentrations, total:HDL cholesterol ratios, and apolipoprotein B Concentrations. Insulin was measured after subjects had fasted for 12 hours. The median triglyceride concentration [150 mg per deciliter (1.7 mmol per liter)], total:HDL cholesterol ratio (6.0), and apolipoprotein B concentration (119 mg per deciliter) were used to define men with either low levels (below the 50th percentile) or high levels (at or above the 50th percentile) for these variables. The results of tests for multiplicative interactions did not reach significance at the 0.05 level for any of the combinations. P values are for comparisons with the reference group, which was assigned an odds ratio of 1.0. To convert values for insulin to pi-comoles per liter, multiply by 6. *Source*: From Ref. 69.

components of metabolic syndrome is the best available approach. However, the development of drugs targeted to reverse insulin resistance is important. The insulin-sensitizing agents thiazolidinediones, which are selective ligands of the nuclear transcription factor peroxisome-proliferator-activated receptor (PPAR), are the first drugs to address the basic problem of insulin resistance in patients with metabolic syndrome and type 2 diabetes (75). The two currently available PPAR agonists, rosiglitazone and pioglitizone, consistently lower fasting and postprandial glucose levels, as well as insulin and free fatty acid levels. Thiazolidinediones also improve dyslipidemias, though no studies are available to explain the precise mechanisms (71). Although nonalcoholic fatty liver disease is not a component of the metabolic syndrome diagnostic criteria, it is frequently present in obese individuals. Thiazolidinediones can improve not only laboratory markers of the liver disease but also a histological picture in these patients (76). Weight gain is an undesirable consequence of thiazolidinediones therapy, though the underlying mechanism is not clear. For example, patients with type 2 diabetes will gain 2 to 3 kg for every percent decrease in glycosylated hemoglobin values (75). The Food and Drug Administration has also included a warning in the prescription information for rosiglitazone (Avandia®) and pioglitazone (Actos®) regarding the possibility of developing anemia and edema.

The PPARα agonists, such as fenofibrate and gemfibrozil, which have cardioprotective effect due to the lipid lowering action, and the glucose-lowering effects of thiazolidinediones have led to a search for dual PPAR agonists (compounds with the combined effects of PPARα and PPAR) (75). According to the Food, Drug, and Cosmetic Act reports of new drug applications, as many as eight dual PPAR agonists are currently under clinical development, including two in phase 3 trials (75). An additional pathophysiological mechanism related to insulin resistance is artherosclerosis leading to cardiovascular disease.

DYSLIPIDEMIA

Atherogenic dyslipedemia (Table 1) is an integral component of metabolic syndrome and is a major contributor to the cardiovascular risks in these patients. Combination of various risk factors seen in metabolic syndrome can lead to a significant increase in the risks of cardiovascular disease. For example, it was demonstrated that addition of dyslipidemia to the presence of diabetes or hypertension results in an increased risk of myocardial infarction by nineteen-fold (77). It is also important to note that an abnormal lipid profile was found to be a more significant risk factor than either hypertension or diabetes alone (77).

The typical lipid abnormalities defined in patients with metabolic syndrome consist of a triad: increased triglycerides, decreased high density lipoprotein-cholesterol, and increased small, dense low density lipoprotein-cholesterol (LDL). The role of LDL in the development of cardiovascular disease is indisputable, but it is necessary to emphasize that patients with metabolic syndrome have far more complex lipid abnormalities. The atherogenic lipid abnormalities associated with

metabolic syndrome are comparable to dyslipidemia found in patients with type 2 diabetes. In patients with metabolic syndrome, the suppression of free fatty acid release from adipose tissue is impaired, secondary to insulin resistance (78). This translates into increased influx of free fatty acids into the liver, the consequences of which are an increase in hepatic production and release of VLDL and triglycerides associated with decreased clearance of these substances, resulting in increase in VLDL and triglycerides levels. Transportation of cholesterol and triglyceride ester between HDL, LDL, and VLDL leads to formation of triglyceride-rich LDL and HDL particles, which become the preferred substrate for hepatic triglyceride lipase. Due to the lack of hepatic lipase, there is poor clearance of small, dense particles of LDL-cholesterol, which are more atherogenic and have higher susceptibility to oxidation. Elevated levels of triglyceride-rich lipoproteins lower HDL-cholesterol by inducing cholesterol exchange from HDL to VLDL via cholesteryl-ester transfer protein. A high proportion of small, dense LDL particles has been classified as a LDL subclass B, or atherogenic lipoprotein phenotype (79).

The atherogenic dyslipidemia of metabolic syndrome is similar to combined hyperlipidemia, and there appears to be an overlap of these two phenotypes (80). The cardiovascular outcomes associated with atherogenic form of dyslipidemia typical in metabolic syndrome patients are much worse, compared to clinical outcomes in patients with isolated elevation of LDL-cholesterol (81). Also, patients having this triad are more likely to have other features of the metabolic syndrome, which puts them at greater risk for cardiovascular events (82).

Another lipid abnormality of interest, which may contribute to an increased risk of cardiovascular disease in patients with metabolic syndrome, is post-prandial hyperlipemia. After food digestion, plasma concentration of chylomicrones increases and these triglyceride-rich remnant particles struggle to be cleared by the liver with endogenous triglyceride-rich proteins, for example VLDL-triglyceride. Due to high levels of VLDL-triglyceride, clearance of chylomycrones is affected and leads to postprandial lipemia. It is known that postprandial hyperlipidemia is associated with endothelial dysfunction and this increase in triglyceride-rich remnant particles in the postprandial state in patients with metabolic syndrome could play a major role in development of atherosclerosis and subsequent development cardiovascular disease (83).

Despite complex pathophysiology of the lipid abnormalities in metabolic syndrome, it is crucial for clinicians to recognize and manage them effectively in an attempt to reduce increased risks of cardiovascular disease.

The current ATP guidelines recommend the use of 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors (statins) in the treatment of metabolic syndrome patients, who have concomitant increase in LDL-C. The statins have been found to have multiple indirect effects on the vasculature, besides the direct cholesterol lowering effect. These positive actions are commonly referred to as pleotropic effects, which include effects on inflammation, coagulability, and adhesion of cells to the vascular endothelium and effects on nitric oxide metabolism. Statins also can improve endothelial function, reduce vascular

inflammation, reduce oxidative stress, decrease thrombosis and platelet aggregation and adhesion of platelets and white cells to the vascular endothelium, stabilize vulnerable plaques, and promote new vessel formation (84). These properties of statins might prove to be clinically useful in reducing the risk of cardiovascular events in patients with metabolic syndrome and diabetes.

Because of the significant increased risk of cardiovasclular event in patients with diabetes and metabolic syndrome, it is important to set aggressive LDL-C goals with an effort to reduce the risk of cardiovascular events. Recent trial data suggest that coronary plaque progression is delayed and the incidence of coronary events is reduced when LDL-C levels are lowered to approximately 70 mg/dl in high-risk patients (85).

The selection of the statin should be primarily based on its effect in reducing the LDL-C. It is important to note that the currently available statins have different effects not only on LDL-C, but also on triglycerides and HDL-C (Table 3) (86,87).

Gastrointestinal upset, muscle aches, and hepatitis are among the most common adverse effects of statins. Hepatotoxicity is very rare in patients taking high doses (approximately 1%) and myotoxicity is even more uncommon (86). Some patients can develop insomnia, bad or vivid dreams, and difficulty sleeping or concentrating. For these patients statin with low penetration in central nervous system should be used for example pravastatin (86).

Combination therapy may often be necessary to combat all lipid abnormalities in metabolic syndrome (88). In the past, lipid-lowering therapy was preferred; bile acid-binding resins are now largely used as adjuncts to statin therapy, especially in patients for whom additional reduction in LDL-C by 10–20% is desired and level of triglycerides is not elevated. Currently available bile acid resins include cholestyramine, colesevelam, and colestipol. These medications are usually given in doses of 4–10 grams twice a day with meals as a suspension in juice or water. The increase in triglyceride concentrations induced by bile acid resins can be a problem, especially in patients prone to hypertriglyceridemia. Due to their mechanism of action based on binding to bile acids in the small intestine and leading to interruption of the enterohepatic circulation of bile acids and increasing the conversion of cholesterol to bile in the liver, these agents can inhibit the intestinal absorption of fat soluble vitamins, including vitamin D, warfarin, digoxin, levothyroxin, thiazide diuretics, folic acid, and statins as well. Also, up to 30% of patients will develop abdominal fullness, gas, and constipation while taking bile acid resins, which could be corrected with dose adjustment and the use of fiber or prune juice in daily diet (89).

Based on the current evidence, the European Consensus Panel recommends that the minimum target for HDL-C should be 40 mg/dL in patients with metabolic syndrome (84).

Nicotinic acid inhibits the mobilization of free fatty acids from peripheral tissue, thereby reducing hepatic synthesis of trigylycerides and secretion of VLDL-C and its conversion to LDL-C. Nicotinic acid has a unique ability to

Table 3 Mean ± SD Percent Changes in Lipids, Apolipoproteins, and Total Cholesterol/HDL Cholesterol and Apolipoprotein Ratios in Patients with and without the Metabolic Syndrome (MS) Pooled Across Doses for Each Drug

	Rosuvastatin 10, 20, 40 mg		Atorvastatin 10, 20, 40, 80 mg		Simvastatin 10, 20, 40, 80 mg		Pravastatin 10, 20, 40 mg	
	MS (n = 165)	No MS (n = 308)	MS (n = 220)	No MS (n = 414)	MS (n = 227)	No MS (n = 421)	MS (n = 186)	No MS (n = 299)
LDL cholesterol	−51 ± 15	−51 ± 13	−44 ± 13	−45 ± 14	−38 ± 13	−36 ± 15	−24 ± 12	−25 ± 12
Triglycerides	−27 ± 19	−21 ± 25	−28 ± 19	−22 ± 26	−21 ± 21	−13 ± 26	−14 ± 22	−7 ± 26
HDL cholesterol	+10 ± 12	+9 ± 11	+7 ± 12	+3 ± 11	+9 ± 10	+4 ± 10	+6 ± 11	+4 ± 10
Non-HDL cholesterol	−47 ± 13	−47 ± 13	−42 ± 12	−42 ± 14	−35 ± 12	−33 ± 14	−22 ± 10	−23 ± 12
Total cholesterol/HDL cholesterol ratio	−43 ± 12	−41 ± 11	−38 ± 11	−35 ± 12	−34 ± 11	−29 ± 12	−22 ± 11	−20 ± 11
Apolipoprotein B	−41 ± 13	−41 ± 13	−37 ± 13	−37 ± 13	−30 ± 13	−28 ± 14	−19 ± 11	−19 ± 13
Apolipoprotein A-I	+8 ± 13	+8 ± 13	+3 ± 13	+4 ± 12	+8 ± 13	+6 ± 11	+6 ± 12	+5 ± 11
Apolipoprotein ratio	−45 ± 13	−45 ± 14	−39 ± 13	−39 ± 14	−34 ± 14	−32 ± 14	−22 ± 13	−23 ± 13

Source: From Ref. 87.

decrease the triglycerides level by up to 30%. The majority of effects of nicotinic acid on triglycerides and HDL-C occur in the low doses. The administration of aspirin (325 mg 30 to 60 minutes before each dose of nicotinic acid for a few days), and taking nicotinic acid at the end of a meal and not taking it with hot liquids can minimize the flushing of the skin, which 10% of patients find intolerable (82). Starting dose is 250 to 500 mg and should be increased monthly by 500 to 1000 mg to a maximum of 3000 mg a day. Hepatitis is more frequent in patients on nicotinic acid than in those who are taking statins, especially in doses of 2000 to 3000 mg. Other side effects include conjunctivitis, nasal stuffiness, loose bowel movements or diarrhea, acanthosis nigricans, and ichthyosis (86).

The agonistic mechanism of actions of the fibric acid derivates (gemfibrozil, clofibrate, fenofibrate) on PPARα was described above in approaches to insulin resistance. The firbrates are considered the most effective triglyceride-lowering drugs, producing as much as 50% reduction. Clofibrate and fenofibrate cause fewer gastrointestinal symptoms than gemfibrozil. Clofibrate can cause erectile dysfunction. Currently fenofibrate is the preferred agent. All fibrates are renally excreted and can accumulate in the serum in patients with renal failure and lead to myositis. Ezetimibe is the first of a new class of lipid-lowering drugs known as intestinal cholesterol absorption inhibitors. It could be administered in once daily doses of 10 mg. The co-administration of ezetimibe with statins offers a well-tolerated and efficacious treatment of lower LDL-C in patients with metabolic syndrome and diabetes (89). The combination of statin and ezetimibe and statin may result in a small increase in the incidence of elevated liver enzyme levels, although cases of severe hepatotoxicity have not been demonstrated (90).

HYPERTENSION

An integral component of metabolic syndrome is a blood pressure of greater than 130/85 mm Hg (Table 1). The relationship between hypertension and metabolic syndrome is emphasized by the fact that even lean hypertensive patients can manifest insulin resistance. Patients with hypertension are several-fold more likely to develop diabetes and cardiovascular disease over a 3- to 5-year period than are normotensive persons (53,86–89). It is also evident that insulin resistance and hyperinsulinemia contribute to the increase propensity for development of hypertension (91–93). It has been postulated that the direct effect of elevated insulin on sympathetic nervous system activity can lead to elevated blood pressure (54).

Impaired insulin signaling through its phosphoinositol 3-kinase and downstream protein kinase B pathways is increasingly recognized as being important for generation of nirtric oxide and other vasodilatory factors with increased insulin-induced renal sodium retention, which contributes to hypertension (53). The generation of nitric oxide is important for insulin mediated glucose utilization and vasodilatation (94–99).

Activation of the tissue renin-angiotensin system seems also to contribute to impaired insulin use in skeletal muscle and adipose tissue and decreases vasorelaxation (54). Angiotensin II can also be produced by adipose tissue and has potent vasoconstictive potential. In the vasculature, angiotensin II results in increased production of reactive oxygen species by stimulation of the NAD(P)H oxidase enzyme, which is expressed in endothelial cells, vascular smooth muscle cells, and vascular adventitial cells (54,99–102). Increased production of reactive oxygen species in turn results in increased nitric oxide turnover by its conversion to peroxynitrite, which also blocks vasodilation (54,103). In hypertensive patients, increased local formation of anigotensin II in adipose tissue was noted and appears to be of considerable interest, given the close relationship between angiotensin II and insulin resistance (104).

There is evidence confirming that insulin resistance and resulting hyperinsulinemia relate with hypertension and coronary artery disease. Untreated hypertensive patients often have higher fasting and postprandial insulin levels than normotensive persons regardless of body mass, as well as direct correlation between plasma insulin concentrations and blood pressure levels (54,86,87).

Genetic predisposition most likely contributes to coexistence of insulin resistance and hyperinsulinemia with hypertension. Changes in glucose metabolism in normotensive offspring of hypertensive parents could support the concept of genetic predisposition for this coexistence (105,106). The fact of increased plasma insulin levels predicting elevated blood pressures in healthy children also supports this concept (107).

Based on the recommendations of the Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure, the goal for blood pressure in patients with diabetes and other high risk individuals (such as those with metabolic syndrome) should be below 120 mm Hg systolic and 80 mm Hg diastolic. Lifestyle modifications described above and dietary changes would be initial steps to control blood pressure. There is some evidence that a low sodium diet helps to maintain lower blood pressure following withdrawal of antihypertensive medications (108). It was also demonstrated that patients who consumed a diet low in saturated fat and high in carbohydrates experienced a significant reduction in blood pressure, even without weight reduction. This diet called DASH (The Dietary Approaches to Stop Hypertension) emphasizes fruits, vegetables, low-fat dairy foods, whole grains, poultry, fish, and nuts, while reducing saturated fats, red meat, sweets, and sugar-containing beverages (109).

In hypertensive patients, increased local formation of anigotensin II in adipose tissue was noted and appears to be of considerable interest, given the close relationship between angiotensin II and insulin resistance (110). Activation of the tissue renin-angiotensin system seems also to contribute to impaired insulin action in skeletal muscle cells and adipose tissue as well as endothelial dysfunction (54).

There is emerging evidence, that insulin resistance and resulting hyperinsulinemia relate to hypertension and coronary artery disease. Untreated hypertensive patients often have higher fasting and postprandial insulin levels than normotensive persons regardless of body mass; as well, a direct correlation exists between plasma insulin concentrations and blood pressure levels (54).

The beneficial effects of renin-angiotensin system blockade by angiotensin enzyme inhibitor or angiontensin receptor blocker on insulin sensitivity and the development of type 2 diabetes in several clinical trials further support a pathopysiological role of the renin-angiotensin system in metabolic syndrome and its cardiovascular complications (Fig. 5) (51,111–113). In addition, treatment with angiotensin-converting enzyme inhibitor with ramipril over 4.5 years was associated with a 25% reduction in the primary outcome of myocardial infarction, stroke, or a cardiovascular death among 3577 diabetic patients (114). It was also demonstrated that angiotensin receptor blockers have benefit in patients with diabetes, reducing morbidity and mortality (115,116).

β-Blocker therapy in patients with metabolic syndrome can also lead to reduction in cardiovascular mortality. Despite the fact that historically β-blockers were withheld from diabetic patients due to the possibility of masking hypoglycemia and increasing insulin resistance, the cardioselective β-blockers should be considered as preferred agents for treatment of hypertension in patients with ischemic heart disease who have metabolic syndrome.

The importance of blood pressure control in reduction of cardiovascular mortality and morbidity, irrespective of the class of drugs used, has been confirmed in a number of large prospective randomized clinical trials (117). Therefore it is of paramount importance that the hypertensive component of metabolic syndrome should be aggressively treated because of the increased risk of cardiovascular events.

FIBRINOLYTIC DYSFUNCTION, ENDOTHELIAL DYSFUNCTION, C-REACTIVE PROTEIN

Patients with metabolic syndrome also have multiple abnormalities, which lead to increased risk of thrombosis. Some of these abnormalities are considered to be driven by adipokines (118–120). Various associated coagulation abnormalities include increased platelet aggregation and activation and elevation of procoagulants, such as fibrinogen and von Willebrand's factor. Defects of fibrinolysis include increased production and activity of plasminogen activator inhibitor-1 (PAI-1) with low levels of tissue plasminogen activator (t-PA) (54,93). PAI-1 has been found to be elevated in patients with type 2 diabetes. The increase in PAI-1 is related to complex interaction between glucose, insulin, and angiotesnisin II and endothelial cell. Increased levels of PAI-1 have been also associated with visceral obesity and hyperinsulinemia (121). Studies of the promoter region of the PAI-1 gene have shown that hyperglycemia stimulates

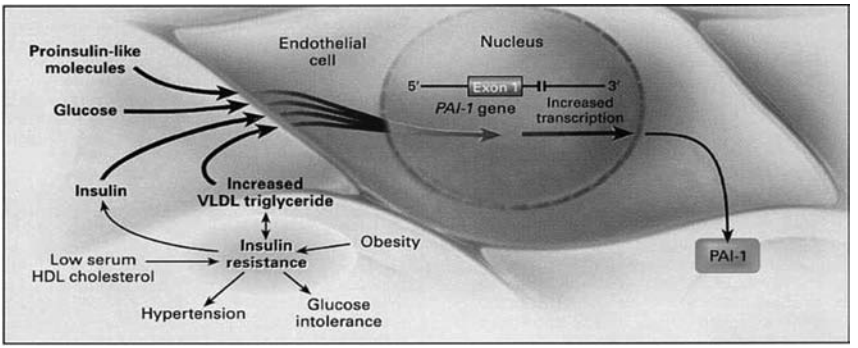

Figure 8 Relation between the synthesis of plasminogen-activator inhibitor type 1 (PAI-1) and the insulin resistance syndrome. The main feature of the insulin resistance syndrome is insulin resistance accompanied by hyperinsulinemia, abnormalities of glucose metabolism, hypertriglyceridemia with low serum high-density lipoprotein (HDL) cholesterol concentrations, hypertension, and obesity. Insulin, proinsulin-like molecules, glucose, and very-low-density lipoprotein (VLDL) triglyceride directly stimulate PAI-1 transcription and secretion in endothelial cells. *Source*: From Ref. 126.

transcription of the gene and reduction of blood glucose usually results in lower PAI-1 levels (Fig. 8) (122).

Increased levels of PAI-1 correlate with increased risk of myocardial infarction (123,124). Firbrinolytic dysfunction increases the propensity to develop arterial thrombosis, which leads to increased incidence in cardiovascular events in patients with metabolic syndrome (125). Plasma concentrations of PAI-1 are highest at night and early morning hours and it has been proposed that the higher incidence of myocardial infarction in the early morning hours could be due to higher plasma PAI-1 concentrations, leading to lower fibrinolytic activity and thrombosis at night (126).

Diabetes and abdominal obesity are risk predictors of both venous thrombosis and an occlusive arterial disease most likely due to existence of an atherothrombotic syndrome secondary to insulin resistance and defective fibrinolysis. Patients with metabolic syndrome were found to have significantly more atherosclerosis compared with patients without metabolic syndrome, independent of their diabetes status (123). These data suggest that the higher risk of cardiovascular events among patients with metabolic syndrome could be related to impaired fibrinolysis and more advanced artherosclerosis (123).

Current evidence also emphasizes that endothelial function is compromised in patients with metabolic syndrome. Endothelial dysfunction has been documented to occur in association with all of the factors that predispose to the development of atherosclerosis and is the initial step in the development of vascular pathology. For clinical evaluation, endothelial cell function can be estimated by measuring changes in blood flow in response to physical or pharmacologic stimuli using invasive or non-invasive techniques. It has been

demonstrated that increase of blood flow in the legs in response to metacholine, which is a stimulator of nitric oxide release, is impaired in nondiabetic insulin-resistant individuals (125).

In patients with insulin resistance, nitric oxide synthesis, which is partially mediated by insulin, is blunted and nitric oxide mediated vasodilation is adversely affected (104). Nitric oxide also inhibits platelet aggregation, leukocytes migration and cellular adhesion to the endothelium, and attenuates vascular smooth muscle cell proliferation and migration. Additional effects of nitric oxide include inhibition of release of cell adhesion molecules and reduced production of superoxide anions. Flow mediated dilatation of the brachial artery, which is nitric oxide dependent, was found to be impaired in metabolic syndrome patients with elevated and normal blood pressure (126,127). It was also found that plasma adhesion molecules are increased in proportion to the degree of insulin resistance in healthy volunteers, which could be related to the decreased nitric oxide production associated with insulin resistance (128).

The degree of endothelial dysfunction was found to be greater in patients with type 2 diabetes compared to type 1 diabetes, suggesting, that besides hyperglycemia, and hyperinsulinemia, the associated dyslipidemia might play an important role (129).

Hyperglycemia was shown to be responsible for endothelial dysfunction in metabolic syndrome. Acute or chronic hyperglycemia can produce impairment of endothelial-dependent vasodilatation (130).

Hyperglycemia-induced activation of protein kinase C (PKC) via increases in the synthesis of diacylglycerol (DAG), followed by activation of phospholipase A2, results in increased production of arachidonic acid metabolites, which have potent oxidizing effects. Reduced nitric oxide synthesis can result from activation of the polyol pathway, which increases the utilization of nicotinamidadenine dinucleotide phosphate (NADPH), an important cofactor in the biosynthesis of nitric oxide (131). Depletion of NADPH, which is essential for the regeneration of antioxidant molecules (such as glutathione, tocopherol, and ascorbate) and cofactor of endothelial nitric oxide synthase (eNOS), leads to depletion of nitric oxide.

Hyperglycemia is also associated with a variety of other molecular changes, including production of advanced glycation end products (AGE), which can increase susceptibility of LDL-cholesterol to oxidation as well as activate the receptors responsible for the release of interleukin-1, tumor-necrosis factor-α and growth factors that can stimulate the migration and proliferation of smooth muscle cells (132).

Endothelial dysfunction plays a role in the development of coronary heart disease and is associated with vascular inflammation and thrombosis, especially in situations with elevated PAI-1 and tPA, which is typical for patients with metabolic syndrome.

The role of inflammation in the process of atherosclerosis is well now established.

C-Reactive protein is an acute-phase reactant, which is clinically used as a marker of inflammation in the body. Mild chronic elevations of CRP concentrations, even when within normal limits, are independently predictive of future cardiovascular events (133,134).

CRP also correlates with every parameter of the metabolic syndrome, including adiposity, hyperinsulinemia, insulin resistance, hypertriglyceridemia, and low HDL cholesterol (119,121).

It was demonstrated that high levels of CRP are related to increased accumulation of visceral and subcutaneous fat deposits measured by computed tomography scan (135).

In postmenopausal women and elderly patients, CRP can be used as a predictor of development of diabetes and accordingly as a predictor of premature coronary artery disease (118,135–138).

It also has been proposed that metabolic syndrome and diabetes mellitus are inflammatory conditions. For example, the risk of developing diabetes and metabolic syndrome in relation to baseline CRP levels in 515 men and 729 women from the Mexico City Diabetes Study revealed that CRP was not a significant predictor of the development of the metabolic syndrome in men, but inflammation was important in pathogenesis of diabetes and metabolic disorders in women, which explains the correlation between CRP and cardiovascular disease (139). West of Scotland Coronary Prevention Study based on the evaluation of 6447 participants concluded that high concentrations of CRP among men with metabolic syndrome can be an independent predictor of both coronary heart disease and diabetes (140). CRP was found to add prognostic information on cardiovascular events when 14,719 initially healthy American women were followed for 8 years in the Women's Health Study (120).

These observations again confirm the role of inflammation in the processes critical to the development of atherothrombosis leading to cardiovascular events.

Furthermore, recent data from the Atherosclerosis Risk in Communities Study (ARIC) also showed that the levels of CRP increase with increasing number of the component risk factors for metabolic syndrome, suggesting that the vascular inflammation in patients with metabolic syndrome is a complex process affected by the number of established risk factors (139).

Based on the current understanding of pathophysiological mechanisms of metabolic syndrome, management of obesity, insulin resistance, and dyslipidemia as described above is crucial in modifying additional cardiovascular risk factors. Although evidence of the benefit of aspirin therapy in primary prevention is not as strong compared to secondary preventions and only limited data is available on protective benefits in individuals with insulin resistance syndrome without diabetes or previously established coronary disease, treatment with aspirin should be strongly considered in patients diagnosed with metabolic syndrome. American Heart Association recommends use of aspirin as prophylaxis in most patients with metabolic syndrome whose 10-year risk for coronary heart disease is $\geq 10\%$ as determined by the global Framingham risk

score (140). The decision to recommend aspirin prophylaxis should rely on individual clinical judgment, taking into account the patient's cardiovascular risk profile, the demonstrated benefits of aspirin on reducing risk of first myocardial infraction, and the side effects that can occur. Clopidogrel is an effective alternative in the approximately 5% of the patients who cannot tolerate aspirin. In addition to antipaltelet activity, aspirin may have other beneficial effects in patients with metabolic syndrome. C-reactive protein can be an independent predictor of both coronary heart disease and diabetes and add prognostic information on cardiovascular events in metabolic syndrome patients (141). It was demonstrated that proinflammatory cytokines and C-reactive protein were significantly reduced after only 6 weeks of aspirin therapy (142).

MICROALBUMINURIA

Vasculature and renal glomerulus have a lot in common structurally and functionally. They are derived from the same progenitor cell line. Vascular smooth muscle cells and mesangial cells produce growth factors (angiotensin II, insulin-like growth facor-1, and cytokines), as well as prostaglandins and nitric oxide to counterbalance many of the effects of growth factors. Microalbuminuria occurs due to pathophysiological changes of glomerulosclerosis, which are very similar to those of atherosclerosis, and include mesangial cell proliferation and hypertrophy, foam cell accumulation, build-up of extracellular matrix, and amorphous materials and evolving sclerosis. All of these would lead to matrix expansion, basement membrane abnormalities, and loss of basement membrane permoselectivity resulting in proteinuria. As a parallel process glomerulosclerosis and atherosclerosis associated with enhanced oxidative stress increase inflammation, impair fibrinolysis and endothelial cell dysfunction, and elevate systolic blood pressure and cause lipid abnormalities (54).

The association between microalbuminuria and cardiovascular risk has been extensively studied in diabetics and non-diabetic hypertensive patients. For example, the large study of 11,343 non-diabetic hypertensive patients with mean age 57 years old from a general population sample in Germany, revealed that 51% were men and mean duration of hypertension was 69 months. Twenty-five percent had coronary artery disease, 17% had left ventricular hypertrophy, 5% had had a stroke, and 6% had peripheral vascular disease. Microalbuminuria was present in 32% of men and 28% of women. When patients, with microalbuminuria were compared with normoalbuminuric patients, the increase in coronary artery disease, left ventricular hypertrophy, stroke, and peripheral vascular disease was demonstrated (143). Further, in patients with coronary artery disease, left ventricular hypertrophy, stroke, and peripheral vascular disease, microalbuminuria was significantly greater than in patients who did not have these complications. It was also shown in this study that microalbuminuria increased with age, severity, and duration of hypertension and hyperlipidemia, and it was associated with higher plasma creatinine values (143).

It is well known that microalbuminuria frequently occurs in patients with diabetes and is a marker of early stage nephropathy. It has been shown to be a strong predictor of cardiovascular events. A systematic review of the literature was done to evaluate the role of this phenomenon and included 264 citations, in which 11 cohort studies were selected for inclusion in the overview, and a total of 2138 non-insulin dependent diabetic patients with mean of 6.4 years of follow-up. This study confirmed that microalbuminuria is a strong predictor of total and cardiovascular mortality and cardiovascular morbidity in patients with non-insulin dependent diabetes mellitus, with the overall odds ratio for all cause mortality being 2.4, and for cardiovascular morbidity or mortality being 2.0 (Fig. 9) (144).

WHO definition of metabolic syndrome has included microalbuminuria as one of the criteria (Table 2). Microalbuminuria in association with obesity can also occur due to the fact that high intake of food rich in protein, may lead to renal hyperfiltration, renal impairment, and finally to microalbuminuria, which also correlates with body mass index, waist-to-hip ratio, and insulin levels, and becomes another cardiovascular risk factor in metabolic syndrome patients (144).

Accelerated atherosclerosis and endothelial dysfunction can be recognized earlier if proteinuria is used as a marker. It was noticed that patients with proteinuria have greater left ventricular mass, greater carotid medial thickening, and endothelial dysfunction, which leads to greater risk of myocardial infarction and mortality (Fig. 9) (145,146).

ABNORMAL URIC ACID METABOLISM

Raised uric acid can be seen in patients who have metabolic syndrome. The major component of metabolic syndrome is insulin resistance, which influences protein metabolism and uric acid as protein metabolism becomes elevated. In patients

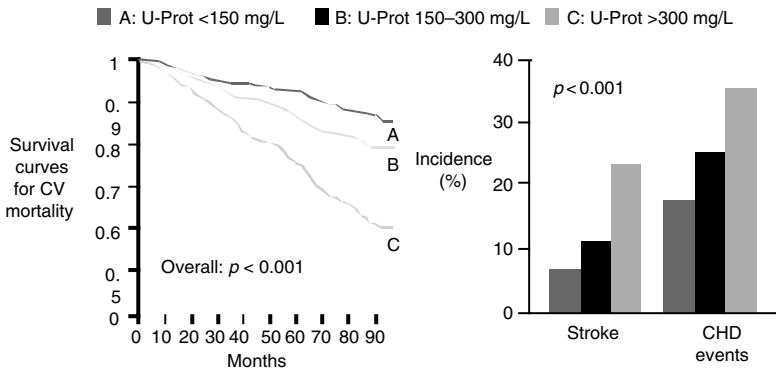

Figure 9 Proteinuria predicts stroke and coronary heart disease in type 2 diabetes patients. *Abbreviation*: U-Prot, urinary protein concentration. *Source*: Ref. 148.

with metabolic syndrome the excretion of uric acid via kidneys is also impaired. The precise role of hyperuricemia in coronary artery disease is controversial. The Framingham Heart Study demonstrated that uric acid does not have a causal role in the development of coronary artery disease or death from cardiovascular disease (147). Contrary to the Framingham data, a cross-sectional population-based study in the First National Health and Nutrition Examination Survey (NHANES I) from 1971–1975 (baseline) and the data from NHANES I Epidemiologic Follow-up Study (NHEFS) suggest that increased serum uric acid levels are independently and significantly associated with risk of cardiovascular mortality (148). In patients with essential hypertension, elevated uric acid levels were associated with increased cardiovascular disease and all causes of mortality (149). Despite the fact that no clear data exist reflecting the relationship between uric acid levels and cardiovascular risks in patients with metabolic syndrome, elevated serum uric acid levels could have a role in increasing these risks.

SUMMARY

The metabolic syndrome represents a clustering of several risk factors linked with marked increase in cardiovascular disease and can be considered a coronary artery disease equivalent. Insulin resistance has been linked to each of the ATP III criteria needed for diagnosis of metabolic syndrome (Table 1). Despite the fact that insulin resistance makes up only one of five criteria for diagnosis of metabolic sydnrome used by WHO (Table 2), the available data indicate that insulin resistance and hyperinsulinemia, even in the absence of overt abnormalities of glucose tolerance, lead to increased cardiovascular disease. Insulin resistance is considered to be a continuous process in which progressive defects in insulin action and insulin release lead to more overt abnormalities of glucose homeostasis. Insulin resistance is an independent risk factor for cardiovascular disease and its presence has been related to macrovascular complications that can occur long before the development of clinical diabetes ("the ticking clock hypothesis") (150).

Obesity is an important component of metabolic syndrome. Visceral adipose tissue has been proposed as the major site of fat deposition associated with the metabolic consequences of obesity that have been related to the cytokine release by the adipocytes (151). Currently, visceral or central adiposity is considered to be the initial physical finding associated with insulin resistance and metabolic syndrome. Increases in visceral adipose tissue lead to increases in free fatty acid flux in portal and systemic circulations, which initiates the cascade of events that are thought to be responsible for insulin resistance and atherogenic dyslipidemia. Visceral adipose tissue may also contribute to other causes of increased atherosclerotic risk, including inflammatory (C-reactive protein), prothrombotic, and fibrinolytic factors. Future assessment of adipose tissue hormonal activity might be helpful in predicting cardiovascular risks. Adiponectin is one of the hormones that is being intensively investigated in

this area. Hypertension is also most likely related to adipocytes production of cytokines, including angiotensin II leading to elevated blood pressure.

Atherogenic dyslipedemia is the central player in developing atherosclerosis, and specific features of lipid disorder in metabolic syndrome put these patients at higher risks. The characteristic lipid disorders seen in this syndrome are hypertriglyceridemia, low levels of high-density lipoprotein cholesterol (HDL-C) and, often, normal levels of low-density lipoprotein cholesterol (LDL-C), which is smaller and more dense than usual.

The evidence described in this chapter from epidemiological and observational studies highlights not only the importance of increasing awareness among clinicians regarding the strong relationship between metabolic syndrome and cardiovascular disease, but also the urgency to intervene and modify the fatal cascade of events in these patients leading to significant increase in mortality and morbidity, which will have a major public health impact worldwide in the coming years (152–154).

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2

Lipoprotein Metabolism and Implications for Atherosclerosis Risk Determination and Treatment Decisions

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INTRODUCTION

The relationship of lipids and lipoproteins to atherosclerosis is not a recent discovery. Nearly 100 years ago, Ignatowosky noted that wealthy patients who consumed large amounts of meat and dairy products appeared to have more arteriosclerosis on autopsy than poor patients who could not afford this rich type of food (1). Soon after this observation, Anitschkow and Chalatow conducted studies on rabbits that indicated that such high fat diets resulted in hypercholesterolemia and subsequent arteriosclerosis (2). With a tremendous amount of foresight, Aschoff suggested in 1924 that arteriosclerosis might be reversible (3). In the 1950s, Dr. John Gofman and colleagues at the Donner Laboratory (University of California) made another major step forward by investigating the relationship of low-density lipoproteins (LDL) to atherosclerosis in the Framingham and Lawrence Radiation Laboratory at Livermore studies (4). His contributions included the association of multiple lipoprotein subclasses defined by Svedberg floatation (Sf) intervals, assessed in the analytic ultracentrifuge. Gofman and colleagues were also well ahead of their time by reporting that high-density lipoprotein 2 (HDL2) (F3.5–9.0) was reduced 32.1% in patients who developed coronary artery disease (CAD). This raised the

possibility of a protective role of HDL and HDL subclasses. An atherogenic index, based on these lipoprotein classes, attempted to quantitate the effect of these lipoproteins on atherosclerosis. With a great degree of prescience, which predated the Adult Treatment Panel guidelines by four decades, it was predicted that if longevity is linked to lipoproteins, then in order to improve longevity, "… a drastic rather than moderate reduction in such parameters is required." Based on the work of Geer and McGill, it was even suggested that alteration in lipoprotein composition might lead to the development of atherosclerosis, even though no alteration in absolute lipoprotein levels occurred. Thus, the field of lipids, lipoproteins, and lipoprotein subclasses within the very-low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), LDL, and HDL regions, and their relationship to atherosclerosis, is at least 50 years old.

In the past 20 years investigators have extended the work of Gofman and colleagues to involve a plethora of investigations that assessed the role of lipoprotein subclasses within the entire subclass distribution and their relationship to atherosclerosis (5). These investigations bridged the gap between basic science and clinical research, and most recently have involved genetics and arteriographic trials (6–8).

Topics involving the clinical relevance of lipoprotein disorders are no longer relegated to a small group of "lipidologists." Among interventional cardiologists there is a growing emphasis on the importance of secondary prevention. Words like "secondary" and "prevention" may not engender the aggressive emotions usually associated with interventionalists, but this segment of cardiology must now embrace what happens after the stents are implanted. For more than 20 years the main concern post-intervention has been the prevention of restenosis. Now, with the advent of drug-eluting stents, this complication is becoming rare, although, it is not eliminated. Recent observations show that cardiac events in the five years after percutaneous intervention are much more likely to be due to progression of disease in untreated segments than to restenosis. This was true even before the drug-eluting stents were introduced. With the compelling evidence that effective manipulation of lipoprotein disorders is possible, the interventionalist must now include a post PCI plan that includes aggressive lipoprotein manipulation. As individualized therapies to address multiple inherited disorders are investigated and become available, there is the opportunity for interventionalists to take a central role in their development and clinical application.

BASIC LIPOPROTEIN METABOLISM

Lipoproteins are a diverse group of spherical particles that can be separated into various categories based on their density. The regions include triglyceride-rich, VLDL and IDL, and the relatively cholesterol rich LDL. High-density lipoprotein (HDL) particles may play a role in what has been termed "reverse cholesterol transport" (9). In general, the production and metabolism of lipoproteins follows a

path of large particles, rich in triglycerols and relatively poor in cholesterol, that undergoes a series of metabolic interactions, which results in more dense particles that are relatively rich in cholesterol and poor in triglycerols. The large triglyceride-rich transport particles, derived from an intestinal source, are termed chylomicrons. The somewhat smaller, triglyceride-rich particles, derived from a hepatic source, are termed VLDL. After a series of interactions with the enzyme lipoprotein lipase (LPL), the particles become more dense and relatively cholesterol rich. An IDL precedes the appearance of LDL, which is normally the greatest source of cholesterol transport among the lipoproteins. Further metabolism involves the interaction of lecithin-cholesterol acyltransferase (LCAT), apoproteins, and neutral exchange factors (10,11). VLDL particles can be produced in both large and smaller forms and through pathways that involve lipoprotein and hepatic lipase (HL), and cholesteryl ester transfer protein (CETP), develops into either large or small LDL particles (Fig. 1) (12,13).

IDL is a relatively triglyceride-rich particle intermediate between VLDL and LDL. IDL is defined as the lipoprotein mass in the Sf intervals Sf 12–20 and has long been linked to CAD risk and arteriographic progression (14). This is of clinical relevance since the most common laboratory method of determining low-density-lipoprotein cholesterol (LDL-C) involves precipitation of apo B-containing lipoprotein particles, measurement of the cholesterol content of the remaining plasma [high-density-lipoprotein cholesterol (HDL-C)] and then calculation of LDL-C with the Friedawald equation (15). When using this method, intermediate-density-lipoprotein cholesterol (IDL-C) is included in the LDL-C number. When IDL and LDL are individually determined in an ultracentrifuge, it has been shown that the natural history of CAD progression is

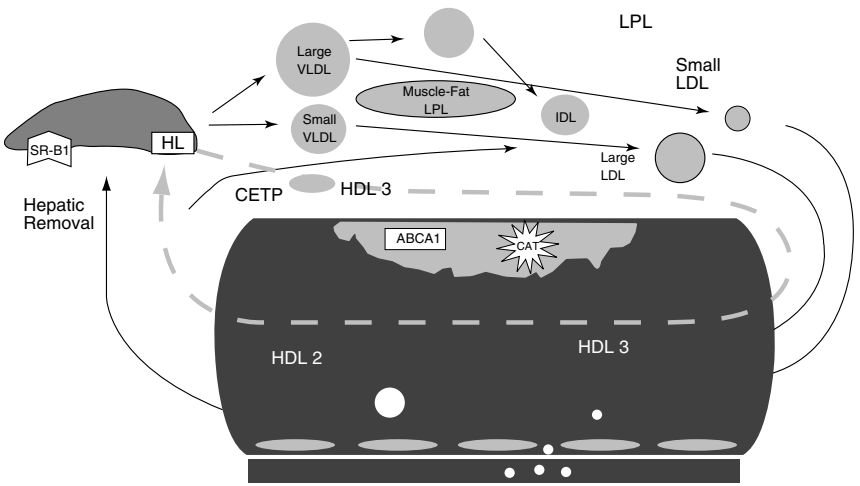

Figure 1 Metabolic pathway of lipoproteins including lipoprotein heterogeneity.

related to IDL and inversely to HDL, but not to LDL (14). It is unclear how much of the atherogeneicity, or treatment benefit, of LDL-C reduction is related to IDL and/or LDL since most clinical research studies employed a calculated LDL-C value that includes IDL. Recently, research using the analytic ultracentrifuge to separate lipoprotein classes has discovered that IDL is significantly associated with atherosclerosis progression as determined by carotid wall intimal media thickness (16).

LDL is not a homogeneous category of lipoproteins but consists of a set of discrete subspecies with distinct molecular properties, including size and density (17,18). In normal subjects, seven major LDL subspecies can be identified. Accurate and reproducible determination of LDL subspecies is made possible by two well-established laboratory methods, gradient gel electrophoresis (GGE), which separates LDL particles on the basis of their differing size, and analytic ultra-centrifugation (ANUC), which separates the particles into 12 regions on the basis of their differing density (19,20). In most healthy people, the major subspecies are large or buoyant, whereas the smaller, denser LDL subspecies are generally present in small amounts (21).

HDL is derived from both intestinal and hepatic sources. Hepatic HDL, in a nascent form, appears as a disk-shaped structure. Intestinally derived HDL is more spherical and varies in its protein composition. Both of these HDL particles are relatively small and cholesterol-poor and can be classified as HDL3. Following interaction with LCAT and LPL in both adipose and muscle tissue, cholesterol ester content is increased and the particle becomes less dense, larger, and classified as HDL2. Based on the relative density obtained in the analytic ultracentrifuge, the more dense, relatively cholesterol-poor form is termed HDL3 (1.125 to 1.21 G per mL) and the less dense, relatively cholesterol-rich form is termed HDL2 (1.062 to 1.125 G per mL) (20,22).

Central to the understanding of lipoprotein metabolism is the action of apoproteins, enzymes, transfer proteins, membrane modulators, and receptors.

Apoproteins

Apoproteins are proteins attached to a lipoprotein particle and are given alphabetical names such as apoprotein A, B, C, D, and E (23). By protruding from the surface of the lipoprotein, they can be recognized by a receptor and assist in uptake or activation of cellular mechanisms. They can also serve as cofactors for specific enzymatic reactions. Each apoprotein probably has more than one function and is in a continuous spectrum of activity. The relative amount of an apoprotein in a lipoprotein particle does not necessarily reflect the biologic importance of the apoprotein. They are identified based on specific antigenic characteristics, and specific apoproteins are associated with various lipoprotein groups. Inherited defects in the amino acid sequence of these proteins can impact normal lipoprotein metabolism by interfering with receptor binding or their actions as cofactors. Some of these apoprotein disorders are relatively common.

The apoprotein content of lipoproteins varies. For example, the relative distribution of apoproteins within a typical HDL particle is: A-I, 46%; A-II, 23%; C-I, 18%; C-II, 2%; C-III, 3%; D, 5%; E, 1% (24). The LDL particle is somewhat unique in that it is in part defined by having only one apoprotein attached, apo B-100.

Apoprotein A can be identified as several forms, including Apo A-I and A-II, and accounts for approximately 70% of the apoproteins on the HDL particle. It is principally associated with the HDL particle. However, A-I is also a constituent of chylomicrons and its synthesis in the intestine is increased after a fatty meal (25). Apo A-I and A-III, along with Apo C-I, are activators of lecithincholesterol acyltransferase (LCAT).

Separation of HDL subclasses can be made on the presence of HDLs with A-I only, or HDL particles with both A-I and A-II attached. Work in France has elucidated HDL subclasses defined as those containing apo A-I only (LpAI) and those containing both apo A-I and A-II (LpAI:II) (26). The LpAI only particle is the HDL subclass most associated with CV protection and is similar to HDL2b. HDL2b, as determined by GGE, is the subclass most associated with cardiovascular protection and has primarily apo A-I as its apoprotein constituent (27).

Apo [a] is an important apoprotein in regard to cardiovascular risk. When attached to apo B and LDL, by a disulfide link, it is termed lipoprotein (a), or Lp(a) for short. The importance of this lipoprotein lies in its very strong association with coronary heart disease and carotid atherosclerosis. Elevated levels may be present in as many as 20–40% of individuals with CAD. The gene is on chromosome 6 and inherited in a dominant fashion, which indicates that approximately 50% of first-degree relatives will express elevated Lp(a) levels (28). This finding may help to explain why some patients with relatively normal blood LDL and HDL cholesterol values still suffer from atherosclerosis (29). This apoprotein is quite large and susceptible to oxidative damage. When oxidized, it is consumed by the scavenger receptor on the macrophage significantly faster than the non-oxidized form (30).

Apo B serves as an identification protein for specific receptors located on hepatic and peripheral cells involved with lipoprotein metabolism (31). Apo B has been identified as primarily two apoproteins that are immunologically distinct. Apo B-100 is produced in the liver and attached to LDL particles. Apo B-48 is derived from the intestines and is approximately half the molecular weight of apo B-100. It is attached to triglyceride-rich particles and not to LDL particles.

Apo C, along with apo A-I, is an activator of LCAT. The hydrolysis of triglycerides by LPL is dependent on apo C-II (32). This is reflected by the substantial elevation in chylomicrons and VLDL seen in persons lacking this apoprotein (33). Apo E plays an important role in hepatic clearance of VLDL remnants and HDL recognition. Apo E can be identified as a number of different

Table 1 Recent Advances in the Understanding of the Role Enzymes, Proteins, Membrane Modulators, and Receptors Play in Atherosclerosis

Name	Function	Recent findings
LPL	TG hydrolysis	Partial defect accounts for substantial amount of plasma TG elevation when exacerbated by lifestyle or diet.
HL	TG and PL hydrolysis	Reconverts HDL2 to HDL3
EL	PL hydrolysis	May be pro or anti atherogenic
LCAT	Esterifies cholesterol	Apo AI is cofactor
ACAT	FC → CE	ACAT1 and ACAT2 discovered
CETP	TG exchange for cholesterol Between VLDL/LDL and HDL	CETP inhibitor in clinical trial
PLTP	Transfers PL from VLDL to HDL	
Paroxonase	Antioxidant	Located to apoAl/HDL2 particles
ABCA1	Transmembrane lipid transport	Genetic disorders discovered
SR-B1	CE uptake from HDL and LDL	May be pro or anti atherogenic

Abbreviations: TG, triglycerides; PL, phospholipids; FC, free cholesterol; CE, cholesterol ester.

isoforms or genotypes that are distinguished on the basis of amino acid or DNA differences (34).

Five major enzymes play a role in basic lipid metabolism: LPL, HL, endothelial lipase (EL), LCAT, and acyl-CoA:cholesterol acyltransferase (ACAT) (Table 1). LPL is a lipolytic enzyme located on the surface of vascular endothelial cells and macrophages (35). It is responsible for Triglycerides hydrolysis. Hepatic lipase (HL) is an enzyme synthesized by hepatocytes and binds to endothelial cells, allowing it to interact with lipoproteins as they traverse the liver (36). Endothelial lipase (EL) is a lipolytic enzyme that uses phospholipids as the substrate (37). LCAT is responsible for the esterification of cholesterol molecules in HDL (38). ACAT serves to convert free cholesterol to esterified cholesterol intracellularly.

Lipoprotein Lipase

LPL is a lipolytic enzyme located on the surface of vascular endothelial cells and on macrophages (39,35). It is responsible for TG hydrolysis and is the rate-limiting step for the uptake of lipoprotein TG and resultant fatty acids into adipose tissue and muscle. Deficiency in LPL activity is often associated with substantial increases in plasma triglycerides and low HDL-C. The relative degree of LPL dysfunction can result in a wide range of triglyceride values that are affected by environmental issues such as diet, body fat, and exercise levels. While mild to moderate elevations in plasma triglycerides is often the result of a polygenic environmental interaction, dramatic elevations in fasting triglycerides, usually greater than 1000 mg/dL, are often associated with inherited defects in triglyceride

metabolism. Normal LPL function is essential for normal triglyceride hydrolysis, and apolipoprotein C-II is a cofactor for LPL action. Apo C-II deficiency results in elevated triglycerides due to reduced LPL activity. LPL deficiency is the most common cause of familial chylomcronemia and is an autosomal recessive trait often presenting in childhood with severely elevated plasma triglycerides, pancreatitis, and abdominal pain, along with eruptive xanthomas and lipemia retinales (40). It occurs in approximately 1:5000 individuals, and the heterozygote state is more common than familial heterozygote hypercholesterolemia and in some populations can be found in 1 in 40 individuals (41). Over 26 mutations in the LPL gene have been identified that can result in a spectrum between mild to complete LPL activity deficiency (42). A specific LPL mutation (Asn291Ser) has been identified in 5% of male CAD patients that results in low HDL-C and may contribute to CAD risk (43).

Hepatic Lipase

HL is an enzyme synthesized by hepatocytes and binds to endothelial cells, allowing it to interact with lipoproteins as they traverse the liver (36). Its major function is to hydrolyze triglycerides and phospholipids in lipoprotein particles. In conjunction with CETP activity, HL is believed to reduce the core of large HDL2 particles and play a role in the conversion of HLD2 to HDL3 (44). Apo A-II may assist in HL activation (45). HL may play a pivotal role in the production of small, dense LDL (46).

Endothelial Lipase

EL is a lipolytic enzyme that exclusively uses phospholipids as the substrate (37). The action of EL releases free fatty acids and creates a small HDL particle. The proatherogenic or antiatherogenic role of EL is underinvestigation.

Lecithin-Cholesterol Acyltransferase

LCAT is the enzyme that catalyzes the esterification of free cholesterol in plasma lipoproteins. The HDL3 subfraction appears to be the main substrate for this esterification reaction and the Apo A-I associated with HDL, and possibly apo A-III, act as cofactors for LCAT (47,48). Human apo Al transgenic mice have been shown to increase HDL-C 6.8-fold with larger HDL particles and increased efflux from cholesterol laden cells (49). This illustrates a potential gene transfection approach for treating low HDL-C.

Acyl-CoA:Cholesterol Acyltransferase

ACAT serves to convert free cholesterol to esterified cholesterol intracellularly through an esterification process. Approximately seven years ago, two different forms of ACAT were described: ACAT1 and ACAT2 (50). These two forms differ in regard to cellular location and potential impact on atherosclerosis (51),

and ACAT1 appears to be expressed in most tissues in the body. In cholesterol-laden cells it serves to prevent intracellular free cholesterol–induced aptosis. This is particularly important for cell survival in macrophages located in atherosclerotic plaques. ACAT2 is located in small intestine enterocytes and hepatocytes. The role of ACAT2 appears to be to esterify cholesterol that is incorporated in VLDL particles, which eventually transform into LDL particles. It has been suggested that inhibition of ACAT2 may be a therapeutic approach to LDL-C reduction. Conversely, inhibition of ACAT1 may be detrimental due to possible disruption of plaque stability due to toxic macrophage death in existing atherosclerotic lesions. The ACAT inhibitor pactimibe, was recently reported not to have any beneficial effect on intravascular ultrasound–determined coronary atherosclerosis progression in humans (52). Future therapies that target ACAT2 may provide a novel means of reducing LDL-C.

Cholesteryl Ester Transfer Protein

CETP mediates the exchange of triglycerides from VLD/LDL particles for cholesterol ester in HDL particles. This activity may be either proatherogenic if it results in the transferred cholesterol ester being taken up by the arterial wall macrophages, or antiatherogenic if the transferred cholesterol ester is removed through the hepatic apo B receptor. Disorders of CETP function led to the development of medications that inhibit CETP and result in an increase in HDL-C (53).

Phospholipid Transfer Protein

Phospholipid transfer protein (PLTP) mediates transfer of phospholipids from triglyceride-rich lipoproteins to HDL (54). It results in conversion of small HDL3 into larger HDL2 particles.

Paroxonase

Paraoxonase is an enzyme initially of interest in the field of toxicology since it is an "A" esterase and hydrolyses organophosphate compounds used as insecticides and nerve gases (55). Paraoxonase is associated with HDL particles, and in sheep most of the paraoxonase activity is associated with the apoAI only particle (56). Thus, part of the protective effect of some, but perhaps not all, HDL particles may be the association of paraoxonase and its putative role in decreasing lipid peroxide accumulation on LDL particles (57).

Membrane modulators are factors that affect the ability of cholesterol to enter or leave the cell. Lipid-free apo A-I, apo A-II, Apo A-IV, apoC, and apoE can cause an efflux of phospholipids and cholesterol (58). This process can be rapid and result in HDL-like particles. This appears to occur primarily in cholesterol-enriched cells such as aortic smooth muscle cells and macrophages, but not erythrocytes (59). The clinical importance of this knowledge involves the

recent reports that the use of apo A products may provide a clinical treatment option in the not-too-distant future (60).

ATP Binding Cassette Transporter 1

ATP binding cassette transporter 1 (ABC1) is a protein that plays an important role in RCT through transmembrane lipid transport via transport channels. This process may serve to "flop" cholesterol and phospholipids from the inner to the outer side of the plasma membrane where it can be picked up by lipid-poor lipoproteins (61).

Scavenger Receptor B1

Scavenger receptor B1 (SR-BI) serves to selectively take up cholesterol esters from HDL and LDL into hepatocytes without taking in the HDL particle itself. Overexpression of SR-BI enhances cholesterol uptake and decreases HDL-C levels (62). Two lipid medications, probucol and atrovastatin, have been reported to increase SR-B1 mRNA and protein expression (63,64). In mice, knockout of the SR-BI gene increased HDL-C two-fold but resulted in increased atherosclerosis (65). This illustrates the complexity of the dynamic RCT process, which is not always reflected by static HDL cholesterol values.

CLASSIC LIPOPROTEIN DISORDERS

Elevated Cholesterol

Elevated total plasma cholesterol was initially identified as a cardiovascular (CV) risk factor through epidemiologic associations that led to the cholesterol hypothesis (66). The cholesterol hypothesis proposed that if total and LDLC were reduced, CV events would be reduced as well. This hypothesis was eventually proven to be correct when the results of the Lipid Research Clinics Coronary Primary Prevention Trial were published in 1984 (67). In this landmark study, a reduction in LDL-C, achieved with cholestyramine, was associated with a statistically significant reduction in clinical events when a 1-tailed t test was used. With the advent of more powerful LDL-C-lowering medications, the cholesterol hypothesis has been verified in both men and women, in subjects with elevated and moderate LDL-C values, and in subjects at risk for CAD and those who have documented CAD. These studies include the Scandinavian Simvastatin Survival Study, the West of Scotland Prevention Study, Cholesterol and Recurrent Events, and the Air Force Coronary Artery Prevention Study (68–71). These studies have consistently shown a 25–35% relative reduction in clinical events. However, review of the actual numbers indicates that a substantial number of subjects who take the active medication continue to have CV events (Fig. 2). This is a reflection of the fact that numerous metabolic issues contribute to CAD events and many are not adequately treated with simple LDL-C reduction alone (72).

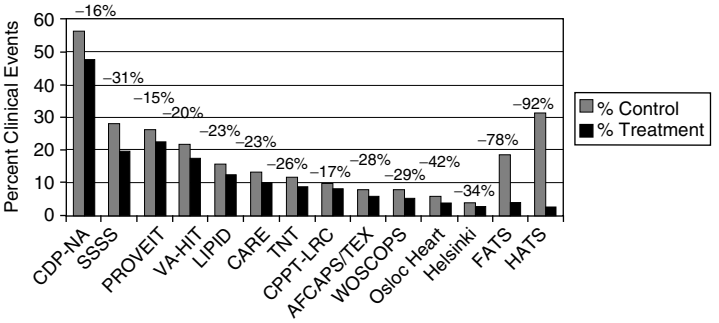

Figure 2 Percent of subjects in the control group (*gray column*) and the treatment group (*black column*) in 14 large clinical trials of cholesterol lowering and coronary events. A consistent approximate 25% reduction in events has been reported in studies using monotherapy. The difference in the height of the blue and red column represents the approximate 25% reduction in clinical events. The height of the red column represents the percent of patients who took the medication yet still had a Coronary heart disease event. The two studies on the right (FATS, HATS) used combination lipid-altering drug therapy and achieved an 80–90% reduction in clinical events. This percent reduction represents the difference in the number of clinical events in the control versus treatment groups. *Abbreviations*: CDP-NA, nicotinic acid arm of the Coronary Drug Project; SSSS, Scandinavian Simvastatin Survival Study; PROVEIT, Pravastatin or Atorvastatin Evaluation and Infection Therapy; VA-HIT, Veterans Affairs HDL Intervention Trial; LIPID, Long-Term Intervention with Pravastatin in Ischaemic Disease; CARE, Cholesterol and Recurrent Events; TNT, Treating to New Targets; CPPT-LRC, Coronary Primary Prevention Trial-Lipid Research Clinics; AFCAPS/TEX, Air Force/Texas Coronary Atherosclerosis Prevention Study; WOSCOPS, West of Scotland Coronary Prevention Study; Oslo Heart, Oslo Heart Study; Helsinki, Helsinki Heart Study; FATS, Familial Atherosclerosis Treatment Study; HATS, HDL Atherosclerosis Treatment Study. *Source:* Modified from Ref. 107.

Recently, the studies PROVE-IT and TNT suggest that LDL-C goals of <70 mg/dL should be considered as new LDL-C goals (73,74). However, close examination of the results of these trials reveals that a large number of patients continue to experience cardiovascular events. The Incremental Decrease in clinical Endpoints through Aggressive Lipid lowering (IDEAL) trial reported a greater reduction in coronary events (-11%) in a group of 4439 subjects treated with 80 mg/day atorvastatin compared to a group of 4449 subjects treated with 20–40 mg/day simvastatin ($p=0.07$) (75). However, this was the difference between 463 (10.4%) events in the simvastatin group compared to 411 (9.3%) in the atorvastatin group. Although these results are statistically (mathematically) significant, the clinical relevance may be viewed as less than significant. A successful approach to cardiovascular event reduction should achieve clinical event reductions of approximately 90%. National Institutes of Health funded studies that employed coronary arteriographic endpoints and used combination

drug therapy have reported clinical cardiovascular event reduction in the 90% range (76,77).

Familial Defective Apo B

A disorder that presents with elevations in LDL-C similar to familial heterozygous (FH) hypercholesterolemia is familial defective apo B (FDB). FDB is a genetic disorder resulting from a single nucleotide mutation at codon 3500 (78). It occurs in approximately one in 500 people in the general population and is associated with LDL-C values between 270–370 mg/dL (79). Unlike FH, LDL receptor function is normal; however, due to the abnormal apo B, only 32% of receptor-binding activity is found. One major difference between FDB and FH is that only one genetic defect has been found for FDB, while approximately 30 mutations have been found for the LDL receptor that results in FH.

Fish Eye Disease

LCAT deficiency, or fish eye disease (FED), is caused by respective mutations of the LCAT gene and associated with low HDL-C (80). FED was initially described in a Swedish family with corneal opacifications that resembled boiled fish; excess amounts of VLDL, IDL, and LDL; and low levels of HDL-C. The functional abnormalities of LCAT are known to cause two diseases characterized by severe corneal opacity: familial LCAT deficiency, which is accompanied with anemia and often renal failure, and FED with few other symptoms (81).

Low HDL-C

Low HDL-C has been identified as a cardiovascular risk factor for many years (82). One problem with attributing independent risk to low HDL-C is the powerful inverse correlation HDL-C has with other risk factors such as triglycerides and small LDL (12). This correlation issue compounds the ability to attribute CV benefit to HDL-C change since most lifestyle and pharmacologic therapies that increase HDL-C also reduce body fat, triglycerides, small LDL, and IDL, each of which is associated with CV risk. Thus, it is often unclear if it is the HDL-C increase that causes the benefit or the triglyceride and small LDL reduction, or a combination of the two. Evidence that HDL-C raising is of CV benefit was presented with the results of the VA-HIT results (83). In this investigation, men with relatively low HDL-C were randomized to gemfbrozil or a placebo and the resulting reduction in CV events was statistically attributed to an increase in HDL-C, which was attributed to gemfibrozil therapy. However, the issue of which parameter was responsible for the cardiovascular benefit remains unclear since gemfibrozil treatment reduces triglycerides and small LDL as well as increases HDL-C (84). From a clinician's standpoint, the argument of statistical independence is less important since clinical trials using combination drug therapy, which both lowers LDL-C, small LDL, and IDL and increases

HDL-C and HDL2, have demonstrated both clinical event and arteriographic benefit (76,77).

Low HDL-C is not rare in the CAD population and as many as 36% of men with premature CAD have been reported to express this trait, which is a broad spectrum of overlapping disorders (85–87). Primary hypoalphalipoproteinemia (HALP) is seen in approximately 4% of CAD patients and, equally important, approximately 50% of the offspring appear to be affected since it is inherited in an autosomal co-dominant pattern (88). In these cases, the HDL particles are particularly small (HDL3), suggesting impaired reverse cholesterol transport and impaired antioxidation capabilities. Low HDL2 has been observed in post–myocardial infarction (MI) patients, even in the setting of "normal" risk factors (89). Low HDL2b has been associated with arteriographic severity and arteriographic progression, particularly in normotriglyceridemic patients (90). In HATS, treatment with nicotinic acid and a statin resulted in a significant increase in LpAI, which was associated with significant arteriographic benefit and reduced clinical events (91).

One inherited example of low HDL-C is a specific LPL mutation (Asn291Ser), which has been identified in 5% of male CAD patients and results in low HDL-C and may contribute to CAD risk (92). Another genetic cause is a polymorphism in the region between the apolipoprotein A-I and apolipoprotein C-III genes that results in abnormally low HDL values (93). In these cases, elevated triglycerides or elevated LDL-C are not common and isolated low HDL is the main contributor to premature CAD. The role of impaired reverse cholesterol transport may be emphasized in different ethnic populations. Males of Asian Indian descent have been found to have abnormally low HDL2b compared to matched Caucasian subjects (94).

Elevated HDL-C generally reflects reduced cardiovascular risk with rare but notable exceptions. Other metabolic disorders such as hyperhomocysteinemia have been associated with CAD even in the presence of elevated HDL-C (95). CETP deficiency results in markedly increased HDL-C values. While elevated HDL-C most often reflects reduced CAD risk, it should be noted that a genetic CETP mutation, resulting in CETP deficiency and associated with HDL-C equal to 205 mg/dL has been reported in a patient with arteriographically documented CAD with angina (96). Sixty percent of the Japanese cases of HALP are associated with CETP deficiency (97). It has been suggested that the HDL observed in CETP deficiency is an atherogenic lipoprotein, as it contains a large amount of CE (98).

Elevated Triglycerides

Elevations in plasma triglyceride levels (hypertriglyceridemia) may be the result of multiple genetic and metabolic issues that can often be exacerbated by environmental issues such as diets rich in simple carbohydrates, excess body fat, and lack of physical activity. Some of the genetic causes that contribute to elevated triglycerides include the apo E 2/2 genotype and partial LPL deficiency

(34,35). Values in excess of 200 mg/dL have been defined as elevated and values >150 mg/dL as grounds for concern (99). Although elevated triglycerides are associated with a predominance of small LDL particles, it is important to appreciate that populations with elevated triglyceride values are a mix of individuals with the small LDL pattern B trait, and those in which the mild to moderately elevated triglycerides (150–250 mg/dL) are not associated with the small LDL trait. In healthy family members, the mean fasting triglycerides in small LDL pattern B subjects is 140 mg/dL while in large LDL pattern A subjects it is 70 mg/dL (100). It may be more physiologically appropriate to define a "normal" triglyceride as less than 100 mg/dL and values in excess of 150 mg/dL as suspicious for the presence of the small LDL trait.

Combined Hyperlipidemia

The combination of elevated triglycerides and elevated LDL-C is termed combined hyperlipidemia, and when a family history of hyperlipidemia or atherosclerosis is present, it is termed familial combined hyperlipidemia (FCH). FCH is associated with a four-fold increased CAD risk (101,102). The variability in phenotypic expression has involved a number of related disorders, including LDL subclass pattern B, hyperapobetalipoproteinemia, familial dyslipidemic hypertension, and syndrome X (103–106). One attribute of FCH involves LDL particles more susceptible to oxidative damage than LDLs from non-FCH individuals (107,108).

ADVANCED LIPOPROTEIN DISORDERS

Many nontraditional lipoprotein disorders are more common than classic elevations in LDL-C, or low HDL-C, and knowledge of these disorders is useful in diagnosing individual patient disorders, estimating risk, and developing individualized treatment plans that are matched to the patient's individual disorder(s) (109). These issues include disorders of IDL and remnant particles and postprandial lipemia, disorders of LDL subclass distribution, disorders of HDL subclass distribution, and apoprotein abnormalities.

IDL and Remnant Particles

Clinical trial evidence for the relevance of IDL dates back to the 1960s. The Framingham and Lawrence Livermore trials revealed a significant relation of triglyceride-rich lipoproteins to atherosclerosis risk (4). Recently, it has been reported that IDL is significantly associated with atherosclerosis progression as determined by carotid wall intima medial thickness (IMT) (16). In a revealing study of the natural progression of CAD assessed with coronary arteriography, it was reported that IDL-C correlated with disease progression while LDL-C did not (14). The standard "indirect" laboratory method of calculating LDL-C results in an LDL-C value that includes IDL-C in the LDL-C number. In this investigation, the

patients were typical CAD patients and not selected for elevated blood cholesterol levels. The somewhat surprising results are clarified when it is understood that the IDL-C and LDL-C were directly determined with ultracentrifugation and thus, the LDL-C number did not include IDL-C as does the standard calculated LDL-C value.

Remnant lipoprotein particles (RLP) are another type of triglyceride-rich lipoprotein that are the result of triglyceride-rich particle metabolism (110). An association of elevated remnant particles to CAD risk has been established and laboratory assays are currently available (111,112). Elevated remnant-lipoprotein-partide cholesterol (RLP-C) levels have been reported to be a significant and independent risk factor for impaired flow-mediated endothelium-dependent dilatation and angiographically proven CAD in patients with the metabolic syndrome (113). Large studies designed to specifically reduce IDL or RLP and investigate the effect on clinical endpoints or arteriographic change, have not been reported. The issue of the independent role RLP may play in CAD risk is complicated by the association of other triglyceride-rich particles, small LDL, and low HDL2 in the setting of elevated RLP.

LDL Subclass Distribution

The small LDL pattern B trait is linked to several metabolic issues that help explain its atherogenicity and has been termed the Atherogenic Lipoprotein Profile (ALP). Pattern B is a term used to describe individuals with a predominance of small LDL particles compared to pattern A, which describes individuals with predominately large LDL particles. Small LDL particles are able to infiltrate the arterial wall approximately 40% to 50% faster than large LDL particles. These particles are more susceptible to oxidative damage, and the HDL subclass that is associated with low CV risk (FIDL2) is reduced in LDL pattern B subjects. Further, this trait is associated with significantly increased blood fats following a meal, increased plasminogen activator inhibitor 1 (PAI-I), low antioxidant lipoprotein content, increased susceptibility to oxidative damage, increased IDL, and increased insulin resistance and risk for the development of Type 2 diabetes (114–117). Elevated plasma triglycerides are often, but not always, associated with LDL pattern B, and epidemiological analysis has now identified elevated triglycerides as an independent coronary heart disease risk factor (118). Thus, the LDL pattern B trait is associated with a plethora of metabolic disorders, each contributing to increased CAD risk.

The dense LDL subclass pattern (ALP or LDL pattern B) is a heritable trait determined by a single major dominant gene (the alp locus) (119,120). The gene for this trait has been localized to chromosome #19 near the LDL receptor, and expression is affected by at least three other loci, the apo AI/CIII/AIV gene cluster on chromosome #11, the manganese super oxide dismutase gene on chromosome #6, and the CETP gene on chromosome #16 (121–123). There is no linkage with the LDL receptor gene. The full expression of this trait occurs

following puberty in men and after menopause in women. Based on Hardy–Weinberg equilibrium, 30% to 35% of the Caucasian population is heterozygous for ALP, and another 5% are homozygous. The dense LDL subspecies is a marker for a common genetic trait that affects lipoprotein metabolism and increases CAD risk.

Low-density lipoprotein subclass distribution determination contributes information to CAD risk determination that is independent of TC and LDL-C. The Boston Area Health Study, the Physicians Health Survey, the Stanford Five City Project, and the Quebec Cardiovascular Study all confirmed that the presence of an abundance of small, dense LDL particles signifies an approximate three-fold increased risk for cardiovascular events (118,124–127). This is clinically important because this common genetically determined CAD risk factor is not reflected by measurement of LDL-C. It helps explain the incidence of CAD in patients who do not have classic hypercholesterolemia.

Seemingly contrary to the abundant evidence that small LDL is a significant risk factor is a report that larger LDL particles are associated with increased CHD risk and not small LDL. This report assessed baseline values only (as a percent distribution) and statistical adjustments for triglycerides and other variables associated with the small LDL trait were required to expose the relationship of CHD risk to larger LDL size (128). Contradicting this report are numerous studies supporting the independent role small, dense LDL plays in CV risk and the recent quantitation of small LDL-C further strengthens the role of small versus large LDL in CHD risk (129). Taken as a whole, the body of evidence supports the conclusion that an abundance of small LDL is a significant risk factor for CHD events. However, this does not exclude the role of larger LDL particles in CHD risk.

Carotid artery wall IMT has been reported to be a useful tool in predicting the risk for MI and stroke in older adults (130). Change in carotid artery IMT is correlated with change in the severity of CAD as assessed by coronary arteriography (131). Evidence that small LDL III plays a role in peripheral vascular disease is present using ultrasonography measure of carotid artery IMT. Investigators in Stockholm reported the relationship of small LDL-III to IMT progression in a group of healthy middle-aged mate subjects (132). Importantly, small LDL III had a significant relationship to IMT thickness ($r = 0.44$, $p < 0.001$) while LDL-C did not. Nicotinic acid is reported to preferentially reduce small LDLs compared to large LDLs (133). In another study utilizing IMT as an outcome variable, the addition of 1000 mg/day nicotinic acid to patients treated with an HMGCoA reductase inhibitor resulted in a significant reduction in carotid IMT-determined atherosclerosis progression (134).

Evidence that LDL heterogeneity is clinically important in determining arteriographic change over time is derived from several investigations. The NHLBI-II was the first investigation to report significantly less arteriographic progression in subjects with reduction in IDL and dense LDL (135). The Stanford Coronary Risk Intervention Project has revealed that individuals with

predominantly dense LDLs in the control group had an approximate two-fold greater rate of arteriographic progression compared to patients with predominantly buoyant LDL, but with multi-factorial risk intervention. The patients with predominantly dense LDL did significantly better than patients with predominantly buoyant LDL in regard to arteriographic benefit (136). The risk of arteriographic progression is independently linked to the smallest of the LDL particles, LDL IVb (137). The St. Thomas Atheroma Regression Study (STARS) investigated the effect of a low fat diet and cholestyramine on arteriographic rates of progression in men with CAD and reported that a reduction in dense LDL was one of the best predictors of arteriographic benefit (138). The Familial Atherosclerosis Treatment Study reported that change in LDL buoyancy was the best predictor of arteriographic outcome in this investigation, which used colestipol + niacin, or colestipol + statin as treatment modalities (139).

The gold-standard laboratory method of determining lipoprotein subclass distribution is based on density as determined in the ANUC (4). This method employs a highly accurate and reproducible ultracentrifugation method that characterizes lipoprotein subclasses by floatation intervals. It is time consuming, expensive, and available only in a limited number of research laboratories. Non-denaturing GGE was developed as a less expensive method of determining lipoprotein subclass distribution (19). A rapid ultracentrifugation method, termed vertical auto profile (VAP), has been used to determine relative floatation index as a determination of change in LDL buoyancy (140). This method determines the cholesterol concentration of multiple lipoprotein fractions based on density. During profile decomposition, peak heights for predefined sub-curves for all classes are simultaneously varied until the sum of the squared deviations between the sum of the sub-curves and the parent profile is minimized using linear regression. A relatively new method used to estimate lipoprotein subclass distribution is nuclear magnetic resonance (NMR) (141). Signals are derived from methyl groups on phospholipids, cholesterol, cholesterol ester, and triglycerides. NMR assumes a constancy of lipid mass contained within a particle of given diameter and phospholipid composition and thus methyl lipid NMR signal. This system uses a library of reference spectra of lipoprotein subclasses incorporated into a linear least-square fitting computer program which works backward from the shape of the composite plasma methyl signal to compute the subclass signal intensities.

National standardization programs do not monitor the accuracy of lipoprotein subclass determination by any of these methods. A split sample assessment of the traditional enzymatic methods of determining lipoprotein cholesterol and the established GGE method of determining LDL subclass distribution, compared to VAP and NMR, revealed significant differences ($p < 0.001$) between methods for total cholesterol, triglycerides, LDL-C, HDL-C, Lp(a), and LDL and HDL subclass distribution (142). Other laboratory methods designed to accurately determine LDL and HDL subclass distribution are under development (143–145). These new methods may provide an accurate, rapid, and

cost-effective means to determine subclass distribution in the hospital and clinical setting.

Individual Versus Group Determination of LDL Subclass Distribution

The presence of predominantly small LDL in large populations is often, but not always, associated with other physiologic parameters and laboratory measurements. Chief among these are fasting triglycerides, HDL-C (inversely), insulin, and body mass index. Use of these surrogate markers provides researchers an inexpensive method of assessing relative differences in large groups who are most likely to have a predominance of small or large LDL. However, within any of these surrogate marker groups, subgroups exist that have a greater or lesser probability of an individual having a predominance of small LDL. In general, the higher the triglyceride value the smaller the LDL size, and the lower the HDL-C the smaller the LDL size (Fig. 3). However, this relationship is most useful clinically when triglycerides are in excess of 250 mg/dL or less than 70 mg/dL and HDL-C is less than 40 mg/dL or greater than 70 mg/dL. The significant relationship between HDL-C and LDL size follows a similar but inverse relationship (Fig. 4). Higher HDL-C values are associated with larger LDL particles and lower HDL-C values with smaller LDL particles. The scatter plots in Figures 4 and 5 are based on 5366 patients with CAD examined at the

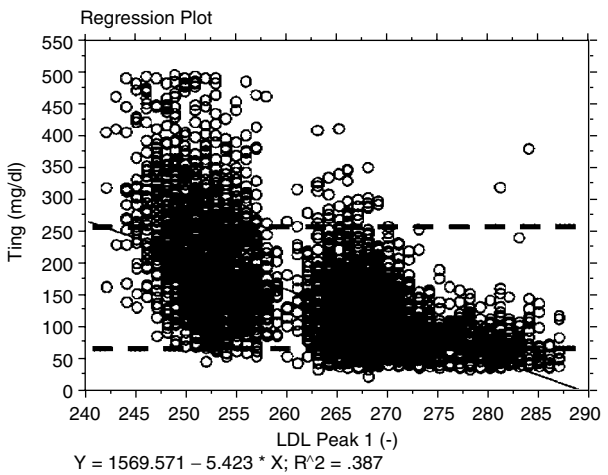

Figure 3 Scatter-plot of fasting triglycerides and LDL peak particle diameter in angstroms ($r = 0.62$, $p < 0.0001$) in 5366 CAD patients seen at the Fuqua Heart Center in Atlanta, Georgia. Large LDL particles have a diameter ≥ 263 angstroms and small LDL particles a diameter ≤ 257 angstroms.

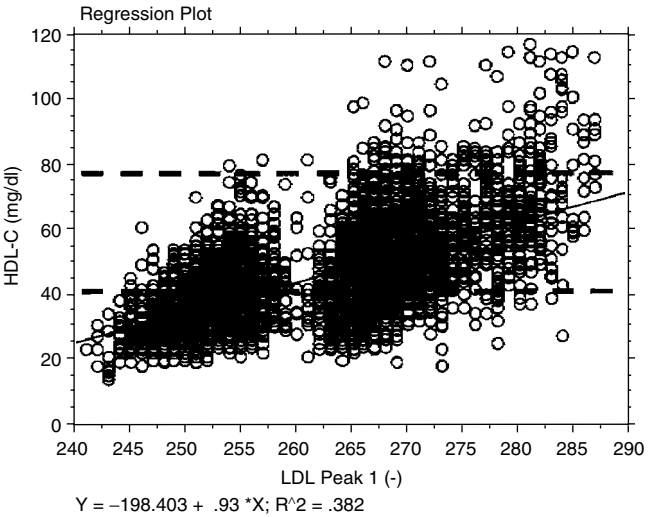

Figure 4 Scatter-plot of fasting HDL-C and LDL peak particle diameter in angstroms ($r=0.62$, $p<0.0001$) in 5366 CAD patients seen at the Fuqua Heart Center in Atlanta, Georgia. Large LDL particles have a diameter ≥ 263 angstroms and small LDL particles a diameter ≤ 257 angstroms.

Fuqua Heart Center in Atlanta, Georgia. In the case of fasting triglycerides, the significant correlation of fasting triglycerides to LDL peak particle diameter ($r=0.62$, $p<0.0001$) reveals a group with fasting triglycerides greater than 250 mg/dL that predominantly express small LDL (<257 angstroms). Likewise, patients with fasting triglycerides less than 75 mg/dL predominantly express large LDL (>262 angstroms). However, when fasting triglycerides are less than 250 mg/dL and greater than 75 mg/dL, a significant overlap exists between individual patients with predominantly large or small LDL particles, which makes fasting triglycerides, in this range, unreliable for clinical decision making in an individual patient. A similar overlap occurs for HDL-C and LDL size as illustrated in Figure 4.

HDL Subclass Distribution

There are three basic methods to determine HDL subclass distribution: density, size, and apolipoprotein content. Based on the relative density obtained in the analytic ultracentrifuge, the more dense, relatively cholesterol-poor form is termed HDL3 (1.125 to 1.21 G per mL) and the less dense, relatively cholesterol-rich form is termed HDL2 (1.062 to 1.125 G per mL) (20). GGE can be used to characterize the distribution of HDL particles based on size (146). Sequential immunoaffinity chromatography can isolate two HDL subclasses defined by their

apo A-I and A-II content as those with A-I only and those with AI and AII (AI:AII) (147). A relationship exists between HDL subclasses as determined by all three methods. HDL2a and HDL3b as determined by GGE contain both apo A-I and A-II, while HDL2b and HDL3c contain apo A-I only (147). The cardiovascular benefit of these particles may also be related to the ability of apo A-I to reduce VCAM-1 expression and damage that results in neointimal proliferation (148). In patients with low HDL-C and documented CAD the Lp(A-I) particle has been correlated with phospholipid transfer protein, which may also play a role in determining Lp(A-I) levels (149).

While low total HDL-C is an established CAD risk factor in epidemiological investigations, differences in HDL subclass distribution (proportion of HDL2 versus HDL3) exist within the "normal" HDL-C range and may contribute to CAD risk in individuals with "normal" HDL-C values. Low HDL2b has been linked to both arteriographically determined CAD severity and arteriographic progression of CAD (150,151). While focus has been mainly placed on low HDL-C and low HDL2b as a risk factor for CAD, it is of interest to note that the cardio protection in high HDL-C patients appears to also be related to differences in HDL subclass. HALP (very elevated HDL-C values) that is primarily HDL2 or HDL A-I only is also associated with decreased HL activity and cardio protection, while those that have high HDL-C values but are primarily composed of A-I:A-II exhibit less protection (152). In the FATS and HATS investigations, increases in HDL2-C, and/or the HDL A-I only particle were one of the best predictors of improvement in coronary stenosis and in HATS were associated with a reduction in clinical events (46). Finally, the inheritance of HDL subclasses has revealed correlations among family members for specific HDL subclasses, which are independent of HDL cholesterol and apo A-I (153).

Lipoprotein a

When apo [a] is attached to apo B and LDL, the particle is termed lipoprotein (a), or Lp(a) for short. Apo [a] is a protein with structural similarities to plasminogen (154). The importance of this lipoprotein lies in its strong independent association with coronary heart disease and carotid atherosclerosis. Elevated levels may be present in as many as 20–40% of individuals with CAD. This finding may help to explain why some patients with relatively normal blood LDL-C and HDL-C values still suffer from atherosclerosis.

Rapid progression of arteriographically quantitated CAD has been reported to be significantly more common in subjects with Lp(a) $>$ 25 mg/dL (155). Lp(a) has been reported to be an independent risk factor for myocardial infarction in young men, is independently associated with arteriographically defined coronary disease, and has been reported to be more closely linked to the extent of coronary atherosclerosis than other lipid parameters (156–158). This inherited disorder appears to increase CAD risk, particularly in the presence of more traditional risk factors such as elevated LDL-C and low HDL-C (159). The presence of elevated

Lp(a) (> fifth quintile) has been reported to significantly increase the future risk of angina approximately three-fold and the combination of elevated LDL-C and elevated Lp(a) increases the risk 12-fold (160). The contribution of elevated LDL-C to increased CHD risk associated with elevated Lp(a) has been observed in several investigations (161–163). The contribution of low HDL-C and elevated Lp(a) to atherogenic risk has also been reported (164).

Most studies investigating the atherogenic potential of elevated Lp(a) have been conducted in large population samples of middle age. Recently the role of elevated Lp(a) in men and women >65 years of age was reported from the Cardiovascular Health Study (165). Those with Lp(a) in the highest quintile had three times the risk of stroke and death associated with vascular events.

Lp(a) isoforms exist that represent a range of different [a] sizes (166). The smaller the [a] isoform, the greater the associated atherogenecity. Lp(a) size has an inverse relationship with Lp(a) concentration so that higher Lp(a) values are associated with small Lp(a) isoforms. The Physician's Health Study has reported that in apo(a) size is an independent predictor of angina regardless of Lp(a) concentration (160). In the Stanford Five City Project, Lp(a) size was found to be predictive of CAD risk in men but not women (167). Lp(a) size difference may help explain why elevated Lp(a) values reflect greater CAD risk in caucasian men compared to African American men. Gender differences may also exist since it has been reported that Lp(a) size is associated with CAD in African American men but not women (168).

Laboratory methods to determine Lp(a) are varied and clinical use hindered by the lack of standardization and accuracy (169). Commercially available methods use antibodies that recognize kringle 4 type 2 repeated epitopes, which are not specific for apo(a) size. A reference ELISA method has been developed that utilizes apo(a) kringle 4 type 9 that is unaffected by apo(a) size (170).

One emerging aspect of Lp(a) atherogenecity involves the potential to adversely interfere with natural thrombolysis (171). Elevated Lp(a) in combination with resistance to activated protein C attributed to the factor V Leiden mutation, and is associated with an odds ratio of 30 for stroke in children. The combination of elevated Lp(a) with any thrombophilic risk factor appears to increase the risk of a thromboembolic event by 2.6 and increases to 6.2 when the Leiden V mutation is present (172).

While the evidence that Lp(a) has a strong link to CAD and PVD is abundant, the clinical evidence that reducing Lp(a) is beneficial is sparse, due to a lack of clinical trials designed to test the hypothesis that reduction of Lp(a) results in a reduction in clinical events. However, some evidence derived from retrospective analysis exists. Reduction of Lp(a) following apheresis has been associated with a significant reduction in restenosis following PTCA (173). Most recently, reduction of Lp(a) in postmenopausal women with elevated Lp(a) has been associated with a significant 17% reduction in CV events and the authors reported Lp(a) to be an independent and modifiable risk factor for CAD events in postmenopausal women with known CAD (174). The

treatment in this investigation was hormone replacement therapy (HRT). Of particular interest in regard to the atherogenecity of elevated Lp(a) associated with elevated LDL-C is the retrospective evidence from FATS suggesting that significant reduction of LDL-C in patients with elevated Lp(a) may retard the rate of arteriographic progression and blunt the adverse affect of elevated Lp(a) (175).

Apolipoprotein B and Hyperapobetalipoproteinemia

LDL cholesterol values are commonly determined by a precipitation method, which results in an LDL cholesterol concentration value but no information in regard to the absolute number of LDL particles. Each LDL particle contains one apoprotein B, and determination of plasma LDL apoprotein B values allows the determination of LDL particle number. It has been suggested that measurement of apo B may be a more reliable indicator of LDL particle number and of more clinical utility than the standard calculated LDL-C value (176). In the setting of normolipemia, plasma apo B values are consistently lower than the LDL-C value. However, the condition described as hyperapobetalipoproteinemia exists in which the apo B value is higher than predicted based on the LDL-C value (177). A remarkable high incidence of hyperapobetalipoproteinemia (81%) has been reported in the post-MI population that exhibits relatively "normal" LDL-C values yet have disproportionately elevated LDL apo B (178). Individuals with this condition have an overabundance of small, dense LDL particles that are similar to those noted in patients with FCH and patients shown to have more progression of CAD demonstrated on arteriography (179). This condition appears to be due to increased apoprotein B-100 synthesis and transmitted as a dominant trait. Incorporation of fatty acids into lipid esters appears to be decreased, which results in abnormal processing of dietary fat and postprandial increases in free fatty acids. Thus, in this relatively common disorder, several abnormalities in lipoprotein metabolism that are not reflected in the LDL-C measurement could contribute to CAD risk.

The clinical importance of determining apo B values lies not only in the identification of hyperapobetalipoproteinemia, but in the association of elevated apo B with other cardiovascular risk predictors that result in identification of a group of patients at extremely high risk for cardiovascular events. The Quebec Cardiovascular Study investigated the relationship of small LDL, elevated fasting insulin values, and apo B in relation to CAD risk. Each abnormality individually contributed to CAD risk as demonstrated in previous investigations. However, in the Quebec Cardiovascular Study, the combination of all three abnormalities (an abundance of small LDL, elevated fasting insulin, and elevated apo B) identified a group of individuals who were at a 20-fold increased risk for cardiovascular events (179). In the new millennium of cardiovascular risk prediction, it is important to assess multiple risk determinants, since interaction between various metabolic

abnormalities has important implications for accurate risk prediction and treatment that are not apparent from standard blood cholesterol measurements.

Apo E Polymorphism

The most common gene affecting LDL cholesterol levels is apo E, which has three major genotypes, or isoforms, designated as E2, E3, and E4 (180). The most common allele, E3, has a frequency of approximately 0.78, while E4 has a frequency of 0.15, and E2 a frequency of 0.07 (181). While these are the most common genotypes, analysis of amino acid substitution has revealed at least 25 mutations in apolipoprotein E. The plasma lipoprotein profile that results from genotype differences relates to the greater fractional catabolic rate of LDL in individuals with the apo E2 genotype compared to those with the common apo E3 (182). This is consistent with the suggestion that hepatic LDL receptor activity is relatively higher in individuals with apo E2 because of decreased uptake of apo E2 containing triglyceride-rich lipoproteins, which results in less suppression of LDL receptors (183). Conversely, fractional catabolic rate of LDL is reduced in individuals with the apo E4 genotype, and this appears to be related to enhanced clearance of apo E4–containing remnants and suppression of LDL receptors. The disease, type III hyperlipoproteinemia, is an example of an interaction of the apo E2 homozygous state with another genetic or environmental factor leading to marked accumulation of triglyceride-rich lipoprotein remnants and accelerated atherosclerosis. Over 90% of individuals with type III hyperlipoproteinemia are apo E2 homozygotes. However, the disease is caused by interaction of the apo E2/E2 state with another genetic or environmental factor because while about 1% of the population expresses the E2/E2 isoform, only 2% of these develop type III hyperlipidemia and most individuals with E2/E2 do not exhibit the abnormal lipid profile.

Apo E isoforms explain part of individual differences in LDL-C response to a reduced fat diet. Men with the apo E3/E4 pattern respond to a reduced fat diet with significantly greater LDL-C reduction compared to men with the apo E3/E3 pattern (184). A differential effect of reduced fat diet–induced reduction in LDL appears to affect large LDL particles greater in individuals with the apo E4 allele and least in E3/E2 subjects, indicating the reduction in large LDL is mediated by an apo E–dependent mechanism. Because of this, diet-induced LDL-C reduction may have a variable benefit in individuals with different E genotypes and LDL subclass patterns (185). Postprandial lipid metabolism differences also exist between E3/E4 and E3/E3 subjects. Subjects with the E4 allele have enhanced postprandial lipemia, which may contribute to increased CAD risk (186).

Apo E isoforms can be of use in CAD risk prediction. The Etude Cas-Temoins sur I'Infarctus du Myocarde (ECTIM) study reported a relative risk for MI of 1.33 ($p = 0.02$) for subjects carrying the E4 allele, which explained approximately 12% of MI cases in the populations studied (187). This finding is consistent with the European Atherosclerosis Research Study in which the

population adjusted odds ratios for the phenotype E4/E3 and E4/E4 were 1.16 and 1.33, respectively, and it was concluded that the apo E polymorphism is one major factor responsible for the familial predisposition to CAD (188). However, the E4 genotype has recently been associated with Alzheimer's disease risk. In families with a history of Alzheimer's disease, the presence of the E4 allele increases the risk for developing Alzheimer's disease, but the interaction is probably complex and may involve interaction with the amyloid B protein precursor gene on chromosome 21q (189). It has been recommended that the Apo E4 test not be used for the prediction of Alzheimer's disease risk (190).

Cholesteryl Ester Transfer Protein Dysfunction

CETP mediates the exchange of triglycerides from VLD/LDL particles for cholesterol ester in HDL particles. This activity may be either proatherogenic, if it results in the transferred cholesterol ester being taken up by the arterial wall macrophages, or antiatherogenic, if the transferred cholesterol ester is removed through the hepatic apo B receptor. Disorders of CETP function led to the development of medications that inhibit CETP and result in an increase in HDL-C (191). In the rabbit model, inhibition of CETP activity with a vaccine or a pharmacologic approach has been shown to reduce atherosclerotic lesions (192,193). Total inhibition of CETP activity in families appears to be associated with increased CVD risk compared to partial inhibition that may reduce CVD risk (194). This may be due to the production of large, cholesterol-rich dysfunctional HDL particles when CETP activity is completely inhibited.

ATP Binding Cassette Transporter 1

Cholesterol efflux from cells is regulated in part by the ABC1 transporter (195). It serves to efflux cholesterol from an intracellular location to lipid-poor apo A-I and forms nascent HDL particles, which can then be converted into mature HDL particles by the action of LCAT. The important role of ABC1 in lipoprotein metabolism and atherosclerosis was discovered through the investigation of the low HDL-C disorder described as Tangier disease and now known to be the result of ABC1 mutations. In order to efflux cholesterol from cells, specific amphipathic helical structures of apoproteins must interact with ABC1. This knowledge has resulted in the production of amphipathic apo A-I mimetic peptides of 18–36 amino acids long that stimulate cholesterol efflux via the ABC1 pathway (196). ABC1 activity can be regulated by several environmental issues including sterols, retinoids, thiazolidinediones, and unsaturated fatty acids (197).

The end result of manipulating ABC1 activity on the atherosclerositic process in humans remains to be elucidated. Overexpression of hepatic ABC1 transporter could increase atherosclerosis by increasing flux into apo B–containing atherogenic particles, or assist in the removal of cholesterol from lipid laden plaques. Overexpression of the ABC1 transporter in mice has been reported to result in decreased diet-induced atherosclerosis (198).

Metabolic Syndrome

The metabolic syndrome combines aspects of lipid disorders, insulin resistance, hypertension, and thrombosis (199). Disorders of lipid metabolism are found in a majority of these patients. Forty-eight percent of metabolic syndrome patients exhibit elevations in plasma triglycerides (>150 mg/dL) compared to 10% of individuals without the metabolic syndrome, and 85% exhibit low HDL-C (<40 mg/dL in men and <50 mg/dL in women) (200). The metabolic disorders associated with the small LDL trait outlined above match many of the characteristics of the "metabolic syndrome." This cardiovascular health threat is rapidly enlarging the population at risk for CAD as can be seen from the startling increase in individuals defined as obese (200). In 1980 14.5% of the U.S. population was determined to be obese and in 2002 this had increased to 31% (201).

ADVANCES IN TREATMENT

Diet

Low fat diets have been recommended as the foundation for treating lipid disorders that involve elevations in LDL-C (99). However, when diets are reduced in fat content, the eliminated fat calories are frequently replaced by calories derived from simple carbohydrates and elevations in plasma triglycerides are often observed. Low fat, high carbohydrate diets have been investigated in regard to LDL and HDL subclass distribution. Dreon and colleagues initially reported that an isocaloric reduction in the percent of fat calories from 46% to 20% resulted in a significant reduction in LDL-C and apo B in subjects with predominantly small LDL, but unexpectedly, 40% of the subjects who exhibited a predominance of large LDL on the 46% fat diet converted to predominantly small LDL on the 20% fat diet (202). This was attributed to the increase of simple carbohydrates in the isocaloric lower fat diet. Further studies by Dreon and colleagues revealed that reduction of total calories from 20% to 10% has no effect on standard lipid measurements or LDL and HDL subclass distribution in individuals classified as very unlikely to carry the small LDL trait based on family history. However, patients who were predominantly large LDL but had the potential to express small LDL revealed no change in LDL-C or total LDL mass, but a significant increase in small, dense LDL balanced by a significant reduction in large, buoyant LDL accompanied by a dramatic reduction in HDL2 (203). This suggests that dietary advice to reduce calories from fat, without attention to the source of replacement calories, may be detrimental to individuals who carry the ALP trait. One popular new diet that reflects a Mediterranean diet influence has been shown to shift LDL subclass distribution towards the less atherogenic large LDL region (204).

Exercise/Weight Loss

Exercise and associated fat weight loss can have a significant effect on reducing small, dense LDL and increasing HDL2 (205). These investigations have indicated that reductions in small LDL, induced by loss of excess body fat, are accompanied by increases in large LDL for no net change in total LDL mass or LDL-C. This effect on LDL and HDL subclass distribution appears to be linked to loss of excess body fat, which can be induced by either dietary caloric restriction or increased exercise caloric expenditure (206).

Nicotinic Acid

Nicotinic acid has been reported to have a differential effect on LDL subclass distribution in subjects classified as having predominantly large or small LDL (207). In response to nicotinic acid, the reduction in small, dense LDL is counterbalanced by less of an increase in large, buoyant LDL, which results in an LDL-C reduction that does not reflect the greater reduction in small LDL (208). Similarly, nicotinic acid has a significant effect on increasing HDL2 that is greater than appreciated from the change in HDL-C. The nicotinic acid–induced increase in HDL2 is due to a reduction in the removal rate of HDL2 particles (209). Niacin selectively and directly inhibits hepatic diacylglycerol acyltransferase 2, but not diacylglycerol acyltransferase 1, thus inhibiting hepatic triglyceride synthesis and VLDL secretion. The recent discovery and characterization of a membrane-bound nicotinic acid receptor (HM74) explains niacin's acute inhibition of adipocyte lipolysis, but the role of HM74 in lowering triglycerides is unclear. Niacin possesses antioxidant, anti-inflammatory, and other beneficial effects on atherosclerosis unrelated to lipid lowering.

Fibric Acid Derivatives (Gemibrozil and Fenoibrate)

In general, fibrates reduce small LDL, particularly when elevated triglycerides are reduced, and they may increase HDL2. However, different fibrates can have different effects on HDL subclass distribution. Gemfibrozil has been shown to reduce small, dense LDL significantly in subjects with predominantly small LDL but has little to no effect on small LDL in subjects who have predominantly large LDL (84,210). A similar effect of fenofibrate on increasing LDL peak particle diameter has been reported (211). This effect of fibrates on LDL and HDL subclass distribution may have implications for the interpretation of the VA-HIT study that associated an increase in HDL-C, attributed to gemfibrozil treatment, with fewer cardiovascular events (83). The Diabetes Atherosclerosis Intervention Study (DAIS) showed that treatment with fenofibrate decreases progression of coronary atherosclerosis in subjects with type 2 diabetes (212). LDL size increased significantly more in the fenofibrate group than in the placebo group ($p < 0.001$) and small LDL was significantly associated with progression of CAD.

HMGCoA Reductase Inhibitors

The effect of HMGCoA reductase inhibitors including pravastatin, lovastatin, simvastatin, and fluvastatin has been investigated using GGE and no significant differential effect on LDL subclass distribution has been reported (213–217). A pilot study of atorvastatin has reported that in patients with elevated fasting triglycerides, atorvastatin has a statistically significant effect on reducing the small LDL subtraction Illa (218). In a small study of patients with chronic renal failure, atorvastatin significantly increased LDL size (219).

CETP Inibition

Inhibition of CETP activity can result in increased HDL-C and may improve reverse cholesterol transport. Partial CETP inhibition appears to provide some CVD protection in animals. Anti-CETP antibodies increase HDL-C and reduce aortic atherosclerosis in rabbits, and the use of the CETP inhibitor JTT-705 increased HDL-C two-fold, which was associated with reduced atherosclerosis in rabbits (220,221). However, the use of JTT-705 in rabbits fed a high cholesterol diet resulted in no atherosclerosis benefit (222).

Two CETP inhibitor agents have been tried in human subjects: JTT-705, and torce trapib. Initial small human trials with torcetrapib have revealed a 16% to 91% increase in HDL-C when doses of 10 mg to 240 mg/day are utilized (223). In patients with low HDL-C ($<$ 40 mg/dL) 120 mg–240 mg/day resulted in a 46% to 106% increase in HDL-C with an associated increase in mean HDL particle size (224).

Lipid Drug Combinations

Combination lipid drug therapy can produce dramatic improvement in the lipoprotein abnormalities associated with combined hyperlipidemia (225). In regard to LDL subclass distribution, combinations of lipid lowering medications that have been reported to have a beneficial effect on LDL subclass distribution include pravastatin + niacin, niacin + resin, and niacin + gemfibrozil (226–228). Combination drug therapy has been used in numerous successful clinical trials including CLAS, SCRIP, SCOR, FATS, and HATS.

Alpha- and Beta-Blockers

Selective and nonselective beta-blocker medications are known to be associated with an increase in triglycerides and a reduction in HDL-C (229). The effect on triglycerides and HDL-C is accompanied by an increase in small, dense LDL counterbalanced by a reduction in large, buoyant LDL particles (230). The reduction in HDL-C induced by nonselective beta blockade is also associated with reductions in the HDL2 distribution (231). The alpha-blocker prazosin is associated with mild reductions in triglycerides and increases in HDL-C, which

are further associated with reductions in small, dense LDL counterbalanced by increases in large, buoyant LDL particles.

Hormone Replacement Therapy

The hormonal components of oral contraceptives exert major effects on plasma lipoprotein metabolism that suggests that hormone replacement therapy in postmenopausal women may impact lipoprotein subclass distribution (232). HRT may be beneficial in postmenopausal women for a variety of reasons and have a differential effect in different subsets of postmenopausal women (233). Treatment with 0.625 mg/day conjugated equine estrogen and 2.5 mg/day medroxyprogesterone in postmenopausal women has been reported to have a significantly greater effect on reducing LDL-C and apo B in postmenopausal women with a predominance of small LDL compared to women with a predominance of large LDL (234). This reduction in LDL-C was accompanied by a significant reduction in small dense LDL, increase in HDL2, and increase in LPL. Postmenopausal women in the highest quartile for Lp(a) have been reported to have a significant reduction in cardiovascular events associated with a reduction in Lp(a) attributed to the HRT (174).

Novel Treatments

Novel therapeutic approaches to optimizing lipoprotein metabolism in order to treat atherosclerosis involve immunization, recombinant HDL, peptide infusion, and gene transfection. In mice, a recombinant adenovirus was constructed in which the human LCAT cDNA was expressed under the control of the human cytomegalovirus immediate/early promoter followed by a chimeric intron (AdCMV human LCAT), which resulted in overexpression of LCAT and a 580% increase in HDL-C and a 149% increase in human apo A-I (235). A CETP vaccine has been used in phase I human trials and reported to develop anti-CETP antibodies in 58% of the human subjects (236). In a rabbit model of atherosclerosis, production of anti-CETP antibodies reduced atherosclerosis by 48% (237).

Human apo A-I transgenic mice have shown a significant inhibition of early atherosclerosis (238). This may be related to alterations in the efficiency of reverse cholesterol transport. Infusion of recombinant apo A-I Milano has been shown in apo E deficient mice to increase cholesterol efflux capacity 60% in one hour and reduce plaque lipid content 50% in 48 hours (239). A small investigation in humans that employed intravascular ultrasound as an outcome variable, reported that infusion of a recombinant apo A-I Milano phospholipids complex significantly reduced atheroma thickness following five weekly injections (240). These novel approaches to therapy illustrate the future role of lipoprotein metabolism manipulation as possible treatment modalities for atherosclerosis.

ROLE OF NONINVASIVE TESTS

While the presence of circulating atherogenic lipoproteins is an expression of a disorder phenotype, it is the development of atherosclerosis disease in the vessel wall that leads to clinical cardiovascular syndromes. Thus, the detection of subclinical atherosclerosis and the noninvasive visualization of plaque composition in the vessel wall are critical in the prevention of cardiovascular disease.

Traditional models of assessing atherosclerosis have grown out of large longitudinal databases and rely on calculating the likelihood of atherosclerosis based on the presence or absence of known atherosclerotic risk factors. Thus, when faced with a given patient, one can calculate the probability of disease by using phenotypic blood markers. However, whether or not atherosclerosis is truly present in an individual cannot be determined from these models. Noninvasive imaging methodologies of atherosclerosis directly visualize disease, thus moving the assessment of atherosclerosis from the paradigm of probability to the paradigm of certainty.

The presence or absence of atherosclerosis can assist with treatment decisions when faced with an asymptomatic individual with a risk factor profile that reflects increased CHD probability. Furthermore, the same noninvasive imaging approaches can be used in individuals to follow the effectiveness of treatment over time. Finally, these imaging endpoints can be used in clinical trials investigating novel therapeutic approaches to modulate atherosclerosis.

Non-Coronary Arterial Beds

Several imaging modalities can be used to visualize atherosclerosis in non-coronary beds. Carotid intima-media thickness (CIMT) is a simple, easy-to-perform method that can be implemented in the outpatient setting and measures the thickness of the posterior wall of the common carotid artery or the internal carotid artery in a standardized fashion. Although not imaging lipoproteins directly, CIMT can visualize atherosclerotic plaques. Both absolute CIMT and change in CIMT over time have been validated in predicting major cardiovascular endpoints (241,242). One of the challenges of CIMT is its dependence on the operator and its relatively low reproducibility, making CIMT an excellent research tool in large populations, but rendering it less reliable in individual patients. Another limitation of CIMT is that it is a one-dimensional evaluation of the three-dimensional process of atherosclerosis.

More recently, cardiovascular magnetic resonance (CMR) has been introduced as a noninvasive modality to image atherosclerosis, since it has many theoretical advantages. First, it is a three-dimensional technique and can therefore more accurately assess atherosclerotic plaque volume. Furthermore, CMR is capable of tissue characterization based on intrinsic signal property differences using different imaging pulse sequences. The most common approach is referred to as a "multi-spectral" approach, where an atherosclerotic plaque is imaged with

a combination of T1-weighted, T2-weighted, and other pulse sequences and different plaque components are evaluated based on the different signal characteristics with different pulse sequences. For example, intra-plaque lipids are characteristically bright on T1-weighted images and dark on T2-weighted images. Moreover, the signal characteristics of lipoproteins are determined by the oxidative state and the surrounding molecular milieu. Such approaches can be used to quantify the relative content of different tissue components in plaques.

This multi-spectral approach has been validated in humans in vivo and has been shown to be quite accurate in classifying human carotid atherosclerotic plaques based on a modified AHA-classification using histology of surgically removed carotid endarterectomy specimens as reference standards (243). The thickness of the fibrous cap in carotid plaques can also be evaluated and has been shown to predict symptomatic patients (244). Injection of gadolinium-based contrast agents can further enhance plaque characterization by highlighting neovascularization and inflammation in plaques (245,246).

More novel plaque imaging approaches rely on molecular or cellular targeting approaches. Fibrin-specific gadolinium-based contrast agents are being developed for thrombus imaging and activated macrophages can be detected by their selective uptake of iron oxide–based MR contrast agents (247,248). While these CMR-based approaches are very promising in non-coronary vascular beds, their implementation is much more challenging in the coronary arteries, although some groups have reported some success (249).

Coronary Arterial Beds

Although intra-vascular ultrasound (IVUS) has made significant contributions to the understanding of plaque remodeling in atherosclerosis, it is invasive and is beyond the scope of this review. Of the noninvasive modalities, cardiovascular computed tomography (CCT) has emerged as a major tool in atherosclerosis imaging in the coronary arteries.

Coronary Artery Calcium

The evaluation of coronary artery calcium (CAC) has been validated in large patient populations, primarily utilizing electron-beam computed tomography (EBCT) approaches. More recently, multi-slice CT, which is much more widely available, has been shown to be equally accurate and reproducible in the assessment of CAC. The predictive value of CAC has been validated in large patient populations and has been shown to have independent and incremental predictive value over the traditional cardiovascular risk factors (250,251). CAC distribution tables have been created and each patient's percentile can be determined based on age and gender. CAC establishes the diagnosis of CAD and moves patients into a secondary prevention category.

Coronary Artery Computed Tomography Angiography

The introduction of the 64-detector CT technology and fast gantry rotation speeds have allowed for greatly improved spatial resolution ($400\mu \times 400\mu$ isotopic resolution) and temporal resolution (~ 160 ms), putting coronary artery computed tomography angiography (CCTA) in competitive range with invasive X-ray angiography. The accuracy of this technology has been recently validated using quantitative X-ray angiography as a reference standard, revealing a sensitivity of 99%, specificity of 95%, positive predictive value of 76%, and negative predictive value of 99% on a per-segment basis (252). The superior sensitivity and negative predictive value make this technique very attractive as an initial imaging tool in the evaluation of patients for obstructive CAD.

A very exciting aspect of CCTA is its ability to detect even non-obstructive, non-calcified atherosclerotic plaques in the coronary arteries. There is tremendous interest in developing strategies directed toward the treatment of these plaques to prevent plaque rupture and subsequent acute coronary syndromes. The 64-detector CCT technology was recently validated against IVUS and was shown to be very accurate in the noninvasive assessment of basic atherosclerotic plaque composition (253). Studies are ongoing at our institution to evaluate the role of CCTA in the assessment of non-obstructive coronary atherosclerotic plaques.

Evaluation of Treatment Effects Using Noninvasive Imaging

CIMT imaging has been used to study the effect of statin treatment on carotid plaque remodeling and showed that more aggressive LDL lowering with a potent statin was more effective in slowing atherosclerosis progression, compared to a less potent statin (254). Furthermore, it has been shown using CIMT that HDL reduction with nicotinic acid resulted in plaque regression, while LDL lowering with a potent statin only induced slowing or halting of progression (255) (Table 2).

CAC evaluation by EBCT has been used to follow treatment with different lipid-modifying medications that have shown minimal or no effect on coronary artery calcification. This suggests that calcification is a late and irreversible process in atherosclerosis. Coronary artery CTA is very promising in the evaluation of changes in plaque characteristics over time. These studies are currently under way.

CMR has been used to study changes in atherosclerotic plaque volume over time in patients treated with simvastatin for two years (256–258). In this study, statin therapy reduced vessel wall area by 15% at 12 months and by 18% at 24 months. Furthermore, there was a significant 5% increase in luminal area at 24 months. Importantly, these changes in vessel wall area were caused by a reduction in maximal wall thickness rather than in minimal wall thickness, indicating a negative (beneficial) remodeling of the atherosclerotic plaque. CMR

Table 2 Recent Advances in the Understanding of the Role of Apoproteins in Lipid Metabolism and Atherosclerosis

Name	Chylomicron	VLDL	IDL	LDL	HDL	Primary function
Al	X				X	Activates LCAT
All	X				X	Influences HDL functional states
AIV	X				X	Satiety signal
B100		X	X	X		Hepatic receptor uptake
B48	X					Dietary fat adsorption
CII	X	X	X		X	Activates LPL
CIII	X	X	X		X	Inhibits LPL
D					X	Transporter of small, hydrophobic ligands
E	X	X	X		X	Ligand for LDL receptors

The primary particle associated with each apoprotein and its primary metabolic function is listed.

is not only able to follow atherosclerotic plaque volume, but also plaque composition over time. In a case–control study from the FATS database it was demonstrated that aggressive triple lipid lowering therapy was associated with significantly fewer plaque lipid components (1% vs. 17%) and significantly more fibrous tissue (84% vs. 77%), compared to placebo (259).

Noninvasive imaging of atherosclerosis is starting to play an important role in the comprehensive evaluation of patients at risk for cardiovascular disease. It complements the evaluation of lipoprotein disorders by providing a phenotypical evaluation of disease expression in the arterial wall. At our institution, we routinely use coronary artery plaque characterization by coronary CTA and carotid arterial plaque characterization by CMR in asymptomatic patients with atherogenic lipoprotein phenotypes.

CONCLUSION

The dawn of the 21st century has witnessed a profound revolution in our understanding of atherosclerosis and the role of metabolic disorders other than the traditional hyperlipidemias. This revolution includes new understanding of the role of apoproteins, metabolic fat processing, and a dramatic expansion of clinical aspects of multiple lipoprotein subclasses that until recently were lumped under one name. This is most clinically applicable in the role small, dense LDL particles and HDL2 play in atherosclerosis progression, stability, and regression. It can no longer be assumed that a “normal” LDL-C reflects a normal CAD risk

Table 3 Prevalence of Metabolic Disorders Contributing to CAD in 1489 Subjects with Established CAD Seen by Invasive Cardiologists

	All	NCEP-NL	NCEP-HR	*p*
N	1489	874 (59%)	615 (41%)	
LDL IIIa + b > 20%	50.1%	44.3%	54.1%	0.005
LDL PPD (A) < 257 A	32.2%	20.4%	43.3%	0.0001
HDL2b < 20%	55.2%	42.1%	73.0%	0.0001
Insulin > 12 uU/ml	23.2%	17.0%	26.4%	0.008
Lp(a) > 25 mg/dl	25.0%	25.1%	22.0%	0.29
Hcy > 14 (umol/l)	10.0%	11.3%	9.2%	0.32
Fibrinogen > 350 mg/dl	37.9%	36.7%	41.5%	0.25
hs-CRP > 0.40 mg/dl	29.5%	27.8%	31.8%	0.42

The prevalence is presented for the entire group and for the group who were at high CAD risk (NCEP-HR) according to ATP-III LDL-C (LDL-C < 100 mg/dL) and HDL-C (HDL-C > 40 mg/dL), and those who were normal risk (NCEP-NL).

status. Disorders other than LDL-C concentrations are common in the CAD population. One thousand four hundred eighty nine patients seen by invasive cardiologists associated with the Fuqua Heart Center in Atlanta, Georgia, were examined in regard to the prevalence of these disorders in patients who met ATP-III LDL-C and HDL-C goals (n = 874) and those who did not (n = 615) (Table 3). An abundance of small LDL was found in 50.1% of the entire population with 44.3% of the patients who met ATP-III goals expressing an abundance of small LDL compared to 54.1% of those who did not. Seventeen percent of the patients who met ATP-III goals exhibited elevated insulin levels compared to 26.4% of those who did not. This higher incidence of small LDL and insulin levels in patients who do not meet ATP-III goals is expected due to the association of the small LDL trait with low HDL-C. However, metabolic disorders not linked to the small LDL trait, nor to plasma cholesterol values, show no difference in incidence in groups who did and did not meet ATP-III LDL-C and HDL-C goals. These issues include elevated Lp(a), elevated homocysteine, elevated fibrinogen, and elevated hs-CRP. In the group that met ATP LDL-C and HDL-C goals, 83.2% had one or more of the metabolic disorders listed above. This has clinical relevance since it has been reported that 45% of patients with CAD, as defined by the presence of coronary calcification, met ATP-III lipid goals (243).

This level of sophistication in the understanding of lipoproteins and CAD is important to clinicians for several reasons. The knowledge allows significantly more accuracy in predicting both primary and secondary risk for clinical events and disease progression or regression. It allows an explanation for the presence of CAD in patients with few to no traditional CAD risk factors. With this knowledge, individual patient treatment goals can be established that create the optimal milieu in which to achieve improved outcomes similar to those reported

in multiple National Institute of Health (NIH)-funded arteriographic investigations. Since many of these disorders are inherited in a dominant fashion, family members can be identified who carry the trait(s) and appropriate noninvasive testing can be recommended. Most importantly, this knowledge allows selection of the most appropriate medication for the individual patient and avoids the issue of treating all patients as if they were in a large clinical trial, an approach that ignores individual variability. Once therapy is initiated, this knowledge allows patient monitoring as lifestyle and pharmacologic treatments take effect and are modified. Intelligent application of detailed knowledge of lipoprotein metabolism and noninvasive test results will contribute to improved patient care now and in the future.

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Hemostatic Risk Factors for Atherothrombosis

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Atherosclerosis predisposes to arterial thrombosis and thrombosis superimposed on a disrupted (ruptured or eroded) plaque is the proximate event that triggers acute ischemic syndromes and sudden death (1,2). Although a number of risk factors for atherothrombosis have been defined through observational and epidemiologic studies, a significant number of atherothrombotic events occur in individuals without traditional atherosclerosis related risk factors (3). A number of studies have also focused on novel risk factors, predominantly related to hemostasis and coagulation, to identify a potential role for primary hemostatic abnormalities and cardiovascular risk (4–7). Normal hemostasis is orchestrated through a closely regulated interaction between prothrombotic and antithrombotic/thrombolytic processes mediated by cellular components, soluble plasma proteins, and endothelium-derived mediators. Genetic or acquired abnormalities that alter the production, activity, bioavailability, or metabolism of specific factors can alter this intricate balance resulting in predisposition thrombotic events.

COAGULATION FACTORS AND ATHEROTHROMBOTIC DISEASE

Fibrinogen

Fibrinogen, precursor of fibrin, is an important coagulation protein that also plays an important role in plasma viscosity and platelet aggregation. Fibrinogen levels

are strongly correlated with traditional vascular risk factors such as age, physical inactivity, hypertension, smoking, and features of the insulin resistance syndrome. Furthermore, fibrinogen is an acute-phase reactant, and the acute-phase response arising from viral infection, inflammatory stimuli, and smoking has been implicated in the development of atherothrombosis. Several prospective studies—Northwick Park Heart study, the Prospective Cardiovascular Munster (PROCAM) study, and the Prospective Epidemiological Study of Myocardial Infarction (PRIME)—of both healthy subjects and those with established vascular disease have identified an association between fibrinogen levels and myocardial infarction, stroke, and peripheral vascular disease (8–10) with an independent relative risk of arterial disease around 2–2.5% in the highest fibrinogen quartile compared with the lowest fibrinogen quartile. Several genetic polymorphisms of the fibrinogen gene have been identified that influence fibrinogen levels as well as other functional aspects of clot formation, but the relationship of these polymorphisms to atherothrombotic vascular disease has in general yielded weak or inconsistent results (4). Recently, results were reported from a detailed individual participant meta-analysis of a large data set with 6944 first nonfatal acute myocardial infarction or stroke events, and 13,210 deaths with cause-specific mortality data, among 154,211 participants from 31 studies (10). The age and sex adjusted hazard ratio per 1 gram increase in usual level of fibrinogen was 2.42 (95% CI: 2.24–2.60) for coronary heart disease, 2.06 (95% CI: 1.83–2.33) for stroke, 2.76 (95% CI: 2.28–3.35) for vascular mortality, and 2.03 (95% CI: 1.90–2.18) for nonvascular mortality (11). The hazard ratios, after adjustment for established risk factors, were reduced to 1.8 for coronary heart disease and stroke. Thus, overwhelming evidence suggests a moderately strong relationship between fibrinogen levels and cardiovascular risk. Although biologically plausible explanations could account for a causal relationship between fibrinogen levels and atherothrombosis, it is also possible that elevated fibrinogen levels reflect the inflammation associated with atherosclerosis rather than reflecting a cause and effect relationship (12).

Factor VII

Factor VII (FVII) is a vitamin K dependent coagulation factor, and its levels are influenced by age, body mass index, and plasma triglyceride levels (4). Several prospective studies have examined the relationship between FVII mediated pro-coagulant activity (FVIIc) and atherothrombosis, but the results have been inconsistent. The Northwick Park heart study found a significant association between FVIIc and coronary heart disease that was stronger than that with cholesterol levels (8); however, subsequent reports, taking into account confounding variables, failed to confirm this finding (13). Similarly, several genetic polymorphisms of FVII gene that influence its levels have been described, but the relationship of these polymorphisms with coronary heart disease have again been weak, negative, or inconsistent (4,14–21).

Factor XIII

This coagulation factor is involved in stabilizing a fibrin clot. Several genetic polymorphisms have been identified in the Factor XIII gene, but once again no consistent relationship of these polymorphisms to atherothrombotic vascular disease has been confirmed; in fact, some of the polymorphisms have actually shown an inverse relationship (22–28).

Factor V/Prothrombin

Several studies have evaluated the relationship between genetic abnormalities in the Factor V and Prothrombin genes and risk of arterial thrombosis. In general, the studies examining the association of Factor V Leiden (1691 G/A) mutation and prothrombin 20210G/A to arterial thrombotic disease have been negative even among young subjects (4). Positive associations have, in general, been observed in studies involving highly selected populations or among children or have considered interactions with environmental risk factors (29,30). Thus, Factor V Leiden mutation was shown to be associated with a 2.5-fold increased risk of nonfatal myocardial infarction among young women predominantly among smokers (31). Similar findings were noted among carriers of the prothrombin 20210A allele who had a 4-fold increase in the risk of myocardial infarction that was again increased more than 40-fold among smokers (32). A combined analysis of Factor V Leiden and the prothrombin 20210A allele in this population showed that the effect of major coronary risk factors was increased 4- to 6-fold by the presence of one of these inherited, prothrombotic risk factors. Subsequently, two case-control studies of men showed an increased risk of myocardial infarction associated with Factor V Leiden and the prothrombin 20210G/A mutations predominantly in the presence of other cardiovascular risk factors (33,34).

Thrombomodulin

Thrombomodulin is an endothelial cell surface receptor for thrombin that accelerates thrombin-induced activation of the natural anticoagulant protein C. Reduced plasma thrombomodulin levels were associated with an increased risk of myocardial infarction in a prospective case-control study (35). The association of certain genetic polymorphisms in the thrombomodulin gene with atherothrombotic disease have been inconsistent (36,37).

Platelets and Atherothrombotic Vascular Disease

Platelet adhesion and aggregation at the site of vascular injury plays an important role in clot formation. Platelet surface receptors such as the glycoprotein IIb/IIIa bind fibrinogen and von Willebrand factor are essential final common steps in platelet aggregation. Genetic polymorphisms of this receptor complex, in particular the presence of glycoprotein IIIa isoforms known as the PL A2, have

been linked to a markedly increased risk for coronary heart disease among young subjects, especially smokers, in some studies, but these findings have not been substantiated in larger studies after accounting for confounding variables (38–48). Similarly, other platelet glycoprotein receptor gene polymorphisms have also yielded inconsistent results (4).

Role of Endothelium-Derived Hemostatic Mediators in Atherothrombosis

Endogenous fibrinolysis is dominantly mediated by endothelium-derived tissue-type plasminogen activator (t-PA) whose action, in turn, is opposed by its inhibitor known as the plasminogen activator inhibitor-1 (PAI-1). Circulating t-PA is mostly complexed with PAI-1, and thus circulating levels of t-PA do not necessarily reflect functionally active t-PA. Elevated levels of both t-PA and PAI-1 have been associated with an increased risk of arterial thrombotic disease in some but not in other studies (13). An imbalance of this fibrinolytic equilibrium is encountered primarily in the insulin resistance syndrome and hypertriglyceridemia (elevated triglycerides are associated with increased PAI-1 levels), which leads to increased plasma PAI-1 and t-PA antigen levels (reflecting inactive t-PA/PAI-1 complexes) with a consequent decrease in fibrinolytic activity (49). Genetic polymorphisms of t-PA and PAI-1 genes have not shown uniform relationship to atherothrombotic vascular disease (4).

A recently identified inhibitor of fibrinolysis is a plasma carboxypeptidase called Thrombin-activatable fibrinolysis inhibitor (TAFI) (50). Plasma TAFI concentrations demonstrate high interindividual variability that is poorly explained by environmental factors (51). Activation of TAFI occurs by the thrombin–thrombomodulin complex and results in prolongation of clot lysis time. Increased plasma TAFI levels have been associated with an increased risk of both deep-vein thrombosis and symptomatic or angiographic coronary artery disease in some studies, whereas others have shown an inverse relationship to risk of myocardial infarction (52–54). In the past few years, several polymorphisms that have been described in the TAFI gene have been identified without a consistent relationship to atherothrombotic risk (4).

Endothelium-derived nitric oxide, produced by the action of endothelial nitric oxide synthase (eNOS) from Arginine, exerts antithrombotic actions in addition to its anti-inflammatory, anti-oxidant, vasodilator, and anti-proliferative effects (55). Several genetic polymorphisms of the eNOS gene have been identified, but their relationship to atherothrombosis has been inconsistent at best (4).

White Blood Cell Count and Atherothrombosis

Inflammation is critically linked to various pathophysiologic events leading to initiation, progression, and destabilization of atherosclerosis (2). A number of epidemiologic studies have shown an association between elevated white blood

cell (WBC) count and coronary heart disease (56–64). Similarly, in acute coronary syndromes, elevated WBC count has been linked to increased risk for fatal and nonfatal cardiovascular events (65–68). A recent study suggested that the cardiovascular risk of elevated WBC counts is carried by increased circulating neutrophil counts and decreased total mononuclear cell counts (lymphocytes plus monocytes) (69). However, some studies have failed to confirm these relationships after correction for confounding risk factors such as smoking (15,70).

CONCLUSION

The relationship between hemostatic factors and risk for arterial atherothrombosis, after correction for all known risk factors and confounding variables, is at best weak and at worst unconvincing. In contrast to venous thromboembolic disease, wherein the role of certain thrombophilia markers is well established, there is little clarity in relation to arterial thrombotic disease except possibly in the case of fibrinogen levels. Although early reports in the literature revealed positive associations, numerous negative studies have followed, and the initial hope that inherited risk factors might contribute significantly to the development of atherothrombotic disease remains largely unconfirmed.

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4

Homocysteine: A Risk Factor for Atherothrombotic Cardiovascular Disease

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The recognition of hypercholesterolemia, tobacco use, hypertension, family history, and diabetes mellitus as risk factors for cardiovascular disease has contributed to the significant decline in cardiovascular morbidity and mortality over the last 50 years. However, we are still far from defining the physiological milieu necessary for the development of cardiovascular disease. The traditional risk factors only explain 50% or less of the variation in atherosclerotic cardiovascular disease. As such, other variables besides the traditional risk factors might offer some insights. Chief among them include homocysteine, a sulfur amino acid formed during the metabolism of methionine. In homocystinuria, an inborn error of this metabolism, homocysteine levels are severely elevated, often to more than 50 times that of normal. The cardiovascular hallmark

of this disease is premature atherothrombosis of the peripheral, coronary, and cerebral vasculature. From this observation, three hypotheses can be proposed: first, that homocysteine directly promotes atherothrombosis; second, that in doing so, the homocysteine concentration in otherwise normal individuals is a measure of cardiovascular risk; and third, that by reducing the homocysteine level, one can diminish this risk. A similar line of reasoning links familial hypercholesterolemia and the established risk factor, low-density lipoprotein, in the general population. Different types of data are needed to prove each of the three hypotheses listed above. First, basic science investigation must establish a coherent mechanism for how homocysteine may cause atherothrombosis in vivo. Second, epidemiological studies must show a strong, consistent, independent association between elevated homocysteine levels and cardiovascular morbidity and mortality. Third, prospective, randomized trials must establish that decreasing the level of homocysteine modifies this risk. Only by fulfilling these criteria can homocysteine join the canon of the classical cardiovascular risk factors of hypertension, diabetes mellitus, hypercholesterolemia, and tobacco use. If it does not fulfill these criteria completely, or does so only partially, homocysteine measurement may still provide prognostic information for patients with unstable angina, acute myocardial infarction, deep venous thrombosis, or stroke.

HOMOCYSTEINE METABOLISM

Homocysteine is a sulfur amino acid formed during the metabolism of methionine, an essential amino acid that is found in dietary protein. Homocysteine is metabolized by remethylation or transsulfuration (Fig. 1) (1). Remethylation is a salvage pathway; homocysteine acquires a methyl group from 5-methyl-tetrahydrofolate to form methionine in a reaction catalyzed by the vitamin B12-dependent enzyme, methionine synthase. 5-methyl-tetrahydrofolate is derived from folate in a cycle catalyzed by methylene tetrahydrofolate reductase (MTHFR). An alternative pathway for remethylation occurs in the liver, where betaine acts as the methyl donor. Transsulfuration occurs during times of methionine excess or cysteine depletion. Homocysteine combines with serine to form cystathionine via a rate-limiting reaction catalyzed by the B6-dependent enzyme, cystathionine-beta-synthase. Cystathionine-gamma-lyase, another B6-dependent enzyme, then catalyzes the hydrolysis of cystathionine to cysteine, which is then further metabolized to glutathione or sulfate (2).

HOMOCYSTEINE: CIRCULATING FORMS

Homocysteine is a four-carbon thiol amino acid that exists in various forms within human plasma depending on the redox status of its sulfhydryl group (Fig. 2). Approximately 1% of circulating homocysteine circulates in the reduced form of the free thiol itself. When this is bound to another molecule of homocysteine, it forms the symmetrical disulfide, homocystine. When bound to

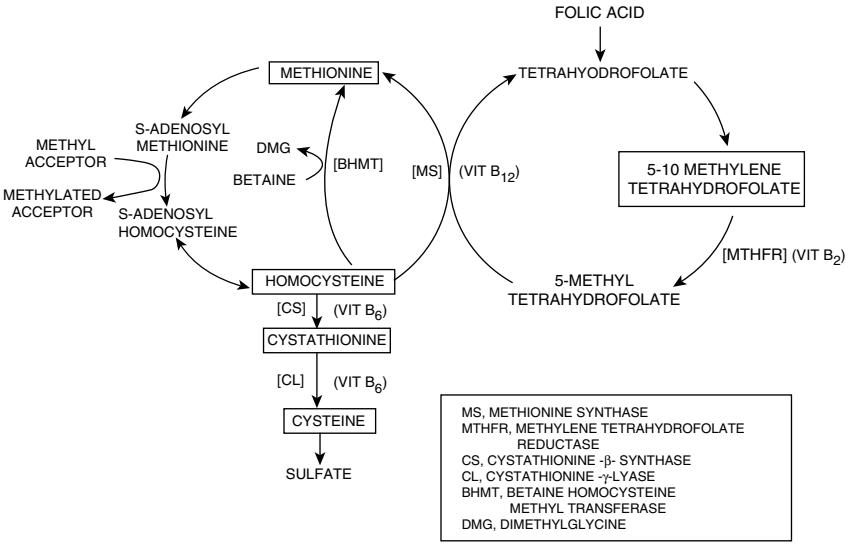

Figure 1 Outline of methionine/homocysteine metabolism. Vitamin coenzymes and substrates: THF, tetrahydrofolate; B_2, riboflavin; B_6, vitamin B_6 as its biological active form, i.e., pyridoxal 5′-phosphate; and B_{12}, methyl cobalamin. Intermediate metabolite: DMG, dimethylglycine. *Source*: From Ref. 1.

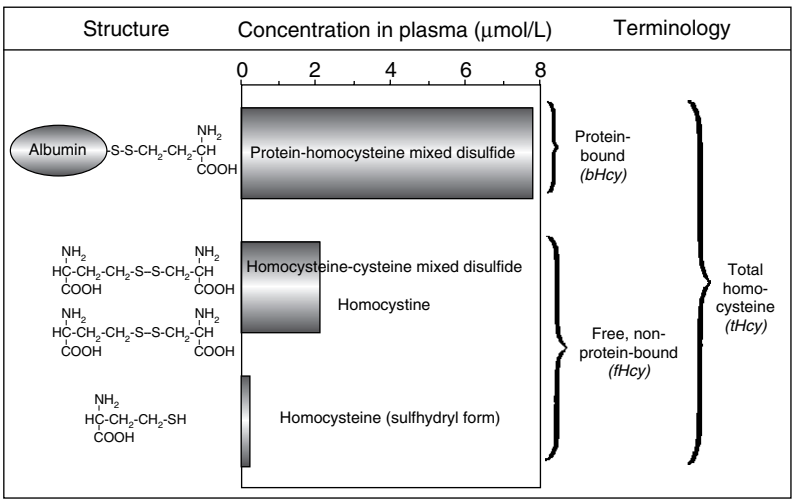

Figure 2 Homocysteine and the major related disulfides in normal human plasma. *Source*: From Ref. 3.

other types of thiols, it forms mixed disulfides, predominantly homocysteine-cysteine disulfide. Roughly 75% of circulating homocysteine is protein-bound, mostly to albumin, by disulfide linkage to protein-cysteine. The term "free" homocysteine refers to those forms of homocysteine that are not protein bound (including disulfides), while "total" homocysteine (tHcy) refers to both protein-bound and free states (3).

The saturation of plasma protein-binding sites only occurs at very high levels of total homocysteine (homocystinuric range >100 micromol) (4). Thus, the relatively large differences in measured total homocysteine between individuals in the general population (on the order of 5 μmol/L) represent very small changes in circulating reduced homocysteine. Serum plasma protein concentration also significantly affects measured total homocysteine given that a large proportion of total homocysteine is protein bound; a positive correlation between tHcy and serum albumin level has been observed (5).

HOMOCYSTEINE: LABORATORY MEASUREMENTS

The clinician must confront three factors when interpreting a laboratory value for homocysteine concentration: the methodologic variability of homocysteine concentration analysis, the biological variability of homocysteine concentration in the subject, and, given these variations, the estimation of patient risk.

Methodologic variability is introduced during sample collection, storage, and laboratory analysis. Food ingestion may change the circulating homocysteine concentration (6,7). Thus, it is recommended that the representative sample be gathered while the subject is fasting. Homocysteine is an unstable compound ex vivo; total homocysteine in whole blood increases at room temperature because of its synthesis and release from erythrocytes. After phlebotomy, levels rise at a rate of 10% per hour in stored blood even with additives such as ethylenediamine tetra-acetic acid (EDTA). Thus, to avoid falsely elevated results, the sample should be promptly placed on ice and centrifuged or stored at 0° C (8).

There are three primary methods for measuring the plasma concentration of homocysteine: electrochemical or fluorescence-detection high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and fluorescence or enzyme immunoassay. In general, HPLC and GC-MS are expensive, cumbersome, and require highly skilled technicians. The immunoassays offer the hope of improved speed and ease.

Analytical quality specifications analysis of these methods by the Centers for Disease Control (CDC), revealed significant inter-laboratory and intra-laboratory variations with none of the laboratories showing optimum performance (9). This variation was less than 10% of the sample measurement; however, this may still present a difficulty for the interpreting physician, because the relationship between homocysteine level and cardiovascular risk may be a graded phenomenon and small differences in levels may have substantial clinical implications.

Even with the accurate measurement of homocysteine concentration, reference ranges must be established so that the clinician can differentiate between normal and pathological levels. Because some studies show that the relationship between total homocysteine and cardiovascular risk is a graded phenomenon, the precise definition of the "upper limit of normal" is unclear. Moreover, many studies that show a strong correlation between total homocysteine levels and risk derive this association not by comparing absolute levels, but by comparing a given cohort's highest and lowest inter-quartile levels. Mean levels of homocysteine differ according to gender and increase with age (10), so that proper reference values must incorporate both of these patient variables.

The patient's clinical situation also affects the measured homocysteine concentration. An acute atherothrombotic event may transiently alter total homocysteine levels because a large proportion of total homocysteine is bound to albumin, which decreases during the acute inflammatory response. In patients presenting with acute coronary syndrome, homocysteine levels do not change, or slightly decrease, between the day of admission and hospital day 2 (11), and then rise significantly thereafter; this rise persists as long as 6 months (12,13). Similar findings have been observed in homocysteine levels measured during the acute and convalescent phases of stroke (14). It is unclear whether homocysteine levels drop initially and then recover, or alternatively, rise from baseline for an undetermined amount of time. This could have a significant clinical impact because the hospital admission for an acute atherothrombotic event is likely to be when the physician assesses a patient's risk factors and the homocysteine level is first measured. It is uncertain how accurately such an admission level reflects future risk given the flux in homocysteine concentrations around this time. Conversely, because of the persistent rise in levels after events, retrospective studies that measure the homocysteine levels of index cases in the initial months after an acute event may overestimate the association between elevated homocysteine and risk.

THE ORAL METHIONINE LOAD

Similar in approach to the oral glucose tolerance test, the oral methionine load is a provocative test to uncover patients who may be at atherothrombotic risk but have a fasting total homocysteine in the normal range. By expanding the definition of hyperhomocysteinemia to include those individuals with a high post-methionine load and normal fasting total homocysteine, it has been stated that as many as 40% of at risk patients are not identified by screening with fasting total homocysteine alone (15). The standard procedure is to administer 0.1 gm/kg of L-methionine orally, usually in fruit juice to mask this amino acid's unpleasant taste. Total homocysteine is measured 4 hours later. An abnormal post-methionine load homocysteine level is generally defined as higher than two standard deviations above the mean, although some cohort studies also define it based on inter-quartile comparison.

Homocysteine metabolism is dependent on two pathways: remethylation to methionine, which is thought to be primary during basal metabolism and periods of methionine scarcity, and transsulfuration to cysteine, a process active during periods of methionine excess. An abnormally elevated response to a methionine load primarily reflects a defect in the transsulfuration pathway. However, the remethylation and transsulfuration pathways may be inter-regulated, so that aberrations of one pathway influence the other (16).

The response to a methionine load depends on age and sex, as well as vitamin status. Women and younger individuals have a statistically significant higher than average post-load homocysteine concentration compared to older males, so any set of standardized reference ranges must take this into account (17).

An important question is whether the hyperhomocysteinemia induced by a methionine load has clinical significance. The magnitude of homocysteine elevation that is induced physiologically by routine dietary stress on the transsulfuration pathway is likely quite small. However, epidemiological data show that patients with an abnormal response to a methionine load despite a normal fasting homocysteine level have an increase in cardiovascular risk (18).

DETERMINANTS OF ELEVATED HOMOCYSTEINE

Plasma homocysteine concentration is dependent on the interplay between genetics, nutritional intake, disease states, and environmental factors.

Genetic causes of hyperhomocysteinemia involve mutations in the enzymes that regulate the metabolic pathways of methionine synthesis. Homozygous cystathionine-beta-synthase (CBS) deficiency is responsible for homocystinuria, in which fasting total homocysteine can reach levels as high as 400 μmol/L. In the United States this disease occurs at a frequency of about 1 in 400,000 births. Approximately 1% of patients with coronary artery disease and hyperhomocysteinemia are heterozygous for the mutation; thus, it is not a major contributor to atherosclerosis in the general population (19). Deficiency of the MTHFR gene also leads to homocystinuria, but this entity is exceedingly rare.

A thermolabile variant of the MTHFR gene has been identified and found to be a major cause of mild to moderate hyperhomocysteinemia. The mutation is highly prevalent: approximately 12% of the white population is homozygote. Homozygotes have approximately 25% (2.6 μmol/L) higher total homocysteine than wild types. Given the large contribution of thermolabile MTHFR to the incidence of hyperhomocysteinemia in the general population, studies to estimate the cardiovascular risk attributable to thermolabile MTHFR have had surprising results in light of the large amount of epidemiological data linking mild to moderate homocysteinemia with cardiovascular disease. Meta-analysis of studies that examine the relationship between thermolabile MTHFR and total homocysteine in patients with and without cardiovascular disease has shown that although this mutation is a major cause of hyperhomocysteinemia, it is not associated with increased cardiovascular risk (20). A similar lack of association

has been observed in studies of thermolabile MTHFR and cerebrovascular disease (21,22).

One likely explanation for this lack of association is that the phenotypic expression of the thermolabile MTHFR mutation may vary according to serum folate levels, being more pronounced in populations characterized by low serum folate levels. A recent meta-analysis by Klerk et al. investigating the mutation's role in the development of coronary heart disease provides evidence in support of this. Overall, homozygous MTHFR individuals were at increased risk for coronary heart disease (CHD) (OR 1.16, 95% CI 1.05–1.28). However, there was significant variation across geographic boundaries with increased risk observed in European (OR 1.14, 95% CI, 1.01–1.28) but not North American populations (OR 0.87, 95% CI 0.73–1.05), where dietary folate fortification protocols implemented by federal governments may have resulted in higher serum folate levels (23).

Nutritional status plays a key role in determining circulating homocysteine levels. For example, approximately two-thirds of the cases of elevated homocysteine in the elderly population may be attributed to B6, B12, or folate deficiency (24). Vitamin B12 and folate are essential co-factors for enzymes involved in the remethylation pathway of homocysteine metabolism. Examination of the Framingham cohort reveals a strong inverse correlation between serum folate and fasting homocysteine level and a weaker yet still significant inverse correlation between vitamin B12 and fasting homocysteine (19). On the other hand, vitamin B6 deficiency disturbs the activity of the transsulfuration pathway of homocysteine metabolism, leading to aberrantly elevated post-methionine load homocysteine levels, not fasting ones. Studies in rats confirm the interactions between vitamin B12, folate and fasting homocysteine, and between vitamin B-6 and post-methionine load homocysteine (25).

HOMOCYSTEINE AND ATHEROTHROMBOSIS: PATHOPHYSIOLOGIC MECHANISMS

The argument that elevated homocysteine is a risk factor for atherothrombosis can be strengthened if a direct, pathophysiologic relationship between homocysteine and atherothrombosis can be elucidated by laboratory investigation. To establish clinical "meaning" by the criteria of evidence-based medicine, it is enough to demonstrate that a positive correlation between a variable and a disease exists and that modification of this variable attenuates or negates this correlation. However, when such information is not fully available, demonstrating a plausible causal mechanism for the association may strengthen the hypothesis.

Homocysteine and Atherothrombosis

Homocysteine has been associated with both atherosclerosis and thrombosis; mild to moderately elevated homocysteine is correlated with peripheral vascular disease, carotid artery stenosis (26), coronary artery disease as assessed by

angiography, and large and small vessel cerebrovascular disease (22,27), while it also is associated with venous thromboembolism (28) and acute myocardial infarction. Ex vivo studies of homocysteine show that the molecule may indeed possess a number of atherogenic and thrombogenic properties that could explain some of these correlations. In culture, homocysteine is directly toxic to endothelial cells (29); stimulates vascular smooth muscle cell (VSMC) DNA synthesis and proliferation (30); increases extracellular superoxide dismutase (31); may promote the oxidation of low-density lipoprotein (32,33); increases collagen deposition (34); and impairs nitric oxide (NO) dependent vasodilation (35). It has also been proposed to play a role in lipid accumulation via induction of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase mRNA synthesis (36). These properties could create an environment of oxidative stress to facilitate the progression of atherosclerosis and the formation of unstable plaques.

In terms of thrombosis, homocysteine in vitro enhances endothelial-cell associated factor V activity (37) and impairs inactivation of factor Va by activated protein C (38); inhibits the binding of anti-thrombin III to the endothelium (39); reduces endothelial binding sites for tissue plasminogen activator (40); decreases cell surface thrombomodulin and protein-C activation (41); induces endothelial tissue factor activity (42); and stimulates platelet activation and aggregation in rats (43).

Homocysteine and Restenosis

Recent studies also postulate a role of homocysteine in restenosis following balloon angioplasty and stenting (44). Restenosis is a major limiting factor in the long-term success of percutaneous coronary intervention (PCI) with plain old balloon angioplasty (POBA) or coronary stenting. In POBA, restenosis is thought to occur from a combination of elastic recoil, negative arterial remodeling, and neointimal hyperplasia (45). In stented lesions, elastic recoil and negative remodeling are limited, and restenosis depends more critically on the degree of neointimal hyperplasia. Neointimal hyperplasia and vascular remodeling is likely a complex response to arterial injury. Circulating homocysteine could exacerbate restenosis by promoting this injury response, possibly through its oxidant and thrombogenic properties, its toxic effects on the endothelial cells and endothelial function, and its stimulatory effects on smooth muscle proliferation.

Animal data supports a possible role of homocysteine in the pathogenesis of restenosis after balloon angioplasty; diet-induced mild to moderate hyperhomocysteinemia in the rat carotid artery balloon-injury model attenuated re-endothelialization after injury and enhanced neointimal formation (46). Folate administration diminished this neointimal response. Continuous intraperitoneal infusion of homocysteine in rats after carotid artery balloon injury also increased intimal hyperplasia compared to controls (47). Recent clinical data also provide support to the notion that homocysteine may impact restenosis associated with

PCI. A small, prospective angiographic study showed no relationship between baseline homocysteine level and the incidence of restenosis at 6 months after POBA stenting; however, the majority of patients had baseline homocysteine levels within the normal range (mean 10.1 ± 3.7 μmol/L) (48). Homozygosity of the thermolabile mutant of the MTHFR was associated with greater target lesion restenosis by angiography and greater intimal hyperplasia area by intravascular ultrasound at 6 months after Palmaz-Shatz stent placement (49). Interestingly, in this study there was no significant difference in total homocysteine levels between MTHFR genotypes. This relationship between thermolabile MTHFR and restenosis was not found in a prospective angiographic study of POBA and stenting (1). Thus, the clinical evidence that homocysteine may play an important role in restenosis is not entirely consistent.

Homocysteine and Endothelial Dysfunction

A number of in vivo studies have analyzed the relationship between homocysteine levels and endothelial dysfunction in healthy volunteers and in patients with cardiovascular disease. The normal endothelium regulates local vascular tone and growth, thrombosis and thrombolysis, leukocyte adhesion, and platelet aggregation. It does so by releasing various factors, including but not limited to NO, Von Willebrand factor, prostacyclin, and interleukins (50). There is evidence that endothelial injury initiates atherosclerosis, and that areas of atherosclerosis demonstrate endothelial dysfunction, manifested by abnormalities in endothelin-dependent relaxation. These abnormalities are likely due to decreased bioavailability of NO. Furthermore, decreased NO has been associated with increased platelet aggregation, leukocyte adhesion, and vascular smooth muscle cell proliferation (51), all of which contribute to the atherothrombotic cascade.

There is laboratory evidence that homocysteine may deplete vascular stores of NO. Sulfhydryl compounds in general form reactive oxygen species by auto-oxidation; some of these species (O2-) combine with NO to form peroxynitrite (ONOO-) and thereby decrease NO bioavailability (52). Furthermore, at a concentration of 50 μmol/L, homocysteine uniquely inhibits the activity and production of glutathione peroxidase, an enzyme that prevents NO inactivation through its anti-oxidant activity (53).

In vivo data show that homocysteine may promote endothelial dysfunction, and that this dysfunction may arise from changes in NO bioavailability due to oxidative stress. Flow-mediated vasodilatation (FMVD), a marker of endothelial function, is significantly less in elderly hyperhomocysteinemic subjects (mean fasting tHcy 19.2 ± 0.8 μmol/L) without clinically evident atherosclerosis than in age- and sex-matched controls (54). The acute hyperhomocysteinemia induced by an oral methionine load significantly impairs FMVD in healthy subjects (55). One week pretreatment with the anti-oxidant vitamin C prior to the oral methionine load prevents this impairment without any change in peak post-load homocysteine levels (56), which supports the hypothesis that homocysteine

decreases NO bioavailability via free radical formation. Furthermore, markers of coagulation and platelet aggregation increase after an oral methionine load in healthy patients, and this increase is blocked by pre-administration of vitamin E and vitamin C (57). In men with known CHD, 8 weeks of treatment with folate and B12 significantly improves FMVD compared to placebo; although total homocysteine levels also improve, after controlling for possible confounders, the improvement correlates independently only with the reduction in free homocysteine (58). This improvement occurs in patients with baseline total homocysteine in both the normal and abnormal range.

What conclusions about homocysteine and atherothrombosis can be drawn from these studies? First, one's interpretation must be guarded because endothelial dysfunction is a surrogate endpoint. No longitudinal study has shown that healthy patients with endothelial dysfunction will develop significant atherosclerosis, although there is other evidence that they are linked (50). The perils of using surrogate endpoints to establish risk, therapy, and outcome are reflected throughout the medical literature, most notably with the paradoxical association between estrogen and low-density lipoprotein (LDL) and endothelial dysfunction, as compared to estrogen and cardiovascular outcomes.

Second, the dysfunction induced by acute hyperhomocysteinemia in healthy volunteers may not be physiologically relevant in patients with chronic, mild-to-moderate hyperhomocysteinemia. The temporal perturbations in homocysteine concentration induced by a regular diet would be much smaller then that induced by the single large methionine bolus of the oral load. However, there is evidence that FMVD can be significantly impaired in healthy volunteers after oral doses one-tenth of that of the standard methionine load (59). Theoretically, then, chronic intake of a diet high in methionine (e.g., red meat) may promote chronic endothelial dysfunction via elevated homocysteine concentration, leading in time to clinical atherosclerosis.

Third, the beneficial effects of anti-oxidants vitamin C and E on endothelial dysfunction and coagulation parameters after a methionine load support the hypothesis that oxidative stress contributes to the pathogenesis of cardiovascular disease. However, in patients at high risk for cardiovascular events, vitamin E supplementation has no effect on cardiovascular outcomes (60).

In regard to the relative endothelial dysfunction found in patients with mild hyperhomocysteinemia *without clinical manifestations* of atherosclerosis, this association may not be causal but simply reflect that endothelial dysfunction and plasma homocysteine are co-markers of risk, reflecting the sub clinical atherosclerosis burden. Homocysteine elevation may be a response to, not a cause of, endothelial dysfunction (61).

The improvement in endothelial function in patients with coronary artery disease after supplementation with folate and B12 lends greater support to homocysteine as a true cardiovascular risk factor, in that modification of the homocysteine concentration favorably alters a surrogate cardiovascular endpoint. However, the significant correlation that was observed was between

improvement of endothelial function and free homocysteine, and this improvement was seen even in patients with normal total homocysteine levels. This makes theoretical sense since the free homocysteine concentration better represents the biologically active component of total body homocysteine. Although this finding is from a single study, it may mean that the monitoring of total homocysteine reduction will be an insensitive measure of response to treatment in prospective trials that examine cardiovascular outcomes after vitamin supplementation. Indeed, supplementation with B vitamins may result in a more pronounced reduction in total homocysteine than free homocysteine (62). Folate may also improve endothelial function in individuals with coronary artery disease through mechanisms independent of homocysteine lowering (63).

In summary, the studies of homocysteine and endothelial dysfunction in humans reinforce the association, but not the causal relation, between homocysteine elevation and atherothrombosis.

The standard weakness of extrapolating ex vivo findings to in vivo actions limits the application of this data to homocysteine's potential pathophysiologic role. Compounding this limitation, these studies use concentrations of homocysteine ranging as high as 10 mmol/L, which is orders of magnitude higher than that found circulating in vivo. The lowest concentration in these studies is 0.1 mM/L, which, although found in patients with homocystinuria, is more than 5-fold higher than the concentration associated with increased atherothrombotic risk in the general population. These studies thus act merely as starting points for further in vivo investigation.

SIGNIFICANCE OF HOMOCYSTEINE CONCENTRATION IN ACUTE ATHEROTHROMBOTIC EVENTS

The possible actions of homocysteine—promotion of endothelial dysfunction, enhanced tissue factor production, increased factor V activity, reduced tissue plasminogen activator and anti-thrombin III binding, and stimulation of platelet activation and aggregation—are all pathophysiologic mechanisms contributing to the thrombus formation that occurs during unstable plaque rupture. Through these pro-thrombotic effects, an elevated homocysteine concentration may potentially cause increased myocardial injury and portend a worse outcome. Theoretically, then, the homocysteine level measured during an acute event itself—for example, in the emergency department for chest pain—may possibly have prognostic significance.

There is data to suggest that homocysteine may contribute to the degree of myocardial damage. Homocysteine concentrations correlate with plasma markers of thrombosis (64) and peak troponin T (65) in patients presenting with acute coronary syndromes. Also in these patients, elevated total homocysteine levels on admission are correlated with higher peak cardiac troponin T (65). However, the data regarding an association between short-term outcomes and admission homocysteine are inconsistent, although it does appear that elevated total

homocysteine on admission for acute coronary syndromes may predict long-term events (66,67).

EPIDEMIOLOGICAL EVIDENCE FOR HOMOCYSTEINE AND ATHEROTHROMBOTIC DISEASE

Retrospective Analysis

McCully first noted the association between homocystinuria and the incidence of accelerated atherosclerosis and thromboembolism (68) through post-mortem examination of patients suffering from varied inborn errors of metabolism that all led to severe hyperhomocysteinemia. A large number of retrospective studies suggest an association between elevated homocysteine levels and atherothrombotic disease including peripheral vascular disease, cerebrovascular disease, and coronary artery disease. However, elevated fasting homocysteine levels have also been shown to be associated with such traditional markers of cardiovascular risk as male gender, advancing age, smoking, high cholesterol, high blood pressure, renal dysfunction, and sedentary lifestyle (10). Thus, an independent relationship of homocysteine with cardiovascular risk is difficult to establish.

The association between homocysteine and peripheral vascular disease in retrospective case-control studies is well established. One meta-analysis suggested an overall odds ratio of 6.8 (95% CI, 2.9–15.8) for peripheral vascular disease and elevated homocysteine (69). In a study of an elderly population taken from the Framingham cohort, elevated homocysteine concentrations were associated with extra-cranial carotid artery stenosis $\geq 25\%$, with an odds ratio of 2.0 in patients in the highest quartile (≥ 14.4 μmol/L) compared to those in lowest (≤ 9.1 μmol/L) (26). In addition, carotid intimal-medial thickness, a surrogate marker for cardiovascular risk, is significantly correlated to plasma homocysteine level (70,71). In nine retrospective case-control studies involving over 1700 patients, the estimated odds ratio for cerebrovascular disease was 2.5 (95% CI, 2.0–3.0) (69). A recent large meta-analysis of 30 studies from 1966 and 1999, the Homocysteine Studies Collaboration, revealed an odds ratio of 0.86 (95% CI, 0.73–1.01) for stroke for 25% lower homocysteine concentrations (72), equivalent to values post-folate supplementation (Table 1) (74). There is some evidence that homocysteine-associated events might be ischemic, not cardioembolic, in nature, caused by large vessel atherosclerosis and/or cerebral microangiopathy (22,27,75).

Retrospective studies have also demonstrated a strong, consistent association between homocysteine and coronary artery disease. Using 15 cross-sectional and case-control studies involving over 4200 individuals, Boushey et al. estimated an odds ratio for a 5 μmol/L increase in fasting total homocysteine of 1.6 in men and 1.8 in women (69). The Homocysteine Studies Collaboration recently compiled evidence from 30 studies and showed an odds ratio of 0.67 (95% CI, 0.62–0.71) for ischemic heart disease (IHD) for 25% lower homocysteine concentrations (Table 1) (72).

Table 1 Summary of Meta-Analyses Studying Relationship of Serum Homocysteine and Ischemic Heart Disease/Stroke

Source	Study types (of studies[a])	Sample size	Variable	Relative risk (95% CI)
Wald et al. 2002 (73)	Retrospective (based on + MTHFR mutation) (72)	16,849	MTHFR + +5μmol/L sHcy[b]	1.21 (1.06–1.39): IHD 1.31 (0.80–2.15): Stroke 1.42 (1.11–1.84): IHD 1.65 (0.66–4.13): Stroke
	Prospective (20)	3,820	+5μmol/L sHcy[b]	1.23 (1.14–1.32): IHD 1.42 (1.21–1.66): Stroke
Homocysteine Studies Collaboration, 2002 (72)	Retrospective (18)	7,761	−3μmol/L sHcy[a]	0.67 (0.62–0.71): IHD 0.86 (0.73–1.01): Stroke
	Prospective (12)	9,025	−3μmol/L sHcy[a]	0.83 (0.77–0.89): IHD 0.77 (0.66–0.90): Stroke

[a] Denotes a 3μmol/L (25%) decrease in sHcy concentration between study groups.
[b] Signifies a 5μmol/L increase in sHcy concentration between study groups.
Abbreviations: sHcy, serum homocysteine; IHD, ischemic heart disease; MTHFR, methylene tetrahydrofolate reductase; CI, confidence interval.

A meta-analysis of studies investigating the relationship between homocysteine and venous thrombosis showed a pooled estimate of the odds ratio of 2.5 (95% CI, 1.8–3.5) for a fasting homocysteine level greater than the 95% percentile, and 2.6 (95% CI, 1.6–4.4) for an elevated post-methionine load homocysteine level (76).

Prospective Analysis

In contrast to the strong and consistent retrospective epidemiological data that mild to moderate hyperhomocysteinemia is associated with a wide spectrum of atherothrombosis, the evidence gathered from prospective studies is rather inconsistent (77). With respect to coronary artery disease, the Physicians' Health Study showed a relative risk of 3.4 for myocardial infarction (MI) in subjects with elevated homocysteine at a 5-year follow-up, but the relationship was no longer significant at 7.5 years (77); in the Multiple Risk Factor Intervention Trial, there was no association detected between homocysteine concentration and heart disease, and homocysteine was weakly associated with C-reactive protein, an inflammatory marker (78); in the Atherosclerosis Risk in Communities Study, total homocysteine was not correlated with the incidence of CHD after accounting for other risk factors (79); and there was no association between plasma homocysteine quartile and the risk of coronary events among cases and controls from a healthy cohort in the Kuopio Ischemic Heart Disease Risk Factor Study (80). In contrast, the British United Provident Association Study found that the relative risk for mortality from IHD was 2.9 when comparing the highest and lowest quartiles of homocysteine concentration (>15.1 and <10.1), and that there was a continuous

dose-response relationship between homocysteine and coronary mortality (81). However, this odds ratio was adjusted for systolic blood pressure and apolipoprotein B, but not for other cardiovascular risk factors. Both a Norwegian study (82) and the Tromso health study (83) showed a graded relationship between mortality and homocysteine concentration.

Prospective studies of homocysteine and cerebrovascular disease provide inconsistent data as well. A nested case-control study of individuals within the British Regional Heart Study cohort—a group of patients ages 40–59 randomly selected from 24 towns in Britain—showed a graded increase in the relative risk of stroke in ascending quartiles of total homocysteine concentration, with an odds ratio of 2.8 in the fourth quartile in relation to the first (84). These patients had no history of stroke at baseline. In a prospective examination of elderly people in the Framingham cohort without history of stroke, individuals with non-fasting total homocysteine concentrations in the highest quartile had a relative risk of 1.82 (95% CI, 1.14–1.92) for stroke over 9.9 years of follow-up compared to individuals in the lowest quartile (85). This association is supported by a cohort study of elderly nursing home patients, which showed by Cox regression analysis a risk ratio for new stroke of 1.079 (95% CI, 1.038–1.121) for each micromole increase in plasma homocysteine in 31 months of follow-up (86). In a Dutch cohort study, high baseline levels of homocysteine were associated with a higher baseline prevalence of stroke, and were associated prospectively with an increased incidence of stroke in normotensive, but not hypertensive, subjects (87). However, in the healthy males (mean age, 59 years) of the Physicians' Health Study, the association between plasma homocysteine concentration and cerebrovascular events was not significant (88). A similar negative finding was reported in a Finnish population-based study of individuals between the ages of 40 and 64 years (89). Two large meta-analyses of prospective studies have recently been published. The Homocysteine Studies Collaboration took data from 30 studies between 1966 and 1999 and the results showed an odds ratio of 0.83 (95% CI, 0.77–0.89) for IHD and 0.77 (95% CI, 0.66–0.90), for stroke (Table 1) (72). Another large meta-analysis of 72 studies by Wald et al. used populations with MTHFR mutation to determine odds ratios in response to the standard of 5μmol/L increase of serum homocysteine (SHcy) (Table 1). The results indicate an odds ratios of 1.23 (95% CI, 1.14–1.32) for IHD, and 1.42 (95% CI, 1.21–1.66) for stroke (73).

The inconsistent results of prospective studies relating homocysteine with atherothrombosis stand in contrast to the strong evidence from retrospective studies. The discrepant findings may be due to several factors:

Confounding bias—The strong association between homocysteine and traditional cardiovascular risk factors (10) raises the possibility that an element of confounding contributes to the retrospective association of homocysteine and atherosclerosis.

Sampling bias—Homocysteine concentrations have been shown to rise up to nearly 40% in the days to months following an acute atherothrombotic event

(12,54). Thus, the sampling of homocysteine concentration in retrospective studies in the days to months after an event could well overestimate the association between homocysteine and risk. In contrast, the deterioration of stored samples could contribute to the lack of significant association found in the prospective studies. The analysis of samples stored over a decade, however, does not support this possibility (90).

Elevated homocysteine as a marker of disease—An alternative explanation for the strong, retrospective association between homocysteine and atherothrombosis is that an elevated homocysteine concentration is a measure of the subject's total atherothrombotic burden and may reflect chronic tissue injury (61). Some studies show higher homocysteine levels with greater degrees of atherosclerosis (91). An association between homocysteine and disease also may be seen in prospective studies if "healthy" cases have "subclinical" atherosclerosis and elevated homocysteine at the time of blood sampling (92).

Lack of power—Prospective studies lack sufficient power because of small sample sizes, generally healthy subjects with lower mean total homocysteine levels, and a low incidence of disease. For example, the Finnish study of cerebrovascular events (89) had a mean homocysteine of 9.0 μmol/L, lower than that of most of the retrospective studies. More recent meta-analyses have achieved a higher statistical power by being able to analyze multiple study populations. This power has enabled a highly significant association to be made between SHcy concentration and IHD as well as stroke (73). However, to fulfill the requirement of a risk factor, the ability to decrease this risk must be provided by prospective therapeutic interventional trials targeting homocysteine levels.

DOES REDUCTION OF ELEVATED HOMOCYSTEINE IMPACT ATHEROTHROMBOTIC DISEASE?

Therapeutic Options for Lowering Elevated Homocysteine

As noted previously, homocysteine concentration is inversely related to the levels of vitamin B12, folate, and to a lesser extent, vitamin B6 (24). The elevated homocysteine in patients with thermolabile MTHFR is manifested predominantly in those with low serum folate (20). One meta-analysis of vitamin supplementation in patients with mild to moderate hyperhomocysteinemia reports that folate supplementation in doses of 0.5 to 5 mg/day significantly reduces total homocysteine concentration (74). A greater degree of reduction is observed in those with higher pretreatment homocysteine concentration (top quintile, approximately 40% proportional reduction) and in those with lower pretreatment folate concentration. After standardization to the approximate average concentrations for Western populations, treatment with folate lowers homocysteine concentrations by 25% (CI 23% to 28%). Supplementation with vitamin B-12 (dosage 0.02–1 mg/day, mean 0.5 mg) produces a small additional effect of about 7% (93). Vitamin B6 treatment alone does not lower fasting total

homocysteine concentrations but does reduce post-methionine load concentrations, although not as much as in combination with folate (94). This finding is consistent with the role of vitamin B6 as a co-factor in the transsulfuration pathway of homocysteine to cysteine. Betaine-dependent remethylation of homocysteine to methionine occurs in the liver. Betaine supplementation in patients with homocystinuria significantly decreases total homocysteine concentration (95). In healthy patients, betaine supplementation significantly reduces fasting homocysteine, but to a far lesser degree than folate (96). Choline, a precursor to Betaine, can serve as a dietary supplement in phosphatidylcholine. This may act as another mechanism to decrease fasting and post-methionine load homocysteine concentrations in serum (97).

Vitamin supplementation may have benefits independent from homocysteine reduction. For example, folate, by stimulating tetrahydrobiopterin regeneration (98) and counteracting homocysteine inhibition of eNOS (99,100), may improve NO availability. In addition, vitamin B6, via its effects on the glutathione anti-oxidation system (101), could assuage the oxidant stress associated with hyperhomocysteinemia (102).

Table 2 summarizes the evidence base of homocysteine-lowering intervention on cardiovascular outcomes.

Impact on Surrogate Outcomes

Several lines of evidence suggest that vitamin supplementation in patients with elevated homocysteine affects surrogate clinical endpoints (Table 2). As noted previously, homocysteine-lowering with folate and vitamin B12 treatment improves endothelial dysfunction in subjects with coronary heart disease (58). Vitamin treatment decreases the incidence of positive stress electrocardiograms in relatives of patients with mild to moderately elevated homocysteine (107). Patients treated with a combination of folate, vitamin B-12, and vitamin B6 show a significant regression in carotid plaque area, even in those with a homocysteine concentration less than 14 μmol/L (108). A recent trial by Marcucci et al. demonstrated a significant benefit of therapeutic correction of hyperhomocysteinemia in renal transplant patients who are at high risk for cardiovascular disease. These high-risk patients were randomly assigned to receive vitamin supplementation (folic acid 5 mg/day, vitamin B6 50 mg/day, vitamin B12 400 μg) or placebo. Carotid intima-media thickness (cIMT) was evaluated after 6 months via ultrasound to serve as a surrogate marker for cardiovascular disease. Patients in the treatment group were found to have a mean cIMT decrease of $32 \pm 13\%$, while in the control group cIMT increased by a mean of $23 \pm 21\%$ (Table 2) (106). This significant discrepancy adds support to the hypothesis that homocysteine may be an important risk factor for cardiovascular disease.

In contrast to the positive studies, a recent study failed to demonstrate any beneficial effect of homocysteine-lowering on inflammatory markers that have been implicated in atherothrombosis. In a double-blind, randomized, placebo-controlled

Table 2 Summary of Randomized Controlled Trials Investigating the Impact of Homocysteine-Lowering Therapy on Cardiovascular Outcomes

Study	Sample size	Study duration	Treatment groups	Outcomes relative risk (95% CI)
Vascular disease: Schnyder et al. 2001 (44)	272 281	6 months	1 mg FA, 10 mg B6, 0.4 mg B12 versus placebo	**0.46 (0.28–0.73): Restenosis** 0.52 (0.28–0.98): MACE 0.48 (0.25–0.94): TLR
Schnyder et al. 2002 (103) (The Swiss heart study)	272 281	1 year	1 mg FA, 10 mg B6, 0.4 mg B12 versus placebo (x 6 months)	**0.68 (0.48–0.96): MACE** 0.62 (0.40–0.97): TLR 0.60 (0.24–1.51): Nonfatal MI 0.52 (0.13–2.04): cardiac death 0.54 (0.16–1.70): mortality
Lange et al. 2004 (104)	316 320	6 months	1 mg FA, 5 mg B6, 1 mg B12 IV[a] x1+ 1.2 mg FA, 48 mg B6, 60μg B12 versus placebo	**1.30 (1.0–1.69): Restenosis** 1.5 (1.0–2.3): MACE
Toole et al. 2004 (105) (The VISP trial)	1827 1853	2 years	2.5 mg FA, 25 mg B6, 0.4 mg B12 versus 0.02 mg FA, 0.2 mg B6, 6 μg B12	**1.0 (0.8–1.3): Recurrent stroke** 0.9 (0.7–1.2): MACE 0.9 (0.7–1.1): mortality
Renal disease: Marcucci et al. 2003 (106)	25 28	6 months	5 mg FA, 50 mg B6, 4 mg B12 versus placebo	32+13% decrease in CIMT 23+21% increase in CIMT
Nonpatient population: Vermeulen et al. 2000 (107)	78 80	2 years	5 mg FA, 250 mg B6 versus placebo	0.9 (0.6–1.3): ABI 1.0 (0.3–4.1): PAD 0.9 (0.5–1.6): carotid stenosis 0.4 (0.2–0.9)+stress ECG

[a] An initial intravenous (IV) bolus dose of vitamins was followed by oral maintenance therapy.

Abbreviations: CI, confidence interval; FA, folic acid; B6 and B12, vitamin B6 and vitamin B12; MACE, major adverse cardiac events (cardiac-cause death, nonfatal MI, revascularization); TLR, target lesion revascularization; TVR, target vessel revascularization; CIMT, carotid intima: media thickness; ABI, ankle-brachial index; PAD, peripheral arterial disease; ECG, electrocardiogram. Primary endpoints are shown in bold fonts.

trial among 381 men and 159 postmenopausal women with homocysteine concentrations of 13 μmol/L at screening, the effect of folic acid supplementation (0.8 mg/d) versus placebo for 1 year was investigated on inflammatory markers—serum concentrations of C-reactive protein, soluble intercellular adhesion molecule-1, oxidized low-density lipoprotein, and autoantibodies against oxidized LDL (109). Despite a 4-fold increase in serum folate and a 25% reduction in homocysteine levels, no changes in plasma concentrations of the inflammatory markers were observed with treatment.

Impact on Clinical Outcomes

The evidence that vitamin supplementation reduces cardiovascular clinical outcomes in patients with elevated homocysteine is incomplete and inconsistent. One recent large, multicenter, double-blind randomized active control study, the Vitamin Intervention for Stroke Prevention (VISP) trial, failed to demonstrate significant risk reduction in recurrent stroke (primary endpoint) or CHD events (secondary endpoint) in high-risk individuals in response to high-dose homocysteine-lowering therapy for 2 years (Table 2) (105). However, this trial was not placebo-controlled but, rather, a head-to-head comparison of daily high-dose vitamin formulations (folic acid 2.5 mg, vitamin B6 25 mg, vitamin B12 0.4 mg) against low-dose daily formulations (folic acid 20 μg, vitamin B6 200 μg, and vitamin B12 6 μg).

Several studies have investigated the effect of homocysteine lowering on restenosis following percutaneous coronary intervention (PCI). A prospective, double-blinded, randomized clinical trial of folate (1 mg/day), vitamin B6 (10 mg/day), and vitamin B12 (400 μg/day) therapy in 205 patients after coronary angioplasty and/or stenting provides the most compelling evidence for the role of homocysteine in post-angioplasty restenosis (Table 2) (44). These patients had baseline normal-to-mild hyperhomocysteinemia (11.1 ± 4.3 μmol/L). Combination therapy with folate, vitamin B12, and vitamin B6 provided a statistically significant 18% absolute risk reduction (19.6% vs. 37.6%, $p = 0.01$) in the primary endpoint of angiographic restenosis (defined as a stenosis of $> 50\%$ at 6-month follow-up). This benefit was primarily observed in patients undergoing angioplasty (10.3% vs. 41.9%, $p < 0.001$), but not stenting (20.6 vs. 29.9%, $p = 0.32$).

The Swiss Heart Study was a randomized, controlled trial that aimed to evaluate the impact of homocysteine-lowering therapy on clinical outcomes (major adverse cardiac outcomes) in 553 patients treated with angioplasty with or without stenting (Table 2) (103). Although homocysteine-lowering therapy did show significant risk reduction in overall adverse outcomes, the benefit was driven primarily by a reduced rate of target lesion revascularization with no significant impact on nonfatal MI or death (103).

In a recent placebo-controlled trial, Lange et al. examined the effect of vitamin therapy at higher doses (folic acid 1.2 mg, vitamin B6 48 mg, vitamin B12 0.06 mg) following an initial intravenous bolus loading dose, on 636 patients following successful coronary stenting with bare-metal stents (Table 2) (104).

The multi-vitamin therapy successfully reduced SHcy levels, but contrary to the previous results by Schnyder et al., the folate therapy actually resulted in significantly increased restenosis and target vessel revascularization rates at 6 months, although clinical endpoints were not affected. The divergent findings of the two studies may be reconciled by considering differences with respect to treatment dose (intravenous loading dose plus higher folate and vitamin B6 maintenance dose in the Lange study), mode of PCI (100% stenting vs nearly 50% in the Schnyder study), lesion length (shorter in Lange et al.), patient population (higher risk in the Schnyder study—more smokers, diabetics and, previous history of myocardial infarction), SHcy levels (lower baseline levels in Schnyder et al.), and angiographic follow-up (only 76% follow-up in Lange et al.). While the risk of high-dose homocysteine-lowering therapy in post-stent patients is of some concern, this is unlikely to have an impact on the overall incidence of coronary restenosis in clinical practice in the United States given the widespread adoption of drug-eluting stents (nearly 85% of all PCIs).

In summary, the evidence generated from intervention studies is inconsistent. More reliable evidence is needed to recommend routine homocysteine-lowering therapy for modifying atherothrombotic vascular risk. Several large-scale studies are currently underway in the United States, Canada, and Europe to examine the effects of lowering blood homocysteine levels on the incidence of heart attacks and/or strokes: the Norwegian Vitamin Interventional Trial (NORVIT), the Western Norway B-vitamin Intervention Trial (WENBIT), the Study of Effectiveness of Additional Reduction in Cholesterol and Homocysteine (SEARCH), the Prevention with a Combined Inhibitor and Folate in Coronary Heart Disease (PACIFIC) Trial, the Vitamins to Prevent Stroke (VITATOPS), etc. One will have to wait for these trial data to accrue before one can confirm or refute the homocysteine hypothesis in atherothrombosis.

CONCLUSION

Severe elevation of homocysteine concentration in patients with homocystinuria leads to a high incidence of premature atherothrombotic events. In vitro and in vivo studies demonstrate a plethora of biologically plausible mechanisms that implicate homocysteine in promoting atherosclerotic and thrombotic vascular disease. Numerous observational studies have also reported on the association between mild to moderately elevated homocysteine levels and vascular risk in both the general population and in those with preexisting vascular disease. In general, the risk for vascular disease is small with prospective, longitudinal studies reporting substantially weaker associations between homocysteine and atherothrombotic vascular disease than retrospective case-control and cross-sectional studies. It is unclear whether a causal relationship exists between homocysteine and cardiovascular risk, if homocysteine is related to other confounding cardiovascular risk factors, or if homocysteine is a marker of existing disease burden. Routine screening for elevated homocysteine is not yet

recommended (1,104). However, screening may be advisable for individuals who manifest atherothrombotic disease that is out of proportion to their traditional risk factors or who have a family history of premature atherosclerotic disease. Vitamin supplementation with folate, B6, and B12 significantly lowers homocysteine concentration and has also been shown to alter surrogate cardiovascular endpoints. Although there is incomplete evidence that vitamin supplementation reduces cardiovascular risk, treatment with low doses is safe and inexpensive. Whether homocysteine is causative in the pathogenesis of atherothrombotic vascular disease will have to await the completion of a number of large, randomized controlled trials studying the effect of homocysteine-lowering vitamins on cardiovascular end points. Until then, the status of homocysteine as a risk factor remains unresolved.

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5

Inflammation, Inflammatory Markers, and Cardiovascular Risk

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INTRODUCTION

Several lines of evidence suggest that inflammatory processes play a critical role in the initiation, progression, and destabilization of atherosclerosis (1–6). Most of the known risk factors for atherosclerosis are believed to trigger inflammatory gene induction/activation in the arterial wall leading ultimately to recruitment, retention, and activation of mononuclear inflammatory cells (monocytes-macrophages, T-cells, mast cells, and dendritic cells) in the subendothelial space. These inflammatory cells contribute to the initiation, growth, and eventual disruption of atherosclerotic plaques leading to thrombosis and acute clinical vaso-occlusive events (1–6). These pathophysiologic underpinnings are supported by accumulating evidence that circulating markers of inflammation can provide important prognostic information across a wide spectrum of clinical settings in a broad array of patient populations, and that such information is often additive and incremental to that obtained from standard markers of cardiovascular risk (7,8). A series of experimental observations further raise the possibility that some of these inflammatory markers such as C-reactive protein (CRP) may not simply be markers but instead may also play a causal role in the pathophysiology of athero-thrombosis through a variety of putative biological actions (9–11).

ROLE OF INFLAMMATION IN ATHEROGENESIS, PLAQUE DISRUPTION, AND THROMBOSIS (FIG. 1)

Atherosclerosis-prone areas include sites of oscillating shear stress where atherogenic lipoproteins appear to be preferentially retained within the arterial wall leading to their oxidative modification and aggregation. According to the prevailing paradigm, a number of stimuli injure or activate vascular endothelium. These stimuli include oscillating shear stress, oxidative stress, elevated levels of atherogenic lipoproteins, modified lipoproteins, hyperglycemia, elevated levels of angiotensin II, low levels HDL, elevated levels of homocysteine, high blood pressure, possibly chronic infections, immune activation, and cigarette smoke. Endothelial activation leads to enhanced expression of several pro-inflammatory genes, which include leukocyte adhesion molecules such as VCAM-1 (vascular cell adhesion molecule) and ICAM-1 (intercellular adhesion molecule) and E-selectin, which in concert with chemokines such as MCP-1 (monocyte chemotactic protein) and interleukin-8, lead to subendothelial recruitment of mononuclear leukocytes (1,12). In the vessel wall, monocytes are exposed to other cytokines such as M-CSF (macrophage colony stimulating factor) induced by retained oxidatively modified lipoproteins, leading to differentiation of monocytes into macrophages. Macrophages ingest lipoproteins through their non-downregulatable scavenger receptors leading to formation of foam cells. Eventually, chemotactic factors and growth factors for smooth muscle cells are induced leading to accumulation of matrix synthesizing smooth muscle cells in the arterial intima leading to the formation of the fibrofatty lesion of atherosclerosis (1,12). Several experimental studies, using gene knockout strategies, have established a key relationship between inflammation and atherogenesis. Thus deficiency of MCP-1, interleukin-8, and M-CSF have been shown to inhibit atherogenesis despite severe hyperpelipidemia in murine models (1,12).

In the clinical setting of atherosclerotic vascular disease, the majority of acute and potentially serious ischemic manifestations are triggered by the superimposition of a thrombus on a disrupted atherosclerotic plaque (2–5,13,14). Atherosclerotic plaques containing a large lipid-rich core and active inflammation are believed to be vulnerable to disruption (so called vulnerable plaques). The integrity of the protective collagen-rich fibrous cap that normally segregates the deeper lipid-rich components of the advanced atherosclerotic plaque from circulating blood, is dependent upon the balance between matrix synthetic and degradative activities within the plaque. While smooth muscle cells synthesize matrix, inflammatory cells may contribute to matrix degradation. Disruption of the fibrous cap is believed to result from excessive matrix degrading activity within the plaque, attributed largely to a family of matrix degrading proteolytic enzymes such as MMP's (matrix degrading metalloproteinase) and other proteases, produced by the inflammatory cells (2–5,13,14). Inflammatory cells in the atherosclerotic plaque are also the major source of tissue factor, a key initiator of the coagulation cascade (2–5,13,14).

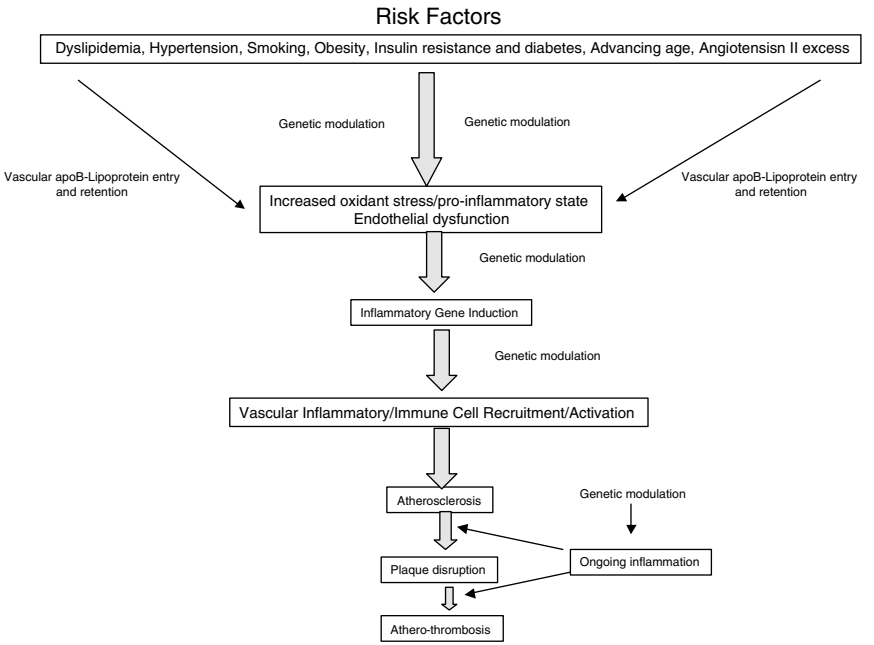

Figure 1 Role of inflammation in atherogenesis, plaque disruption, and thrombosis.

Thus lipid-rich regions of atherosclerotic plaque are impregnated with tissue factor derived from apoptotic inflammatory cells, accounting for its enhanced thrombogenicity (2–5,13,14). Taken together, a considerable amount of evidence implicates inflammation in initiation and destablization of atherosclerosis.

SYSTEMIC MARKERS OF INFLAMMATION AND CARDIOVASCULAR RISK

As the inflammatory paradigm of atherothrombosis is becoming a mainstream pathophysiologic concept, investigators have been examining the value of several systemic markers of ongoing inflammation as potential predictors of cardiovascular risk. These systemic markers include CRP, interleukin-6 (IL-6), tumor necrosis factor alpha, CD 40 ligand, myeloperoxidase, serum amyloid A, soluble cell adhesion molecules (VCAM-1 and ICAM-1, P-selectin), metalloproteinases such as MMP-9, pregnancy-associated protein (PAP), and lipoprotein-associated phospholipase A-2 (LP-PLA2), myeloperoxidase, and adipocytokines such as the anti-inflammatory cytokine adiponectin (Table 1) (7,8,15–28).

Table 1 Systemic Inflammatory Markers

C-reactive protein
Lipoprotein-associated phospholipase A-2
Interleukin-6, Interleukin-1
Pregnancy-associated protease
Matrix metalloproteinase-9
Soluble adhesion molecules: vascular cell adhesion molecule, intercellular adhesion molecule, P-selectin
CD 40 ligand
Tumor necrosis factor alpha
Myeloperosidase
Adiponectin (anti-inflammatory cytokine)

C-Reactive Protein (CPR)

Among the various markers, considerable attention has been devoted to circulating levels of CRP as a risk indicator. CRP is an acute phase protein with a plasma half life of 19 hours, named for its ability to precipitate the C-polysaccharide of *Pneumococcus*. It is a member of the pentraxin family of calcium dependant ligand-binding plasma proteins involved in the innate immune system produced almost exclusively by liver in response to IL-6 after tissue injury, infection, or other inflammatory stimuli. The human CRP molecule consists of five identical non-glycosylated polypeptide chains each containing 206 amino acids (29). The CRP levels in blood can be measured accurately and reproducibly down to very low levels using recently developed high sensitivity assays. It is a stable molecule with a long half life and does not exhibit circadian variation. Subjects in the general population tend to have stable CRP levels characteristic for each individual except for occasional increases associated with minor or subclinical infections, trauma, or inflammation. Twin studies show a highly significant genetic basis for CRP levels which is independent of age and body mass index. The levels of CRP may also be regulated by genetic variations (30–32).

The precise mechanisms of CRP elevation in vascular disease are not well understood and several potential mechanisms have been suggested, which include: (1) increased release of IL-1 and IL-6 from inflammatory foci within atherosclerotic plaque leading to hepatic overproduction of CRP; (2) myocyte damage resulting from acute vascular occlusion resulting in an acute phase response; and (3) systemic stimuli (such as chronic infections) that create chronic inflammation that leads to increased CRP levels as well as to vascular inflammation.

C-Reactive Protein and Vascular Disease Risk in Subjects with No Known Vascular Disease or with Subclinical Vascular Disease

Several prospective studies have shown that, among individuals with no known cardiovascular disease or known pre-clinical vascular disease, elevated CRP levels

measured by high sensitivity assays, are associated with an increased future risk of cardiovascular events (18,33–42). These predictive relationships were shown to persist even after correction for other known risk factors such as smoking status, lipoprotein profile, and fibrinogen levels and were also observed in women. Ridker and colleagues have recently demonstrated that the adverse prognostic value of elevated CRP levels is additive to that of lipoprotein variables (43).

The recent findings of the Reykjavik study (44), supplemented by a meta-analysis of all 22 prospective studies, have indicated that overall an elevated CRP provides only a modest incremental prognostic value over other traditional risk factors with an overall adjusted odds ratio of about 1.5 (95% CI: 1.25 to 1.68) for the highest third compared to the lowest third of CRP values (44). In contrast the corresponding odds ratio was 2.4 for elevated cholesterol and 1.9 for smoking (44). Recent studies have also suggested that CRP levels may not be an independent predictor of first cardiovascular events in elderly patients, which is in contrast to most studies that have involved middle-aged patients (45). Elevated CRP levels have also been noted with obesity, insulin resistance, type II diabetes (24,46–52), and depression (53). Elevated CRP and IL-6 levels were recently shown to predict an increased risk of future development of type II diabetes in the Women's Health Study (54).

C-Reactive Protein and Vascular Risk in Patients with Known Chronic Vascular Disease

Similarly, in a prospective European Trial involving 2121 patients with angina, elevated CRP levels at baseline were associated with an increased risk for future non-fatal myocardial infarction or sudden cardiac death with an odds ratio of 1.81 for patients in the fifth quintile of CRP (55).

C-Reactive Protein and Vascular Risk in Patients with Unstable Angina Syndromes

Following an initial description by Berk et al. in 1990, several studies involving patients with unstable angina/non-q myocardial infarction, elevated CRP was shown to be associated with increased short-term risk of recurrent ischemic events (56–60). Furthermore, the prognostic value appeared to be additive to that of cardiac specific troponin in the TIMI study and incremental to other risk factors including troponin-T in another study (59,60). In addition to short-term outcome, adverse prognostic implications of elevated CRP levels prior to discharge on long-term outcome was also shown (61–63). These findings have been contradicted by other studies that failed to observe any significant prognostic implications of an elevated CRP in patients with unstable angina.

C-Reactive Protein and Vascular Risk in Patients with Acute Myocardial Infarction

Acute myocardial infarction is associated with elevation of CRP levels, at least in part related to myocardial necrosis and a secondary inflammatory response.

Several studies in patients with acute myocardial infarction have shown that elevated CRP levels are associated with increased risk of cardiovascular complications including cardiac rupture and that successful reperfusion is associated with a fall in elevated CRP levels beyond that predictable on the basis of infarct size reduction (58,64–66).

C-Reactive Protein and Therapeutic Interventions

In as much as CRP levels are elevated in presence of modifiable risk factors such as obesity, insulin resistance, and cigarette smoking, emphasis on vigorous risk modification is appropriate in such cases to reduce the pro-inflammatory state and cardiovascular risk.

Elevated cardiovascular risk associated with elevated levels of CRP in apparently healthy subjects was shown to be attenuated by use of aspirin in the Physicians Health Study (18). The group in the lowest quartile of risk (based on CRP) had only a 14% relative risk reduction, whereas the group in the highest quartile of risk experienced a robust 56% relative risk reduction with aspirin. It is interesting to note that aspirin in conventional antithrombotic doses does not reduce circulating CRP levels. Several studies have shown that CRP levels fall with initiation of statin therapy, often independent of LDL lowering, consistent with their anti-inflammatory effects (67–70). Although some of the studies have suggested that the decrease in CRP with statins occurs independent of LDL lowering, additional studies are needed to definitively prove whether CRP lowering is or is not related to lipid (both LDL and non-LDL lipid fractions) modifying effects of statins. Furthermore, data from the secondary prevention CARES trial showed that pravastatin was most effective in reducing cardiovascular risk in the subgroup with elevated CRP levels (67). Most recently, retrospective analysis of the AFCAPS/TEXCAPS primary prevention trial also showed that increased cardiovascular risk associated with elevated CRP levels was reduced by statin therapy (lovastatin), even in subjects with average or below average cholesterol levels (71).

Since nearly half of all myocardial infarctions occur in subjects with average or below average cholesterol levels, these tantalizing observations if confirmed in prospective trials would provide a useful way of selecting a relatively high-risk subset of such individuals who could benefit from statin therapy despite average or below average cholesterol levels. Analysis of two recent trials of low (pravastatin, 40 mg) and high dose statin (atorvastatin, 80 mg) therapy in patients with established coronary heart disease showed that patients achieving a reduction in CRP levels had better overall clinical outcomes across all levels of LDL cholesterol, with the best outcomes achieved among patients with LDL below 70 mg/dl and CRP lowering to levels below 2 mg/L (72,73). These intriguing observations raise an interesting question: Should CRP monitoring supplement LDL monitoring in patients receiving statins or, for that matter, other risk modifying therapies? Answers to this question must await additional prospective trials such as the JUPITER trial, which is currently ongoing.

C-Reactive Protein and Pathogenesis of Athero-Thrombosis

It has been argued that downstream cellular effects of CRP may directly contribute to athero-thrombosis thus serving as a risk factor in addition to being a risk marker. Recent data suggest that CRP may be also produced by vascular wall cells where it may have pro-inflammatory effects and stimulate macrophage uptake of LDL, thereby contributing to the pathogenesis of athero-thrombosis (9,10,74–78). Immunoreactive CRP has been identified in atheromatous plaques and aggregated CRP binds to LDL, whereas native CRP binds to oxidized and aggregated LDL leading to complement activation (29,75,76,79–82). Complement activation, known to be potentially involved in atherogenesis, may be one potential mechanism by which CRP could contribute to atherogenesis. Other potential cellular mechanisms that may contribute to pro-atherogenic effects of CRP include its ability to stimulate LDL uptake by cells facilitating foam cell formation, induce tissue factor expression in monocytes in culture, and induce the expression of pro-inflammatory molecules in cell culture (77,83–85). Other investigators have attributed these pro-inflammatory effects of CRP in cell culture to contaminants such as azide and endotoxin present in the commercial preparations of CRP (29,86). Murine experiments have both supported as well as refuted pro-atherogenic effects of over-expression of CRP (87–91). Thus, the issue of pro-atherothrombotic effects of CRP and their relevance to human disease remains uncertain and further investigation of this intriguing concept is warranted.

Inflammatory Markers Other than C-Reactive Protein: Lipoprotein-Associated Phospholipase A-2 as a Risk Marker

Although CRP has been the most studied of systemic inflammatory markers, other proteins that are involved in inflammatory cascades such as IL-6, serum amyloid A, and soluble leucocyte adhesion molecules such as VACM-1 and ICAM-1, P-selectin, PAP, and Lp-PLA2, myeloperoxidase, and adiponectin have also been evaluated as potential biomarkers of vascular risk.

Lipoprotein-Associated Phospholipase A-2 as a Risk Marker

Among these markers, Lp-PLA2 has undergone fairly extensive evaluation as a risk marker as well as a potential risk factor for cardiovascular events (92). Lp-PLA2 (also known as platelet activating factor acetylhydrolase or PAF-AH) is a subtype of the phospholipase A2 superfamily that hydrolyzes phospholipids. Recent epidemiologic data have suggested that Lp-PLA2 levels using an immunoassay for its mass (PLAC test) may identify individuals at increased risk for cardiovascular events including stroke (92). An analysis of data from the primary prevention statin trial, the West of Scotland Coronary Prevention Study (WOSCOPS), demonstrated a modestly increased risk associated with increasing levels of Lp-PLA-2 after multivariate analysis that was independent of CRP, fibrinogen, white cell count,

and classical risk factors including smoking (93). Similar results were observed in the ARIC (atherosclerosis risk in communities) study where both elevated CRP and Lp-PLA2 independently and additively contributed to increased risk for cardiovascular events with hazard ratios ranging from 1.78 for Lp-PLA2 elevation, to 2.53 for CRP elevation, and to 2.95 for the combined elevation of Lp-PLA2 and CRP (94,95). Similar results were observed in the MONICA (monitoring trends and determinants in cardiovascular disease) cohort study (96) and the Rotterdam study (97). A smaller prospective nested case control study, however, failed to show a relationship between Lp-PLA2 and cardiovascular events after multivariate adjustment (98).

Several small prospective studies have also identified a modest relationship between the angiographic or electron beam computed tomographic evidence of coronary artery disease and Lp-PLA2 levels (92). Elevated levels of Lp-PLA2 have also been noted in a relatively small cohort of patients with acute coronary syndromes where an elevated level of Lp-PLA2 has been linked to increased cardiovascular risk (92).

Lipoprotein-Associated Phospholipase A-2 as a Contributor to Atherothrombosis

As in the case of CRP, it remains unclear whether Lp-PLA2 has pro-atherothrombotic effects in addition to being a marker of inflammation. In mice, Lp-PLA2 is mostly associated with HDL and experimental observations suggest an anti-atherogenic role for Lp-PLA2 in mice (92). However in humans, Lp-PLA2 is mostly associated with LDL and pro-atherogenic effects have been attributed to it, leading to the development of inhibitors of Lp-PLA2 activity as potential anti-atherosclerotic agents; these inhibitors are currently undergoing clinical evaluation (92). As in the case of CRP, statin therapy has also been shown to reduce Lp-PLA2 levels (92).

Adiponectin (An Adipose Tissue Derived Anti-Inflammatory Cytokine) and Cardiovascular Risk

Recently, a novel circulating cytokine, derived predominantly from adipocytes, called adipnectin, has been described (99). Adiponectin has anti-inflammatory, insulin-sensitizing effects and athero-protective actions (99). Circulating levels of adiponectin are generally reduced in obesity and metabolic syndrome and appear to be inversely related to future risk of myocardial infarction in healthy subjects largely independent of lipids, CRP, and other known risk factors (17). Additional studies are needed to define the role of this novel anti-inflammatory cytokine in cardiovascular disease protection and risk prediction.

CONCLUSION

A body of evidence implicates inflammation in the pathophysiology of atherothrombosis and thus systemic markers of inflammation could provide additional prognostic information in patients at risk for or with established disease. Extensive investigation of CRP has identified it as a lead candidate although other markers such as Lp-PLA2 and others could supplement or supplant CRP. The overall incremental value, over and above known risk factors, appears to be statistically significant but biologically modest in magnitude, perhaps belying the complex pathophysiology of atherothrombosis and the gaps in our knowledge. Continued investigation and refinements in biomarkers could, in the future, establish a clinically relevant role for inflammatory markers for prediction of risk and perhaps for monitoring the efficacy of therapy as well.

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6

Chronic Infections as Risk Factors for Atherothrombosis

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INTRODUCTION

Atherothrombotic vascular disease is the leading cause of death in most of the western nations and predicted to become the leading cause of death in the world over the next decade. A number of risk factors have been identified that increase the risk of atherothrombosis and they include family history of premature vascular disease, dyslipidemia, smoking, hypertension, insulin resistance and diabetes mellitus, obesity, atherogenic diet, hyperhomocystenemia, estrogen deficiency, and lack of physical activity. However, these conventional risk factors do not account for all of the attributable risk of atherothrombotic vascular disease. A search for additional risk factors has led to the idea that chronic infections may be an important risk factor. Chronic infections as potential culprits in the vascular inflammatory response have received renewed interest since the critical role of inflammation in the evolution, progression, and destabilization of atherothrombosis has been recognized (1–3). From a historical perspective, the idea that infections may contribute to atherosclerosis was suggested by several authors around the beginning of the 20th century (4,5). In fact, in 1911, Frothingham stated that "The sclerosis of old age may simply be a summation of lesions arising from infectious or metabolic toxins" (6). Based on examination of 400,000 sections from 40 necropsy cases, Leary coined the term "abscess" to

describe atheromatous plaques containing leucocyte infiltration (7). Based on recent seroepidemiologic data, human pathology, biological plausibility, experimental models, and pilot clinical trials, a potential causal link between chronic infections and atherothrombotic disease has received much attention in recent years (8–10).

VIRAL INFECTIONS AND ATHEROTHROMBOSIS

Experimental observations of Fabricant et al. suggested a link between cytomegalovirus (CMV) infection and atherosclerosis (11,12). In noncholesterolemic chickens, an avian herpes virus (Marek's disease virus) induced atherosclerotic lesions and increased cholesterol ester accumulation in aortic smooth muscle cells. Furthermore, virus-induced atherosclerosis could be prevented by a vaccine derived from turkey herpes virus (11,12). These early observations have been supported by experimental findings in rat models of atherosclerosis or accelerated allograft atherosclerosis (13) and in atherosclerosis-prone transgenic mice (14,15). CMV infection induces human arterial smooth muscle cell proliferation, possibly by inactivating p53, a pro-apoptotic tumor suppressor gene that stimulates cholesterol esters accumulation in smooth muscle cells and induces a prothrombotic phenotype in endothelial cells. These observations provide biologic plausibility to the potential causal link between CMV infection and atherothrombotic and proliferative vascular disease (16,17). However, seroepidemiologic data and data from direct examination of vascular tissue from humans have been less than persuasive for native atherosclerosis and negative or at best inconsistent for restenosis and transplant vasculopathy (18–25). Thus, data to definitively link CMV to atherosclerosis or restenosis are lacking. Other herpes viruses, hepatitis A and B virus, and the influenza virus have also been implicated in some but not in other studies. However, the evidence is indirect and far from established (26–28). Influenza epidemics were associated with significant increase in cardiovascular death (29) and in many patients the acute myocardial infarctions are preceded by an upper respiratory infection (30,31). The relationship of influenza infection and acute manifestations of coronary artery disease meet most of the Hill's criteria of causality: strength of association, consistency, temporal sequence, coherence, biologic plausibility, and experimental evidence. The criterion which is not met is analogy, which is the weakest criterion. Furthermore, observational studies have suggested protective effect of influenza vaccination against cardiovascular events, a 67% reduction in the risk of myocardial infarction, and a 50% reduction of risk in cardiac arrest and stroke (32). In a small randomized study by Gurfinkel and colleagues, cardiovascular death occurred in 2% of vaccinated versus 8% of the control patients ($p = 0.01$) (33). These data need to be confirmed in large, prospective trials.

BACTERIAL INFECTIONS AND VASCULAR DISEASE

Several bacteria have been implicated with atherothrombosis and include *Chlamydia pneumoniae, Helicobacter pylori* and *Porphyromonas gingivali* (2,3,10).

CHLAMYDIA PNEUMONIAE

C. pneumonia has received a lot of attention as a putative culprit in atherothrombosis. *C. pneumoniae* is an intracellular organism responsible for upper respiratory infections, pneumonia, and sinusitis. The prevalence of infection with *C. pneumoniae* increases with age so much so that up to 80% of people 65 years of age or older have evidence of exposure (34,35). *C. pneumonia* infection introduced through the respiratory tract increases early foam-cell type atherosclerotic lesions in normocholesterolemic and mildly hypercholesterolemic rabbits (36–39). *C. pneumoniae* infection augmented and accelerated atherosclerosis only in presence of hypercholesterolemia in murine models in some (40,41), but not in other studies (42). Although azithromycin treatment was shown to reduce augmented atherosclerosis from *C. pneumoniae* infection in cholesterol-fed rabbits (38), no such benefit was observed with azithromycin in murine atherosclerosis excacerbated by *C. pneumoniae* infection (43). The ability of *C. pneumoniae* or one or more of its structural components such as the heat shock protein 60 to induce pro-atherogenic, pro-oxidant, pro-inflammatory, and pro-thrombotic reponses in cells relevant to atherothrombosis (smooth muscle cells, endothelial cells, and macrophages and T-cells) provides biological plausibility to the potential causal link between *C. pneumoniae* and vascular disease (44).

Several seroepidemiologic studies, mostly retrospective, have, in general, shown a 2-fold or more risk of coronary or cerebrovascular disease among seropositive compared to seronegative individuals (18,23,45–51), although other prospective studies have failed to demonstrate a convincing relationship (52–54). These studies used different criteria for seropositivity, different and subjective methods of antibody assay, and had statistical biases introduced by subgroup analysis. *C. pneumonia* has been detected in human atheromatous or aneurysmal tissue by immunocytochemistry in approximately 50% of specimens (range: 40–100%), by polymerase chain reaction (PCR) in 0–60% of specimens, and has been isolated from one carotid endarterectomy specimen, one coronary artery from a transplant recipient, and 16% of coronary atherosclerotic tissue removed at atherectomy (55–62). Two large retrospective case control studies have examined the relationship between prior use of antibiotics and risk of myocardial infarction. The larger of the two studies showed that the frequency of use of tetracyclines or quinolones in the three preceding years was lower among cases of myocardial infarction than controls (63), whereas the other, smaller study failed to find a relationship between the prior use of erythromycin, tetracycline, or doxycycline and first myocardial infarction (64).

Small pilot intervention trials involving anti-chlamydial antibiotics (azithromycin or roxithromycin) have been reported. Two of these studies, conducted following an index event of acute myocardial infarction or unstable angina, suggested reduction in recurrent coronary events with antibiotic therapy (65,66), but a longer term follow-up showed loss of initial benefit at 6 months in the ROXIS trial (67). The ACADEMIC randomized trial showed no clinical benefit of a 3 month course of azithromycin treatment on clinical outcomes although reduction in serum markers of inflammation was demonstrated (68). These studies used different regimens and durations of treatment, were small in size, and were not powered to provide definitive evidence for or against the hypothesis.

Another small, randomized trial showed no overall clinical event reduction in azithromycin-or amoxicillin-treated patients compared to placebo group; however, a post-hoc analysis combining the two antibiotic arms suggested a clinical benefit compared to placebo (69).

A large, randomized trial ISAR-3 involving 1100 patients undergoing angioplasty and coronary stenting (PCI) showed no prevention of restenosis with 30 mg daily of roxithromycin for 4 weeks. Post-hoc analysis suggested a significant reduction in restenosis in the subgroup with high anti-*C. pneumoniae* antibody titres (70). Similarly, 3 months treatment with doxycyline did not influence adverse clinical event and restenosis rate after PCI (71).

Three large trials using antichlamydial antibiotics, azithromycin (AZACS trial), roxithromycin (Antibio trial), and most recently PROVE IT-TIMI 22 with gatifloxacin in patients with acute coronary syndrome have been published (72–74). Although they differed in selected antibiotic, treatment regimen, and duration of follow-up from 6 months to 30 months, no effect of the active treatment arm was shown (Table 1).

Two large trials tested the effect of azithromycin in chronic coronary artery disease patients. WIZARD trial tested a 3 month course of azithromycin in patients with previous myocardial infarction and serologic evidence of *C. pneumoniae* infection. ACES trial tested weekly treatment with azithromycin for one year, with 4 years of follow-up (75,76). Both failed to show any benefit, even in several predefined subgroups of patients (Table 2). The WIZARD study suggested a transient benefit early during treatment, a finding that was not supported by other studies.

There are several potential explanations for the negative outcome of the clinical trials: (1) incorrect hypothesis, i.e., infection does not play a role either in

Table 1 Infectious Agents Implicated in Vascular Disease

1	Viruses	CMV; *Herpes simplex* 1 and 2, *Hepatitis A*, Influenza
2	Bacteria	*Chlamydia pneumoniae*, *Helicobacter pylori*, *Porphyromonas gingivalis*

Table 2 Clinical Endpoints in Trial of Antibiotics for Secondary Prevention of Coronary Artery Disease

Trial (Ref.)	Year	No. pts.	Indi-cations	Therapy	Follow-up	Endpoint (C/Rx)
ISAR (70)	2001	1020	Post PCI	Roxithromycin 1 months	D, MI 1 year	7% vs 6%, p=0.45
AZACS (72)	2003	1450	ACS	Azithromycin 5 days	D, MI, R 6 months	14% vs 15%, p=0.606
ANTIBIO (73)	2003	872	AMI	Roxithromycin 6 weeks	D 1 year	6.5% vs 6.0%, p=0.739
WIZARD (75)	2003	7724	Chr CAD	Azithromycin 3 months	D, MI, R, AP 3 years	14% vs 15%, p=0.23
PROVE-IT (75)	2005	4162	ACS	Gatifloxacin 18 months	D, MI, R, AP 2 years	23.7% vs 25.1%, p=0.41
ACES (76)	2005	4012	Chr CAD	Azithromycin 12 months	D, MI, R, AP 4 years	22.3% vs 22.4%, p=NS

Abbreviations: D, death of any cause; MI, nonfatal myocardial infarction; R, revascularization; AP, hospitalization for angina pectoris.

acute atherothrombotic events or in the chronic progression of the disease, (2) the advanced stages of the disease are not influenced by the antibiotic therapy, and least likely and (3) the selection of the antibiotic therapy and duration was inadequate. As on the other hand the observations for the role of the inflammation in the atherogenesis continue to accumulate, these findings suggest that we should rethink the strategy and not abandon the hypothesis altogether. The current conclusion, though, should be that the standard antibiotic therapy for *C. pneumoniae* does not favorably influence the outcomes related to coronary artery disease (77).

HELICOBACTER PYLORI

Seroepidemiologic data linking *H. pylori* infection to atherothrombotic vascular disease are overall not persuasive. Smaller studies suggested a positive relationship (18,78–80), while the larger studies failed to demonstrate a relationship (18,81–84). More recent studies suggested that only strains of *H. pylori*, which express the cytotoxin-associated gene A, have a link with atherosclerosis (85). Attempts to demonstrate *H. pylori* in human atherosclerotic tissue have in general proven fruitless (83). Although 1 of 39 carotid plaques was shown to contain *H. pylori* DNA, contamination could not be excluded (84). One small intervention trial reported recently showed no clinical benefit of anti-*H. pylori* drug regimen compared to placebo (69). There are no good experimental data for the

relationship between *H. pylori* infection and atherosclerosis in animal models and no experimental data in vitro to demonstrate biological plausibility favoring a pro-atherogenic role. Thus, at the present time, the balance of evidence does not lend a strong support for a causal role for *H. pylori* in atherothrombosis.

PERIODONTAL DISEASE, *PORPHYROMONAS GINGIVALIS, STREPTOCOCCUS SANGUIS,* AND ATHEROTHROMBOSIS

Periodontitis is a common chronic inflammatory disease in the periodontal tissue leading to destruction of the bone surrounding the teeth, and is responsible for tooth loss in adults. Several studies have suggested an increased risk of coronary heart disease or stroke with periodontal disease or tooth loss (86–92), whereas others have raised questions about this link (93). Direct pathological demonstration of culprit pathogens, *Bacteroides forsythus*, *Porphyromonas gingivalis*, *Prevotella intermedia*, *Streptococcus sanguis* among others, have not been reported in human atherosclerotic tissues. Elevated levels of fibrinogen and Factor VIII have been reported with periodontal infection in small studies (89). Biological plausibility is suggested by the fact that periodontal disease may predispose to atherothrombosis given the abundance of pathogens involved, local production of pro-inflammatory and matrix-degrading molecules, and its association with other risk factors such as fibrinogen levels and white blood cell (WBC) counts. Of the two common pathogens involved in periodontal disease, *S. sanguis* can enhance platelet aggregation and *P. gingivalis* may increase risk of thrombosis by production of platelet aggregation, associated protein, and stimulation of Factor X (87,88). A recent experimental study has shown accelerated atherosclerosis with *P. gingivalis* infection in apo E null mice (94). No intervention trials in animals or humans have been reported.

Thus the overall evidence implicating periodontal disease and atherothrombosis remains inconclusive.

POTENTIAL ROLE OF MULTIPLE INFECTIOUS ORGANISMS

The concept of "total pathogen" burden (Fig. 1) in which instead of a single pathogen, multiple pathogens are involved in the pathogenesis of atherothrombosis, was recently suggested (95). This hypothesis is supported by cross-sectional studies and prospective studies wherein the risk of coronary heart disease events increased with the number of chronic infections as assessed by positive serology to specific pathogens CMV, *Hepatitis A*, *H. pylori*, *herpes simplex* 1 and 2, and *C. pneumoniae* (95,96). Increased pathogen burden was associated with increasing levels of circulating C-reactive protein believed to reflect inflammation. Similarly, the Bruneck prospective, population-based survey suggested that a history of chronic infections (respiratory, urinary, dental, and others) amplified the risk of carotid atherosclerosis independently of other known risk factors. The risk was highest in patients with a prominent inflammatory response revealed by elevated

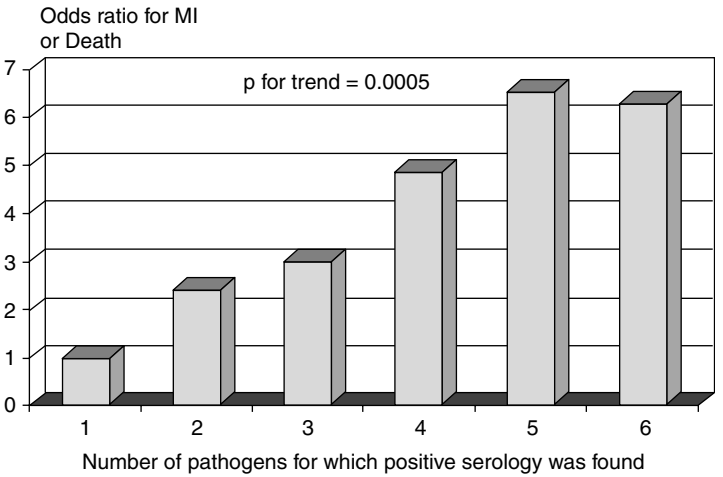

Figure 1 Relationship between pathogen burden at baseline and subsequent risk of myocardial infarction or death in a prospective cohort of 890 patients undergoing coronary angiography. Pathogen burden is defined as immunologic evidence for prior infection with one or more of the following pathogens: Cytomegalovirus, Herpes simplex virus-1, Herpes simplex virus-2, Hepatitis A virus, Chlamydia pneumoniae, Helicobacter pylori. *Source*: Adapted from Ref. 96.

circulating markers (soluble adhesion molecules, endotoxin, human heat shock protein 60, and antibodies to mycobacterial heat shock protein 65) (97). Lack of association between chronic infection with multiple agents and endothelial dysfunction suggested that these agents are not implicated as early etiologic triggers but may be involved at later stages of the atherosclerosis (98).

These studies provide support for the concept that chronic infections from a diverse group of pathogens and the consequent inflammatory response may increase the risk of atherothrombosis.

POTENTIAL MECHANISMS BY WHICH INFECTIONS MAY PREDISPOSE TO ATHEROTHROMBOSIS

Infection may be linked to atherothrombosis by at least two different mechanisms.

Direct Infection of Cells of the Vessel Wall and Pro-Atherothrombotic Effects

Infectious organisms can infect one or more types of cells relevant to atherothrombosis (monocytes-macrophages, endothelial cells, vascular smooth muscle cells) leading to a host of changes that could promote atherosclerosis, plaque

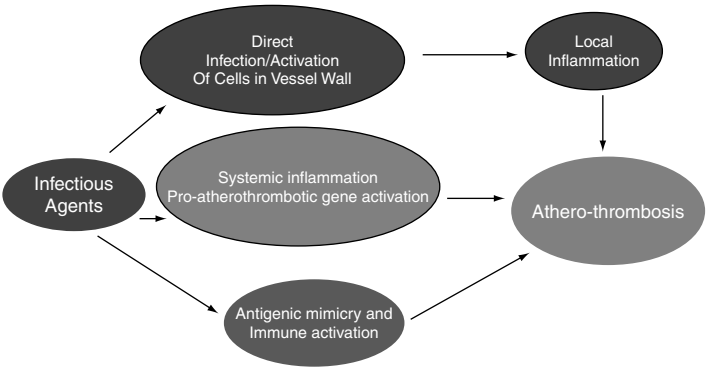

Figure 2 How chronic infections may contribute to atherothrombosis.

disruption, and thrombosis. The procoagulant effects, enhanced scavenger receptor expression and foam cell formation, enhanced expression of adhesion molecules and inflammatory cytokines, enhanced expression of metalloproteinases, and enhanced migration, survival, and proliferation of smooth muscle cells may be some of the proposed mechanisms (Fig. 2) (99–102).

Remote Effects of Infection

Antigenic Mimicry and Immune Activation

Experimental studies have suggested that breakdown of immune tolerance and development of an autoimmune response to aberrant presentation of otherwise hidden endogenous antigens may be provoked by infectious organisms carrying homologous antigens. This immune response may lead to vasculitis and myocarditis and possibly even atherosclerosis through molecular or antigenic mimicry (103). Heat shock proteins may be such antigens since bacteria contain them, viruses use them when budding from host cells, and they are highly conserved across prokaryotes and mammalian species (104–112). In this instance, an infection at a site remote from the vessel wall may be sufficient to create immune-mediated vascular injury without requiring actual infection of the vessel wall (112).

Pro-Inflammatory Effects of Remote Infections

Infections remote from the vessel wall may induce systemic inflammation by inducing pro-inflammatory circulating cytokines and/or changing the normally protective and anti-inflammatory high density lipoprotein (HDL) into a pro-atherogenic and pro-inflammatory HDL, thus contributing to atherothrombosis by an indirect mechanism also not requiring infection of the vessel wall (113–114).

SUMMARY

The hypothesis that infectious organisms may contribute to atherothrombosis, directly or indirectly through immune activation and systemic inflammation, is currently a focus of intense scrutiny. A number of recent large scale, randomized trials of antibiotics have been disappointing in terms of lack of benefits of anti-chlamydial therapy. It remains unclear as to whether negative therapeutic trials indicate: (1) lack of a significant pathophysiological role of one or more infectious organisms, (2) application of therapy too late in the course of the disease evolution ("horse being out of the barn" analogy), or (3) incorrect choice of antimicrobial therapies or other unknown factors.

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7

Novel Psychosocial Factors and Cardiovascular Disease

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INTRODUCTION

Cardiovascular disease remains the largest contributor to morbidity and mortality in developed countries (1). Advances in prevention and treatment of cardiovascular disease in the last 40 years resulted in an approximate 40% decline in cardiovascular disease mortality (1). Despite this remarkable success, it is sobering to note that this decline appears to have abated in recent years (2), paradoxically despite continued improvement in risk factor modification (3).

‡ Deceased.

Similarly, it has also become increasingly clear that up to 50% of patients with established coronary artery disease (CAD) will have recurrent cardiac events such as myocardial infarction and cardiac death despite aggressive management of traditional risk factors (4). Both these lines of evidence suggest that development of a better understanding of novel risk factors and newer therapies aimed at these nontraditional risk factors are needed.

Multiple studies have demonstrated that psychosocial factors are associated with elevated risk of cardiovascular disease in both patients with established disease (5,6) as well as non-diseased subjects (7,8). The spectrum of psychosocial factors is diverse, ranging from acute, such as outbursts of anger and mental stress which can trigger pathophysiologic responses and an acute event, to chronic psychologic risk factors such as hostility, depression, or exhaustion that correlate with higher rates of CAD (9,10). Psychosocial factors such as work stress, financial stress, and stressful life events have been shown to be associated with myocardial infarction in the large INTERHEART study which included male and female patients from a wide range of countries and ethnicities (11). While it appears clear from these data that there is a simple and direct relationship between psychosocial factors and cardiovascular disease, not all studies have demonstrated such positive findings (12,13), and the magnitude of risk is variable between studies (5–8). Given the complex nature of human behavior, it is likely that the relationship between psychosocial factors and cardiovascular disease are mediated at multiple levels along the pathophysiologic mechanisms responsible for cardiovascular disease events (Fig. 1). These interactions may explain some of the lack of consistency between studies, and point to the need for continued mechanistic understanding as an aid to designing effective psychosocial intervention strategies.

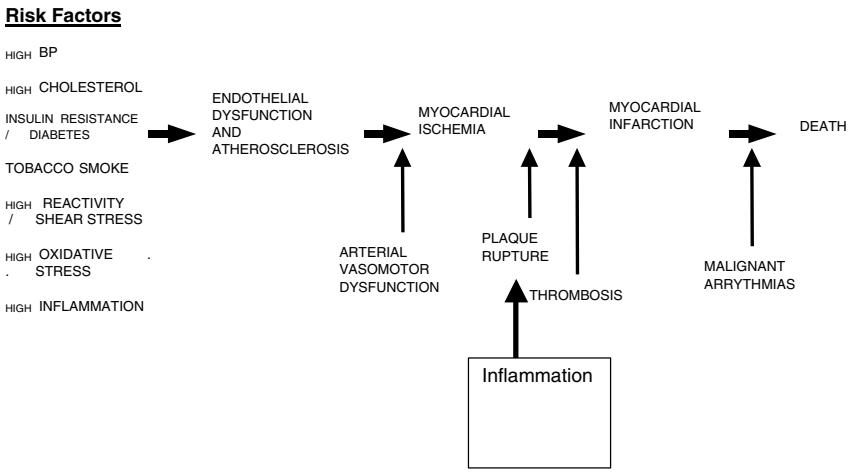

Figure 1 Pathophysiologic mechanisms of cardiovascular disease.

DEPRESSION AND CARDIOVASCULAR DISEASE

The lifetime prevalence of major depression in the general population is 15% (14), while among coronary disease patients the prevalence is even higher (17–27%), particularly among the elderly (14–18). The presence of depressive symptoms or major depressive episode has been prospectively linked to fatal and nonfatal cerebral and cardiovascular events (15,17–28) in both populations, with (15,23–26) and without diagnosed coronary disease (18–22). This increased risk holds true in individuals with a remote history (>10 years) of depression before onset of coronary artery disease and appears to be independent of other coronary risk factors (20). Not all studies have demonstrated linkage between depression and cardiovascular events, however (27). The majority of studies show an elevated risk of CAD endpoints with risk ratios ranging from 1.5 to 4.5 over a minimum of 4.5 years (17–23).

Studies that have specifically examined depression and the outcomes of recurrent cardiovascular events, revascularization procedures, and death in patients with established coronary disease demonstrated relative risks as high as 7.8 (CI: 4,9–12). In a 6-month post-infarct follow-up, Frasure-Smith showed depression (Diagnostic and Statistical Manual of Mental Disorders-III diagnosis) was an independent risk factor for mortality (relative risk 4.29, CI: 3.14–5.44) (17) and depression remained an independent mortality predictor at 18-month follow-up (relative risk 1.5, CI:1.3–10.1) (23). The Cardiac Arrest in the Cardiac Arrhythmia Pilot Study (CAPS) involving coronary patients with significant ventricular arrhythmias showed elevated all-cause and cardiovascular mortality at one year among those patients with evidence of depressive symptoms on the Beck Depression Inventory (24). Longer term outcomes utilizing self-reporting of depressive symptoms in 1250 post-infarct patients showed significantly increased cardiovascular mortality over 15.2 years in those with higher Zung Self-Rating Depression scores (25).

While the methodology and measurement tools in the previous studies are quite variable, there is a consensus of evidence that depressive symptomology is associated with increased cardiovascular morbidity and mortality. Mechanisms to explain this increased risk may be due to an increase in the incidence of sudden cardiac death in patients with depression (23), mediated by alterations in the autonomic nervous system as indicated by decreased heart rate variability (29,30), consistent with impaired vagal tone and increased sympathetic tone (30,31). Additional mechanisms may include depression-related alterations in platelet function and reactivity (32,33). Prior work has demonstrated significantly increased binding at the IIb/IIIa complex, indicating increased platelet reactivity among depressed patients compared to controls (34). Other work has shown that chemotactic factors including PF4 and ß-TG are significantly higher in patients with coronary disease and depression, as compared to those with coronary disease alone or control (35). Preliminary data, including results from the

Sertraline Antidepressant and Heart Attack Randomized Trial (SADHART), suggest that some of these platelet effects may be reversible with selective serotonin reuptake inhibitor treatment (36–38). Increased conversion of prothrombin to thrombin is noted in depressed patients (34). Finally, the poorer outcomes of depressed patients may be due to poorer compliance with medications and/or healthier lifestyles.

While recognition and treatment of depression is important in and of itself, ongoing work is testing the hypothesis that treatment of depression reduces cardiovascular morbidity and mortality. The safety of treatment of recurrent depression with sertraline in 369 patients with unstable angina and recent myocardiaI infarction (MI) has been established in the SADHART trial (35). The Enhancing Recovery in Coronary Heart Disease Patients (ENRICHD) randomized 1165 post-infarct patients with depression or perceived low social support to treatment with psychotherapy and pharmacotherapy for non-responders (39). While there was not a statistically significant reduction in mortality in those randomized to treatment, those with improved symptoms had increased survival as compared to non-responders (21.2% vs 10.4%) (39).

SOCIAL SUPPORT

Social isolation or lack of a social support system is associated with an increase in all-cause mortality, with relative risks ranging from 1.46–3.80 (6,40–42) Among male survivors of acute MI in the Beta-Blocker Heart Attack Trial (6), the risk was further enhanced in patients with increased measures of life stress. Prior work has demonstrated that the elevated risk associated with lack of social support is independent of left ventricular function and coronary anatomy (43), enhanced among those with diminished economic resources (43), relevant in the elderly (42), and linked with emotional support (42). Mechanisms behind the link between social support and cardiovascular disease are likely multifactorial, and may include limited access to and utilization of healthcare, poor compliance to healthy lifestyles, associated adverse pathophysiology, or combinations of these.

PSYCHOSOCIAL FACTORS AND TRADITIONAL CARDIOVASCULAR RISK FACTOR CLUSTERING

Traditional cardiovascular risk factors, as outlined in the Framingham Study, include cigarette smoking, hypertension, diabetes mellitus, dyslipidemia, family history of premature coronary disease, and sedentary lifestyle (12). It is clear that psychosocial factors directly correlate with a higher prevalence and clustering of traditional cardiovascular risk factors. Psychosocial stress may result from and contribute to high-risk behaviors that then result in the clustering of smoking, inactivity, and obesity. Mortality, for example, correlates with higher blood pressure, cardiac reactivity, blood cholesterol, and cigarette smoking, as well as poor diet and exercise habits (44). Despite the strong correlation between the

hostile personality trait and traditional risk factors, the lack of evidence supporting this as an independent risk factor is probably accounted for by incompleteness and inaccuracy of traditional risk factor data collection and statistical covariance analyses. Similar clustering results have been found using psychosocial factors measures such as hostility (45), depression (46), and low socioeconomic status/social support (43). Indeed, adverse psychosocial factors likely both cluster together (e.g., hostility-depression-lack of social support), and are identifiers of clusters of traditional cardiovascular risk factors (39).

PSYCHOSOCIAL FACTORS AND ATHEROSCLEROSIS

Not surprisingly, most of the above-mentioned psychosocial factors have also been demonstrated to correlate with measures of atherosclerosis. Carefully controlled animal primate studies using cynomolgus monkeys have clearly demonstrated the roles of psychosocial stress (47) and social isolation (48), created by varying cage rotations/restrictions, in atherosclerosis measured at necropsy. Notably, it is the dominant male monkeys that appear most susceptible to the stress-induced atherosclerosis (49), in some ways parallel to the human Type A behavior and hostility work. Type A behavior personality (47), hostility (50), depression/hopelessness (51), and job stress (52) have been shown to correlate with atherosclerosis in humans using measures such as coronary angiography or carotid artery intima-media thickness. In general, these human studies have adjusted for the traditional cardiovascular risk factors, suggesting that the psychosocial factors provide additional independent risk. Pathophysiologic links between pyschosocial factors and atherosclerosis independent of traditional cardiovascular risk factors may include inflammation-induced LDL-cholesterol oxidation, elevated shear stress and adverse catecholamine, and reproductive hormonal changes which result in increased endothelial damage leading to atherosclerosis (53).

PSYCHOSOCIAL FACTORS AND ENDOTHELIAL FUNCTION

There is evidence that endothelial dysfunction is one of the earliest signs of atherosclerosis, such that the balance of local arterial mediators results in a loss of functional dilation (54). Recent evidence also suggests that arterial vasomotor dysfunction plays an important role in acute cardiac events and death. Inappropriate vasoconstriction, or lack of coronary artery dilation in response to an increased demand, is present in stable coronary heart disease (55–57). This is related to dysfunctional endothelium due to the underlying atherosclerosis and contributes to the genesis of myocardial ischemia. Arterial vasomotor function is mediated by both endothelial function and other local regulators as well as systemic autonomic nervous system activity.

Preliminary work in animals and humans suggests that psychosocial factors influence endothelial function. Psychosocial stress created by frequent cage

rotation produced endothelial dysfunction in a primate model, even in the absence of diet-induced atherosclerosis (57). Notably, a gender difference was noted, in that the dominant male monkeys had the greatest endothelial dysfunction (58), whereas female subordinates demonstrated the greatest abnormalities (58), suggesting that behavioral and physiological gender differences may have implications for cardiovascular disease. One human angiographic study has demonstrated a relationship between reported anger and coronary endothelial dysfunction (59). Previous work evaluating a possible surrogate of endothelial function, peripheral vascular reactivity, has demonstrated links to psychosocial stress in monkeys (57) and hostility in humans (60), as well as progression of atherosclerosis in humans (61). Pathophysiologic links between psychosocial factors and endothelial function may involve direct endothelial damage due to catecholamine and blood pressure surges, resulting in intimal damage, inflammatory-induced free radicals blocking nitric oxide synthesis, and activated platelet-triggered endothelial reactivity (62).

PSYCHOSOCIAL FACTORS AND MYOCARDIAL ISCHEMIA

Myocardial ischemia results in the setting of atherosclerotic cardiovascular disease when myocardial blood flow demand outstrips the supply. Mechanistically, this is triggered by both increases in demand mediated dominantly by increases in heart rate and blood pressure, and reduced supply mediated by coronary artery vasoconstriction, typically during physical and/or mental exertions (63). Prolonged myocardial ischemia results in myocardial infarction, most typically as a consequence of obstructive thrombus formation. It is well documented now that laboratory mental stress triggers increased blood pressure demand (64), coronary artery vasoconstriction, and reduced blood flow (65), with resultant myocardial ischemia (63). Parallel ambulatory studies in coronary artery disease patients in daily life show similar results (66), and have been extended to demonstrate that both intensity of mental effort and negative mood correlates with myocardial ischemia. Patients with documented mental stress ischemia had more exaggerated hemodynamic rest and stress responses to mental stress (67). Both ambulatory (68) and laboratory (69) studies have demonstrated that hostility correlates with ischemia in humans.

PSYCHOSOCIAL FACTORS AND PLAQUE RUPTURE

Current pathophysiological understanding suggests that acute cardiovascular events such as myocardial infarction and unstable angina are precipitated by atherosclerotic plaque rupture, where the cholesterol crystals and cellular debris are extruded into the coronary artery lumen and the subendothelial collagen components are exposed to circulating blood, promoting intracoronary thrombus (70). While it is possible that plaque rupture is serendipitous, increasing lines of evidence suggest that it is more likely triggered by both internal and external

events (71). Specifically, it has long been noted that these acute cardiovascular events occur in a circadian rhythm, reflecting sympathetic nervous system activity (72). Moreover, anger, and mentally stressful triggers were identified as triggers in a carefully designed study of myocardial infarction antecedent behaviors (71). Pathophysiologic links between psychosocial stress and plaque rupture may include surges in heart rate, blood pressure, and sympathetic nervous system activity, as well as weakening of the collagenous plaque cap from inflammatory processes, all of which may contribute to plaque instability (73).

PSYCHOSOCIAL FACTORS AND THROMBOSIS

It is likely that atherosclerotic plaques rupture relatively frequently and that a determining factor to progression to an acute cardiovascular event is the propensity for thrombus formation and the balance between pro- and anti-thrombic factors. Traditional risk factors clearly play a role in promoting thrombus. Data demonstrate that dyslipidemia (74), diabetes (75), and cigarette smoking (76) promote thrombus formation, via platelet- and non-platelet-mediated mechanisms. Preliminary data suggest that psychosocial factors also play a role in thrombus promotion. Pathophysiologic links appear to include a relationship between hostility and increased platelet reactivity (77), Type A/hostility, and reduced bleeding time and prostacyclin formation (78), elevated PAI-1 levels, and depressive symptoms (32), as well as enhanced platelet aggregation by mental stress related to increases in catecholamines and sympathetic nervous system activity (79).

PSYCHOSOCIAL FACTORS AND LETHAL ARRHYTHMIAS

Approximately half of the cardiovascular deaths experienced in the U.S. annually occur suddenly, due to a malignant ventricular arrhythmia (ventricular fibrillation) as the culmination of atherosclerotic plaque rupture/ischemia/infarction (Fig. 1) (1). We currently have no treatment for these patients other than the autonomic defibrillators implanted in the few who survive. Electrical stability and ventricular fibrillation threshold are influenced by many factors, including the autonomic nervous system. It is clear that lowered levels of parasympathetic nervous system tone and increased levels of sympathetic nervous system tone promote ventricular fibrillation in myocardial substrate at risk. Animal work in dogs demonstrates that anger, produced by restraining the dog, lowers ventricular fibrillatory thresholds measured directly by electrophysiological testing (80). More indirect measures are needed for human work. Heart rate variability, obtained from electrocardiographic recordings (81) and baroreflex testing (82), are noninvasive estimates of autonomic nervous system tone that are responsive to both acute (83) and chronic (29) conditions. Multiple lines of evidence indicate that psychosocial factors influence the autonomic nervous system measured by heart rate variability or baroreflex testing, including mental stress (83),

depression (84), and anxiety (85). Because both heart rate variability and baroreflex measures have been demonstrated to predict future cardiovascular events (86,87), these tools provide particularly rich insight into the interplay between psychosocial factors and cardiovascular disease.

PSYCHOSOCIAL FACTORS AND INFLAMMATION

The progression of coronary artery disease can be viewed as a "response to injury" (88) that is promoted by inflammatory processes. At present, little is known about the interaction between psychosocial factors and inflammatory processes in progressive atherosclerosis. Several lines of evidence indicate that inflammatory processes play a crucial role in plaque formation (89). Both acute and chronic psychological factors may promote the expression of adhesion molecules (90). These adhesion molecules (e.g., intracellular adhesion molecule-1) may cause monocytes and T-cells to adhere to the vascular endothelium, followed by monocyte infiltration and conversion to macrophages. Two main responses emerge from the penetration of macrophages and T-cells into the vascular wall: (1) activation of the cytokine cascade, and (2) release of growth factors (e.g., insulin-like and platelet-derived growth factor). Cytokines and growth factors will accelerate smooth muscle cell proliferation from the intimal layers of the vessel wall and promote progression of atherosclerosis. It is of interest that both episodic (91–93) and acute psychological risk factors for coronary syndromes are reported to affect circulating levels of T-cell, B-cells, and aspects of the cytokine cascade. A major challenge in this area of research relates to documenting the clinical significance of elevated levels of the inflammatory measures. Furthermore, the relationship between inflammatory processes and the progression of coronary artery disease is not fully understood, and it is not clear to what extent circulating inflammatory measures reflect the processes occurring at local atherosclerotic plaques. Nonetheless, the relationship between psychosocial factors, measures of inflammation, and cardiovascular disease may reveal new insights and opportunities for intervention.

PSYCHOSOCIAL INTERVENTION TRIALS

Psychosocial interventions that have been designed to address psychosocial factors could lower recurrent coronary artery disease events. At least 23 psychosocial intervention trials have evaluated the alteration of cardiac events following behavioral interventions in patients with established coronary artery disease (93–114). Small sample sizes, resulting in low statistical power, have often been a problem among these trials. Other problems include the lack of a uniform definition of "psychosocial stress," a wide divergence of clinical approaches toward psychosocial intervention among the trials, and, in some trials, a lack of true randomization due to the behavioral study design. Nevertheless, three meta-analyses of 12 (115), 23 (116), and 11(117) controlled

stress-management intervention trials involving 1484, 3180, and 3485 patients, respectively, demonstrate that psychosocial stress intervention has a beneficial impact on reducing recurrent cardiac events/death by 50–70%.

When only fully randomized trials which report recurrent cardiac events such as myocardial infarction and death as outcome variables are included, a summarized odds ratio demonstrates a significant 50% reduction in cardiac events among the intervention patients (118). One of the largest randomized trials performed in post-infarct patients, also demonstrated a statistically significant 51% one-year reduction in cardiac death (95). This trial employed simple stress management techniques performed by general nurses, and the overall mortality reduction was due primarily to a 61% reduction in out-of-hospital sudden cardiac death. Long-term follow-up of the patients in this trial has further demonstrated that withdrawal of the stress management at the end of the trial resulted in a disappearance of the beneficial mortality reduction (118). A more recent trial, from the same investigators using a similar environmental stress intervention, demonstrated no significant effect on cardiovascular events in the total group, and evidence of an adverse effect in women randomized to the intervention (119). These trials used stress-reduction interventions which included nurse visits and home help if indicated at the time of increased stress as evaluated at monthly phone checks. Another recent large trial (106), which used psychologic counseling and stress management training sessions for seven weeks following a cardiac event, demonstrated a reduction in recurrent events that was evident at six-month follow-up, but not persistent at 12-months follow-up. These later studies represent considerably larger studies with improved trial design features compared to the previous literature, and therefore cannot be easily discounted. Future trials might consider equipping patients with skills or tools to utilize during times of stress to test if this would result in better long-term outcomes.

At present, it is unknown if all patients or just those with high psychosocial stress might benefit from psychosocial stress interventions. Although essentially all patients with established coronary disease experience some psychosocial distress related to their disease, some suffer more seriously (119), and one intervention study has suggested that patients with high psychosocial stress appear to benefit the most, in terms of mortality reduction, from stress management (120). Specific stress management techniques, such as group support, yoga, biofeedback, and meditation, have not been tested in randomized clinical trials with adequate sample sizes to assess cardiovascular events. In planning these and other future psychosocial intervention trials, consideration should be given to these prior clinical trial results for optimization of successful and valid results.

SUMMARY

Psychosocial factors appear to be risk factors for cardiovascular disease morbidity and mortality. Multiple studies have demonstrated that psychosocial

factors are risk factors for cardiovascular disease in both patients with established disease (5,6) and non-diseased subjects (8). Current literature also suggests that psychosocial factors contribute to coronary artery disease via complex interactions along a pathophysiological chain of events, including adverse effects on traditional risk factor clustering (45), atherosclerosis (50), endothelial function (59,65), myocardial ischemia (63,66), plaque rupture (72), thrombosis (76,77), and lethal arrhythmias (29,84).

It is clear that psychosocial factors contribute to coronary artery disease morbidity and mortality via complex interactions. Continued efforts designed at understanding the behavioral and physiologic associations between psychosocial factors and cardiovascular disease are needed. Current psychosocial factor intervention trials suggest that the magnitude of risk reduction associated with intervention may be similar to that of other proven therapies for coronary artery disease, such as lipid-lowering (120), antiplatelet therapy (121), beta-blocker medication (122), and bypass surgery (123). Continued efforts are aimed at designing effective psychosocial interventions offer, promise for further reductions in cardiovascular disease morbidity and mortality.

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8

Genetic Risk Assessment Strategies for Coronary Artery Disease

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INTRODUCTION

Coronary artery disease (CAD) remains the leading cause of death and premature disability in the United States and other industrialized countries (1). Individuals with genetic predisposition to atherosclerosis have substantial risk for developing CAD, especially at early ages (2). As a result, they may have the most to gain from preventive interventions (3). This chapter will review the role of genetics in the development and progression of CAD, the available genetic risk assessment strategies for CAD, and clinical application of genetic risk information for CAD prevention including recommendations for risk factor modification and early detection, and the role of genetic counseling and education.

ROLE OF GENETICS IN DEVELOPMENT AND PROGRESSION OF CAD

The accumulation of atherosclerotic plaque in an artery wall is a chronic disease that begins early in life (4). This process appears to be initiated and/or facilitated by chronic injury to the endothelium (5). Plaques may become symptomatic when they are large enough to restrict blood flow leading to tissue ischemia. Acute coronary syndromes such as unstable angina, myocardial infarction (MI), and sudden death occur when thrombus forms on a thrombogenic plaque or when

unstable plaques rupture or ulcerate, leading to thrombus formation and possible vessel occlusion (6,7).

CAD is a complex disorder due to many risk factors. Multiple biochemical processes are involved, including lipid and apolipoprotein metabolism, inflammatory response, endothelial function, platelet function, thrombosis, fibrinolysis, homocysteine metabolism, insulin sensitivity, and blood pressure regulation (2). Each of the biochemical processes associated with CAD is comprised of enzymes, receptors, and ligands, which are encoded by our genes. Variations in these genes can alter the function of the constituents within a metabolic pathway. These genetic variations interact with each other and with non-genetic factors, resulting in variable susceptibility to the development and progression of atherosclerosis and thrombosis (2). Non-genetic risk factors for CAD include exposures, such as tobacco smoke, and behaviors (e.g., exercise, and dietary patterns), many of which may be culturally determined. Like genetic factors, environmental and behavioral risk factors often aggregate in families.

Dozens of candidate genes have been associated with CAD or MI (8), although some associations have conflicting results [e.g., angiotensin converting enzyme, methylenetetrahydrofolate reductase (MTHFR), platelet glycoprotein receptor IIIa, and factor VII] (9–18). The variable results may be due to chance, to errors in estimating the frequency of polymorphisms in the case or control group, to not matching the race/ethnicity of cases and controls, or to studying related but distinct phenotypes such as the presence of atherosclerosis versus the occurrence of MI. Investigations utilizing genome scan approaches have found novel genetic loci associated with CAD, which might provide additional insight to genetic factors contributing to atherosclerosis and coronary events (19–23). There are also numerous studies that have found genetic associations or linkage with related disorders such as hypertension (24–29), obesity (30–38) diabetes (39–49), lipids (50–53), and oxidative stress (54).

GENETIC RISK ASSESSMENT STRATEGIES TO ASSESS CAD AND MI SUSCEPTIBILITY

CAD is a complex disorder. This generally means that the manifestations of CAD arise from the interaction of several predisposing genetic and/or environmental factors (Fig. 1). Therefore, global risk assessment has been recognized as an effective approach in preventing CAD and its manifestations (55). Through global risk assessment a more accurate estimation of absolute risk can be determined based on the summation of risks contributed by each risk factor. Subsequently, the intensity of managing modifiable risk factors can be adjusted by the severity of the overall risk.

Most people will be served well by existing global risk assessment methods and prevention guidelines. However, genetic susceptibility to CAD is not adequately addressed by these methods, and underestimation of risk and missed opportunities for prevention can result for people who are genetically

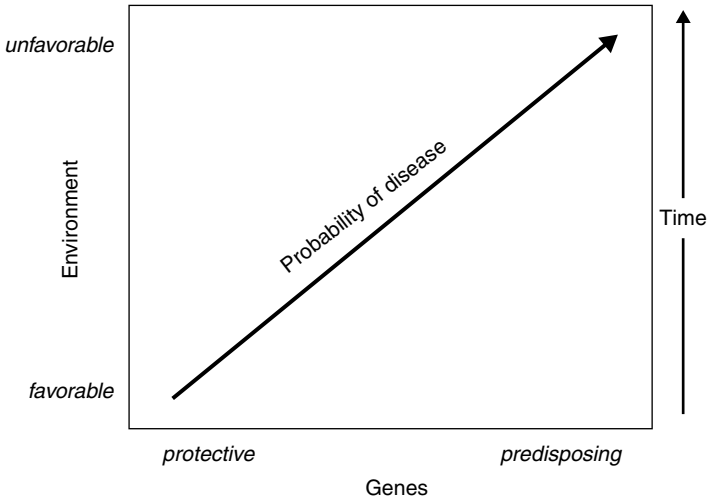

Figure 1 Characteristics of genetic susceptibility to coronary artery disease. Consultation for genetic risk assessment and specialized risk reduction should be considered for individuals with at least one of the above characteristics. CAD, coronary artery disease; close relative, first and/or second degree relative from the same lineage.

predisposed to CAD. The Framingham Risk Score is a widely used risk assessment method for prediction of CAD risk (56). It considers the established risk factors of gender, age, smoking, total cholesterol, LDL cholesterol, HDL cholesterol, and diabetes, but not family history of CAD or related disorders. The National Cholesterol Education Program Expert Panel (57) provides algorithms for treatment of lipid disorders in adults. The established risk factors of hypertension, diabetes, smoking, gender, age, and minimal family history information (parental history of MI before age 55) are used to determine risk and recommend lipid-lowering treatment. However, the risk associated with additional family history of CAD or related disorders is not included.

Family History Collection and Interpretation

The systematic collection and interpretation of family history information is currently the most appropriate screening approach to identify individuals with genetic susceptibility to CAD and MI. Family history of CAD and related conditions reflect the interactions of genetic, environmental, cultural, and behavioral risk factors shared among family members.

Family history of CAD is a significant risk factor for CAD. On average, there is a 2 to 3-fold increase in risk for CAD in first-degree relatives of affected individuals (58–62). Having two or more first-degree relatives with CAD is associated with a 3 to 6-fold increase in risk (63,64). The earlier the age of onset

the greater is the risk of CAD to relatives (63–66). In addition, the risk of disease is typically greater in relatives of female cases compared to male cases, suggesting greater genetic burden in female cases (61,66–68).

Much of the familial aggregation of CAD might be explained by the familial aggregation of established risk factors such as elevated LDL cholesterol, low HDL cholesterol, and diabetes (66). In a recent analysis of the Third National Health and Nutrition Survey, adults with a parental history of CAD were more likely to have multiple risk factors (OR for four or five risk factors compared with none was 2.9, 95% CI, 1.4–6.3) (66). Yet even after adjusting for these established risk factors, family history remains a significant independent risk factor for CAD (65,66,68–75). An explanation for this remaining risk may be familial aggregation of emerging CAD risk factors including hyperhomocysteinemia (76), C-reactive protein (CRP) (77), elevated fibrin D-dimer, tissue plasminogen activator, fibrinogen (78), and insulin resistance (79). In addition, the interactions of the genetic, environmental, cultural, and behavioral risk factors shared by family members may be too complex to assess with usual statistical methods.

The estimated accuracy and prevalence of a family history of CAD and related disorders are high enough to justify using family history for risk stratification and targeting screening and prevention to the level of familial risk. Several studies have shown that family history reports of CAD in first-degree relatives are generally accurate with sensitivity estimates from 67% to 85% (63,80,81). The relatively high sensitivity values indicate that family history can be used with some confidence to stratify risk above average. The specificity estimates for family history reports are more than 90% (63,80,81), indicating a lack of over-reporting disease in relatives. Similar sensitivity and specificity estimates are seen for diabetes and hypertension (81). Prevalence rates of a positive family history of CAD are substantial, with estimates ranging from 14% among high school students (82) to 29% among healthy adults in their mid-thirties (11% having high familial risk and 18% having an intermediate familial risk) (83).

Individuals with familial risk for CAD can be identified by asking targeted family history questions, including the number of relatives affected with CAD, their age at diagnosis, gender, degree of relationship to each other and the patient, and the presence of other conditions in the family such as stroke, hypertension, lipid abnormalities, and diabetes (83). With this information, stratification into different familial risk groups is possible, which can inform prevention activities (Fig. 2) (83). Pedigree analysis, which involves collection and interpretation of more comprehensive family medical history, is performed in the setting of a genetic evaluation for individuals with high familial risk of CAD or for those who may have Mendelian forms of cardiovascular disease.

Biochemical Testing to Assess CAD and MI Susceptibility

Tests to assess genetic risk for CAD are primarily biochemical analyses that measure the different pathways involved in development and progression of

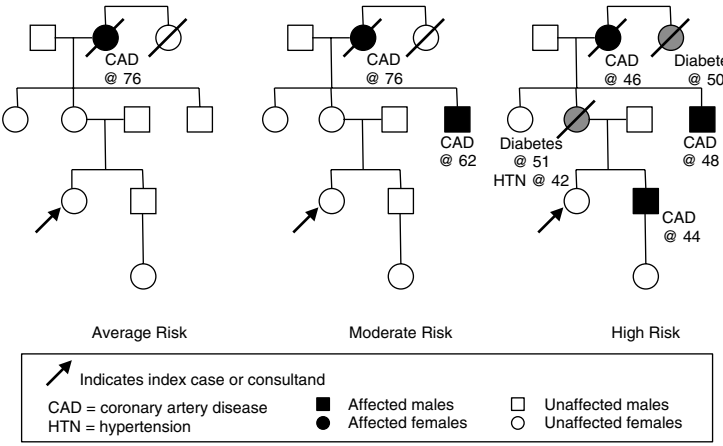

Figure 2 Examples of risk stratification with pedigree analysis. Average risk is associated with environmental risk factors with a small contribution of susceptibility genes. Moderate risk is most likely due to several susceptibility genes of small effect interacting with environmental risk factors. High risk is associated with many susceptibility alleles of small effect interacting with environmental risk factors, or rarely, single gene disorders associated with high risk.

coronary atherosclerosis. Several of these identify established risk factors, such as increased LDL cholesterol, decreased HDL cholesterol, and diabetes, which are known to be causally related to CAD (55). Many others are considered emerging risk factors that are strongly associated with CAD (84–93), but for most, a causal relationship with CAD has not been determined. Examples of emerging risk factors for CAD include small dense LDL particles, hyperhomocysteinemia, CRP, interleukin-6, and factors involved in fibrinolysis such as plasminogen activating factor inhibitor-1 and fibrinogen. Although treatment strategies exist for many emerging risk factors (see below), treatment for most of them has not so far been associated with primary prevention of CAD events. Nonetheless, measuring these risk factors can result in more accurate risk stratification. Currently, recognizing a higher level of CAD risk because of emerging risk factors allows patients and clinicians the opportunity to intensify the treatments that have been proven effective for CAD prevention.

Hyperhomocysteinemia

Extreme elevations in plasma homocysteine (>200 μmol/L), owing to deficiency of cystathione beta synthase or other key enzymes involved in homocysteine metabolism, cause premature cardiovascular disease. More modest elevations of homocysteine (>10 to 15 μmol/L) are associated with increased risk for cardiovascular disease (93). Homocysteine may increase the risk for cardiovascular disease by decreasing endothelium-dependent vasodilation, increasing

platelet adhesiveness, activating certain clotting factors, and inhibiting fibrinolysis by promoting lipoprotein(a) binding to fibrin (94). Homocysteine levels are increased by deficiency of the B vitamins that are cofactors for enzymes involved in homocysteine metabolism, including folic acid and vitamins B6 and B12. Homocysteine also increases with declining renal function, pernicious anemia, thyroid dysfunction, psoriasis, certain malignancies, anticonvulsant therapies, certain oral contraceptives, methotrexate, niacin, fibrates, and metformin (95,96). Homocysteine levels can often be lowered to a desirable range with folic acid and vitamins B6 and B12 (97–99). Lowering homocysteine with B vitamins has been shown to decrease the incidence of major cardiovascular events in a double-blind placebo-controlled trial in 533 subjects with coronary stenosis (100). However, another trial comparing high versus low-dose B vitamins in 3680 patients with ischemic stroke (101), and a controlled trial of folate alone for patients with CAD (102) showed no effect of vitamin supplementation on subsequent coronary events or stroke, even though baseline homocysteine levels were associated with increased risk in these prospective studies. The preventive effect of vitamins *before* development of symptomatic atherosclerosis is unknown.

Lipoprotein(a)

Lipoprotein(a) is a lipoprotein particle composed of an apolipoprotein B-100 particle covalently linked to an apolipoprotein(a) particle. Apolipoprotein(a) is homologous to plasminogen and may compete with plasminogen, thereby limiting fibrinolysis (103). Lipoprotein(a) has also been implicated in foam cell formation, endothelium-dependent vasodilation reduction, and LDL cholesterol oxidation promotion (104). Levels of lipoprotein(a) are strongly genetically determined (105,106). Lipoprotein(a) increases slightly with age and at the time of acute illness; also, females have greater values than males, with values increasing after menopause (107). The distribution of levels varies widely among racial and ethnic groups (107). Most of the associations with CAD have been found in Caucasians. Levels >20 to 30 mg/dL are considered high. Lipoprotein(a) levels can be reduced with niacin (108). Diet and exercise have no effect on lipoprotein(a) levels (109,110). In post-menopausal women, estrogen replacement therapy can lower levels (111), and in men, testosterone can lower levels (112). Reduction in lipoprotein(a) attributed to estrogen has been associated with a reduction in cardiovascular events in women (113). However, hormone replacement therapy with either estrogen for women or testosterone for men is not the standard of care for reducing CAD risk. Aggressive LDL cholesterol lowering appears to abolish the CAD risk associated with elevated lipoprotein(a), even with unchanged lipoprotein(a) levels (114). Thus this should be the treatment goal for high-risk individuals with elevated lipoprotein(a).

Atherogenic Lipoprotein Phenotype

Atherogenic, small, dense LDL cholesterol particles, reduced fraction of HDL2b, low HDL cholesterol, elevated triglycerides, and excess apolipoprotein B are characteristic of the atherogenic lipoprotein phenotype (ALP). ALP occurs in up to 25% of middle-aged men (115) and is associated with a 3-fold increase in CAD risk (116,117). ALP can be improved with regular exercise, loss of body fat, restricted intake of simple carbohydrates and alcohol (118), medical therapy including niacin and fibrates (119,120), and avoidance of β-blockers if possible (121). Fish oil supplementation also improves the lipid profile associated with ALP (122). Modifying ALP with the above measures, particularly niacin alone or in combination with other lipid-lowering therapy, has resulted in regression or prevention of progression of coronary atherosclerotic lesions, and reduced coronary risk (123).

Insulin Resistance

Insulin resistance is associated with many traditional and emerging risk factors (hypertension, hypertriglyceridemia, small LDL cholesterol particles, decreased HDL cholesterol, elevated PAI-1, fibrinogen, and CRP). It can be considered a risk factor predisposing to CAD (55). An estimated 24% of adults in the United States have the metabolic syndrome associated with insulin resistance (124). The third report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (ATPIII) (57) highlights the importance of treating patients with the metabolic syndrome to prevent cardiovascular disease. Insulin resistance can be effectively treated with lifestyle changes and metformin (125).

C-Reactive Protein

Many studies have shown a strong association between CRP and future cardiovascular events (126,127). Measurement of high sensitivity CRP is a useful clinical marker of inflammation related to atherosclerosis (127). Statin drugs used for cholesterol lowering have been associated with reduction in high sensitivity CRP (128). This may be due in part to the anti-inflammatory effects of these drugs. A recent report has shown that HRT increases CRP levels (92), suggesting a possible mechanism for increased CAD risk due to HRT. Thus, high sensitivy CRP (hs-CRP) could become a target of therapy for reducing CAD risk. However, at this time, measurement of hs-CRP is used primarily to stratify risk and guide recommendations for modification of other risk factors.

Thrombophilia

Several factors involved in promotion of thrombosis and inhibition of fibrinolysis are associated with CAD. Among this group of CAD risk factors, fibrinogen is one of the most important. Fibrinogen levels are modifiable through smoking cessation, aerobic exercise, weight loss, fibric acid medications, and omega-3

fish oils (129,130). Antiplatelet medications such as aspirin and other forms of anticoagulants might also reduce the thrombotic risk associated with elevated fibrinogen.

DNA-Based Testing to Assess Susceptibility to CAD and MI

There are over 30 Mendelian disorders (single gene disorders) that feature CAD or MI (Table 1) (131,132). Genetic tests for many of these Mendelian disorders are available and include DNA-based tests and biochemical analyses (131). These conditions are generally associated with a substantial risk for CAD and MI at young ages. For most of these Mendelian disorders, personal and family history characteristics are crucial for identifying individuals at risk. Specifically, early-onset CAD is usually present in multiple family members, and family members may have associated conditions such as stroke, diabetes, thrombophilia, or cholesterol abnormalities. Thus, collection and interpretation of family medical history is central to providing access to genetic testing services that are available for diagnosis of Mendelian forms of CAD and MI.

Despite the success of identifying susceptibility genes for multifactorial, non-Mendelian forms of CAD and associated conditions, the risk associated with any one of these gene variants is generally of small magnitude and by itself has little clinical significance (133). Before testing for low-risk susceptibility genes has widespread clinical application, additional studies are needed to assess the prevalence and penetrance of these genotypes, as well as the effect of other genes and environmental factors on their expression. Furthermore, the clinical utility of DNA-based testing for CAD susceptibility compared to other risk assessment strategies, including familial risk assessment and assessment of biochemical risk factors, must be proven. Nonetheless, testing for many CAD susceptibility genotypes is available. Examples below describe the potential benefit and limitations of DNA-based testing for CAD susceptibility in the clinical setting.

Cholesterol Ester Transfer Protein

Kuivenhoven and co-workers (134) found a significant association between variation at the cholesterol ester transfer protein (CETP) locus and angiographic progression of coronary atherosclerosis in men with CAD. Furthermore, there was a dose-dependent relation between one specific CETP gene polymorphism (TaqIB) and the efficacy of pravastatin in slowing the progression of atherosclerosis. Although this CETP association with CAD progression was significant, the finding has limited clinical utility. Although individuals with the B1B1 genotype derived the greatest benefit, treatment with pravastatin improved the outcome for all study subjects, abolishing any differences based on CETP genotype.

Table 1 Mendelian Disorders Featuring Coronary Artery Disease and Myocardial Infarction

Disorder	Mode of inheritance	OMIM entry
Abdominal obesity-metabolic syndrome	MF	605552
Apolipoprotein(a) polymorphism/ LPA excess	AD	152200.0001
Apolipoprotein A-I deficiency	AD, AR	107680.0011 107680.0012 107680.0013 107680.0015 107680.0017 107680.0022
Atherosclerosis susceptibility/ atherogenic lipoprotein phenotype (ALP)	AD, MF	108725
Coronary artery dissection, spontaneous	AD	122455
Cerebrotendinous xanthomatosis	AR	213700
Fabry disease	XLR	301500
Familial combined hyperlipidemia	AD, MF	144250
Familial defective apo B	AD	144010
Familial hypercholesterolemia	AD	143890
Familial hypercholesterolemia, autosomal recessive	AR	603813
Familial partial lipodystrophy	AD	151660
Familial pseudohyperkalemia due to red cell leak	AD, AR	177720
Fibromuscular dysplasia of arteries	AD	135580
Heparin cofactor II deficiency	AD	142360
Homocystienemia	AD, MF	603174
Homocystinuria	AR	236200
Homocysteinemia/homocystinuria due to *N*(5,10)-methylenetetrahydrofolate reductase deficiency	AR	236250
Hyperlipoproteinemia, type III	AR with pseudo-dominance	107741
Methylcobalmin deficiency, cbl G type	AR	250940
Niemann-Pick disease, type E	AR	257200
Progeria	AD	176670
Protein C deficiency	AD	176860
Pseudoxanthoma elasticum	AR	264800
Pseudoxanthoma elasticum, autosomal dominant	AD	177850
Sitosterolemia	AR	210250

(Continued)

Table 1 Mendelian Disorders Featuring Coronary Artery Disease and Myocardial Infarction (*Continued*)

Disorder	Mode of inheritance	OMIM entry
Spontaneous coronary dissection	AD	122455
Tangier disease	AR	205400
Vitamin B12 metabolic defect, type 2	AR	277410
Vitamin B12 metabolic defect with methylmalonic acidemia and homocystinuria	AR	277400
Werner syndrome	AR	277700
Williams syndrome	AD	194050

Abbreviations: OMIM, On-line Mendelian Inheritance in Man (131), a periodically-updated reference to inherited disorders associated with alterations in single genes; AD, autosomal dominant; AR, autosomal recessive; MF, multifactorial; XLR, X-linked recessive.
Source: From Ref. 8.

Apo E

The ApoE4 allele has been associated with CAD in several populations (135–137). ApoE2/E2 homozygous individuals are at risk for type III hyperlipoproteinemia, which is associated with an increased risk for atherosclerosis. In addition, apoE genotyping could come to play a role in recommending lipid-lowering diets (122,138–141). Forty percent of the individual variation in response of LDL cholesterol levels to a low-saturated fat diet is familial (142). This might be due in part to the apoE locus. Several studies have shown that carriers of the apoE4 allele tend to be more responsive to the LDL-lowering effects of low-fat dietary interventions compared to non-carriers (138–141). Carriers of the apoE2 allele may be particularly susceptible to unfavorable changes in lipids and to coronary heart disease when they are exposed to diets high in saturated fat (141).

The apoE genotype influences the responsiveness to fish oil supplementation in subjects with an ALP (122). Individuals with an apoE2 allele displayed favorable changes when given fish oil, including a marked reduction in the postprandial rise in triglycerides and a trend toward increased lipoprotein lipase activity compared to non-E2 carriers (143). ApoE4 carriers had an unfavorable response compared to E3/E3 homozygotes with a significant increase in total cholesterol and a trend toward a reduction in HDL cholesterol (122).

Despite these important associations relating response to diet and the apoE genotype, clinicians must proceed with caution when considering this particular genetic test as a means to assess CAD risk. The apoE4 genotype is also associated with increased risk for Alzheimer disease (144). The American College of

Medical Genetics (ACMG) and the American Society of Human Genetics (ASHG) have not endorsed apoE testing for diagnosis or prediction of Alzheimer's disease (145). Therefore, patients should be informed of the association of apoE genotype with Alzheimer's disease if considering apoE genotyping for cardiovascular risk assessment.

Methylenetetrahydrofolate Reductase

Homozygosity for the MTHFR C677T mutation has been associated with elevated levels of homocysteine (146); homocysteine levels are associated with CAD risk (93). A recent meta-analysis of case-control studies demonstrated a significantly higher risk of CAD associated with the MTHFR C677T genotype, especially in the setting of low folate status (147). The ASHG/ACMG statement regarding measurement and use of total plasma homocysteine recommends that the basis for elevated homocysteine levels (greater than 15 μM) be determined before treatment, because inappropriate supplementation of folate, Vitamin B12, and pyridoxine has some possibility of causing harm (148).

Prothrombin G20210A

In a study of postmenopausal women, risk of MI was significantly increased (OR = 10.9, 95% CI, 2.15–55.2) in those with the prothrombin G20210A mutation who also had hypertension and were taking hormone replacement therapy (HRT) (149). Women with the prothrombin mutation had only a mildly increased risk of MI if they did not use HRT. Those without the prothrombin G20210A mutation were not at substantially increased risk for MI even if they used HRT. These findings suggest a potential benefit of prothrombin G20210A mutation testing in women at high risk for MI who are considering use of HRT. However, decision-making regarding HRT use is complex and it is uncertain how much value such testing would add in the clinical setting.

Platelet Glycoprotein Ia/IIa Receptor

Smoking is a significant risk factor for CAD and MI. However, individuals with specific genotypes have greater risks for MI associated with smoking. One example is the Gln-Arg192 polymorphism of the human paraoxonase gene (150). Another is the 807T allele of the platelet glycoprotein Ia/IIa receptor (151). Homozygosity for the platelet glycoprotein Ia/IIa receptor 807T by itself is associated with about a 3-fold increase in risk for MI, smoking alone with a 4-fold increase in risk (151). These two risk factors interact with a greater than multiplicative effect, yielding an odds ratio of 25 for MI among individuals who were homozygous for the 807T allele and also smoked (151). Although knowledge of increased risk due to high-risk alleles might be expected to improve smoking cessation efforts, this has not been demonstrated. Furthermore, the absence of these risk alleles does not allow one to smoke with impunity, since smoking very likely increases risk for MI through other mechanisms and it is associated with other hazardous health effects.

Platelet Glycoprotein IIIa Receptor

Several studies have identified a strong association between the platelet glycoprotein receptor IIIa (GPIIIa) A2 allele and extensive CAD or occurrence of coronary thrombosis (12–14). However, other studies have failed to demonstrate an association with CAD or MI (15–17). Cooke and colleagues (18) argue that differences in aspirin use might account for some of the discrepancies in studies investigating this polymorphism, because aspirin has been shown to inhibit the increased platelet aggregation observed with this polymorphism. Aspirin very likely has other beneficial effects in the prevention of CAD and acute coronary syndromes. Its use is recommended for both primary and secondary prevention of CAD (152). Thus, the clinical utility of genotyping GPIIIa would be limited since it seems unlikely that this test alone will distinguish who will benefit from chemoprevention with aspirin.

5-Lipoxygenase Polymorphisms

5-lipoxygenase converts dietary fatty acids to leukotrienes, potential inflammatory mediators of atherosclerosis. In a cross-sectional study of 470 healthy, middle-aged people, carotid artery intima-media thickness (measured as a marker of atherosclerosis) was increased in the 6% of people with a variant genotype (either of 2 polymorphisms) in the promoter region of the 5-lipoxygenased gene (153). The increased thickening, adjusted for other risk factors, was comparable to the increase seen with diabetes. Those with these polymorphisms also had doubled levels of CRP. Higher dietary intake of polyunsaturated n-6 fatty acid increased the effect of the gene variant, whereas higher intake of n-3 fatty acids (e.g., from fish oils) lessened the effect (153). Although this observation suggests a genotype-diet interaction that could identify people more likely to respond to fish oils for prevention of atherosclerosis, clinical use of 5-lipoxygenase genotyping would have to await prospective studies showing that individualized treatment prevents CAD.

α-Adducin Variant

A population-based case-control study of patients treated for hypertension found a significant interaction between the α-adducin gene variant, Trp460, and diuretic therapy on the risk of MI or stroke (154). The α-adducin gene variant was identified in more than one third of the participants. The risk of MI or stroke in individuals with the wild-type genotype did not depend on the type of antihypertensive therapy. However, in carriers of the α-adducin variant, diuretic therapy was associated with a lower risk of MI and stroke than other antihypertensive therapies (odds ratio, 0.49; 95% CI, 0.32–0.77). Other traditional cardiovascular disease risk factors did not influence this interaction. These results suggest a role for genotyping hypertensive individuals for the α-adducin variant allele, Trp460, to determine benefit from diuretic therapy.

However, these findings need to be confirmed in other studies, and other benefits and risks of diuretic therapy need to be considered before such testing translates to clinical practice.

Alcohol Dehydrogenase Type 3

Alcohol consumption has been associated with reduced risk of CHD. People with an alcohol dehydrogenase type 3 (ADH3) allele metabolize alcohol more slowly. This genetic variant in men is also associated with a lower risk of MI (RR = 0.65; 95% CI, 0.43–0.99) (155). A significant interaction between this allele and alcohol intake has been found. Those who are homozygous for this allele and drink at least one drink a day have the greatest reduction in risk for MI (RR = 0.14; 95% CI, 0.04–0.45) and the highest HDL cholesterol levels (for interaction P = 0.05). Again, this finding has limited clinical utility since all men in this study appeared to benefit from consuming at least one drink per day regardless of their genotype. In addition, many other variables need consideration when counseling about alcohol intake.

Estrogen Receptor-α Gene

Herrington and colleagues (156) have shown that sequence variation of the estrogen receptor-α gene (IVS1-401 C/C genotype) is associated with the magnitude of increase in HDL cholesterol levels when estrogen or combination HRT is administered to women with CAD. However, this response has not yet been linked to variation in the risk of cardiovascular disease.

APPROACH TO INDIVIDUALS WITH HIGH FAMILIAL RISK

The following paragraphs will review the process of genetic evaluation for an individual referred because of personal or family history characteristics suggestive of a strong genetic susceptibility to CAD or MI (Table 2). The process includes: (1) genetic counseling and education, (2) risk assessment using personal and family medical history, physical examination, laboratory testing,

Table 2 Characteristics of Genetic Predisposition to Coronary Artery Disease (CAD)

- Early onset CAD: men age < 55 and women age < 65 years
- More than one close relative[a] with CAD, especially female relatives
- Multiple atherosclerotic vessels (e.g., coronary, carotid, aorta) with multifocal involvement (i.e., angiographic severity)
- Presence of multiple CAD risk factors in family members with CAD
- Presence of related disorders in close relatives (e.g., diabetes, stroke, hypertension, peripheral vascular disease)

[a] Close relative, first- or second-degree relatives.

and screening for early detection of CAD, and (3) recommendations for risk factor modification.

Genetic Counseling and Education Regarding CAD Susceptibility

An important goal of genetic evaluation for CAD is the development of individualized preventive strategies based on the genetic risk assessment, and the patient's personal medical history, lifestyle, and preferences. Genetic counseling is critical for delineating a patient's motivation and likely responses to learning of a genetic risk. Through genetic consultation patients will be educated about the role of behavioral and genetic risk factors for CAD, their mode of inheritance, and the options for prevention and risk factor modification. This communication process ensures the opportunity to provide informed consent, including discussion of the potential benefits, risks, and limitations of genetic risk assessment, and options for prevention (157).

Although family history of CAD has been shown to be a significant predictor of CAD risk, a recent report has shown that this familial risk does not translate to spontaneous improvement in lifestyles of at-risk relatives (158). In the Coronary Artery Risk Development in Young Adults study, CAD risk factors were assessed over two consecutive 5-year follow-up periods among 3950 participants aged 18 to 30 years. Kip and colleagues (158) found that the occurrence of a heart attack or stroke in a young adult's immediate family member did not lead to self-initiated, sustained change in modifiable risk factors. These results argue that primary care clinicians may need to actively intervene in people with a family history of CAD, where the opportunities for prevention are substantial (3).

Because most of the established and emerging risk factors for CAD aggregate in families, a family-based approach to risk factor modification ought to be an effective strategy, and this has been demonstrated in a few studies (159–161). Lifestyle changes, such as dietary modification, weight control, and smoking cessation, are likely to be more effective when delivered to the family than to an individual because family members can influence each other and provide ongoing support to one another.

Risk Assessment

Review of the personal medical history should include diagnoses of CAD, MI, peripheral vascular disease, stroke (including transient ischemic attacks), thrombosis, arrhythmia, heart failure, pulmonary disease, diabetes, and hypertension. Medical records, particularly procedure reports, are reviewed for confirmation. The Review of Systems will focus on cardio-respiratory function, including questions regarding angina, shortness of breath, dyspnea on exertion, paroxysmal nocturnal dyspnea, pedal edema, claudication, and exercise tolerance. Inquiry regarding tobacco exposure, history of alcohol use, exercise, and diet should also be performed.

During genetic consultation, a pedigree is constructed by obtaining demographic and medical information for all first and second-degree relatives, including current age or age at death, cause of death if deceased, history of CAD, other forms of heart disease, and related conditions such as stroke, peripheral vascular disease, aortic aneurysm, hypertension, diabetes, and lipid abnormalities, and associated risk factors such as smoking. Additional questioning can be helpful regarding procedures that might have been performed such as coronary artery bypass surgery, angioplasty, echocardiogram, or pacemaker placement. When available, medical records and autopsy reports of family members are reviewed to verify diagnoses and document test results. The family history should include ethnicity and country of origin since certain conditions might be more prevalent in certain ethnic groups. For example, the prevalence of insulin resistance is high among individuals of Native American admixture (162,163).

Once this information is collected, pedigree analysis is performed to determine the most likely mode of inheritance (i.e., Mendelian versus multifactorial) and the risk of disease to the patient and to unaffected relatives. If a Mendelian disorder is suspected, this analysis helps to elucidate the differential diagnosis. This process can inform recommendations for appropriate diagnostic tests as well as individualized management and prevention strategies. For example, an inherited susceptibility to thrombosis may be suspected in a pedigree that features multiple affected relatives with early onset of CAD, stroke, and other thromboembolic events (164). Testing of thrombotic markers might reveal important risk factors in the family. Recommendations can be made to avoid factors that may aggravate that risk such as use of oral contraceptives, HRT, and prolonged periods of immobility, and for prophylactic use of anticoagulants in high-risk situations.

A physical examination focused on CAD risk should include blood pressure in the arms and the ankles. In addition to identifying hypertension, these measurements can be used to calculate the ankle/brachial blood pressure index (ABI). Values <0.9 are correlated with atherosclerosis. In addition, a blood pressure of 130/85 or greater is a criterion for the metabolic syndrome (57). Weight and height should be obtained and body mass index should be calculated. This can be helpful in identifying a need for achieving an ideal weight and monitoring diet and exercise interventions. Waist circumference should be obtained, as it can be a factor in identifying the metabolic syndrome (57). Evaluation of lipid disorders should include examination of the eyes, assessing corneal arcus and lipemia retinalis. Examination of the skin should include assessment for xanthelasma and tendonous xanthomas. The cardiovascular exam should include careful assessment of the heart and lungs, as well as listening for bruits at major vessels in the neck, abdomen, and groin, and palpation of the aorta and distal pulses. Any abnormalities can be followed up with additional studies, such as ultrasound. Physical signs of Mendelian disorders that feature cardiovascular disease, for example, Marfan syndrome, Ehlers-Danlos syndrome type IV, pseudoxanthoma elasticum, and Fabry disease, should also be sought.

Laboratory testing to detect traditional and emerging risk factors for CAD includes fasting lipid panel, lipoprotein(a), LDL cholesterol particle size, HDL cholesterol fractionation, apolipoprotein B, hs-CRP, glucose, and homocysteine measurements. The ALP can be identified if there is a preponderance of small, dense LDL cholesterol, decreased fraction of HDL2b ($<15\%$), elevated triglycerides, and elevated apolipoprotein B. ALP can be effectively treated with lifestyle changes and/or medications (niacin or fibrates) as reviewed above (118–120). Fasting insulin can be checked if there is evidence of impaired glucose tolerance. The metabolic syndrome can be identified if at least three of the following criteria are met: blood pressure $>130/85$ mm Hg, waist circumference >102 cm in men and >88 cm in women, HDL cholesterol <40 mg/dL in men and <50 mg/dL in women, triglycerides of 150 mg/dL or greater (57). If the metabolic syndrome is present, or if there are signs of insulin resistance or impaired glucose tolerance, oral glucose tolerance testing should be considered for detection of diabetes (Fig. 2).

DNA-based testing may be considered in specific situations for high-risk individuals. MTHFR mutation analysis for the C677T allele can be performed if hyperhomocysteinemia is detected. Factor V Leiden (FVL) mutation analysis can be performed for premenopausal women with other high-risk factors for MI who are considering use of oral contraceptives. If the FVL mutation is identified, oral contraceptives may be avoided because of an associated risk for MI in premenopausal women (164). Prothrombin G20210A mutation analysis can be considered for high-risk, postmenopausal women considering HRT. The combination of HRT and the G20210A mutation are associated with risk for MI (149). ApoE genotyping can be considered if there is a question about the diagnosis of type III hyperlipoproteinemia, or if the apoE genotype would significantly influence dietary recommendations.

Early detection strategies for CAD might be useful to further stratify risk in asymptomatic individuals at increased risk for CAD (55), especially if the identification of subclinical atherosclerosis will alter recommendations regarding risk factor modification or adherence to risk-reducing strategies. Noninvasive tests such as carotid artery duplex scanning to measure intima-media thickness, ABI, electron beam CT (EBCT) to detect coronary artery calcification, ultrasound-based endothelial function studies, magnetic resonance imaging techniques, and testing for hs-CRP offer the potential for measuring and monitoring atherosclerosis in asymptomatic people. Several of these methods are highly valid and predictive of CAD events (e.g., ABI, carotid intima-media thickness, and EBCT) (55). Once a higher risk is confirmed with these methods, aggressive medical therapies for primary prevention can be recommended.

The EBCT is the most popular of these early detection methods. There is consistent evidence that coronary calcification correlates with the presence and degree of plaque at autopsy, by intravascular ultrasound (165), and by angiography (166,167). Coronary calcification is also correlated with nonfatal infarction and need for subsequent coronary revascularization in both

asymptomatic individuals (168–170), and patients undergoing coronary angiography (171). A prospective study has shown that EBCT identifies a high-risk group of asymptomatic subjects with clinically important silent ischemia as demonstrated by stress myocardial perfusion tomography (SPECT) (172). Abnormal SPECT was seen in 11.3% of patients with coronary calcium scores of 101–399, and 46% with scores of 400 or greater. Until recently, however, the added value of the coronary calcium score beyond the usual risk assessment methods had not been demonstrated. In a recent study of sibships at high risk for hypertension, a coronary artery calcium score above the 70th percentile was significantly associated with occurrence of coronary events, over an average of five years, after adjusting for Framingham risk scores (OR = 2.8; 95% CI, 1.2 to 6.4) (173). Thus, for individuals with a greater than average CAD risk (for example, those with a significant family history), the coronary calcium score obtained with EBCT has potential to detect advanced but asymptomatic coronary atherosclerosis, leading to recommendations for aggressive risk factor modification. At least one study has shown that knowledge of coronary calcium scores positively influenced behavior in self-referred subjects (174), although additional outcomes research regarding the utility of this approach is necessary. In addition, low coronary calcium scores may be valuable in defining a lower CAD risk (55), which could provide some reassurance to individuals assigned a high risk because of their family history. Risk factor modification could be relaxed somewhat for them, on the basis of this imaging.

Risk Factor Modification

Genetic information about CAD risk has value in guiding decision-making regarding lifestyle and other disease management and prevention strategies. Individuals with a strong genetic susceptibility to CAD, as determined by family history and the presence of established and emerging risk factors, may derive the greatest benefit from traditional preventive strategies such as smoking cessation and screening and treatment for elevated cholesterol and blood pressure. Individuals with CAD might also benefit from targeting emerging risk factors with specific interventions and lifestyle changes. However, for the most part, evidence regarding primary prevention of clinical cardiovascular events in individuals who have effectively modified emerging risk factors is lacking and prospective clinical trials are necessary. Therefore, it is crucial to discuss these potential benefits and limitations with any patient undergoing assessment of emerging CAD risk factors.

Cholesterol lowering is an important clinical strategy in both primary and secondary prevention of CAD (57). Use of cholesterol-lowering agents has been effective in reducing atherosclerosis incidence, disease progression, and CAD mortality (174–180). In high-risk individuals, hypercholesterolemia should be treated initially with lifestyle changes, and if necessary, with lipid-lowering medications to achieve a risk-appropriate LDL cholesterol value. However, even

when there is effective lipid lowering, a substantial proportion of individuals will develop CAD or have progression of their disease (181). Therefore, considering treatment of additional biochemical risk factors in high-risk individuals is a reasonable approach.

If there are small LDL cholesterol particles, then niacin should be considered in doses of up to 3 to 4 g a day (119,120). This can be used in combination with a statin drug if LDL cholesterol is elevated. Niacin can also be prescribed in similar doses to treat elevated lipoprotein(a) levels (108), or if estrogen replacement therapy is an option, this can be considered (111). Niacin can also raise HDL cholesterol (182), as do exercise (183) and moderate alcohol intake (184). With niacin therapy, monitoring of transaminases, uric acid, and blood glucose should be performed, as abnormalities can arise (185). Transaminases and creatinine kinase levels can also increase with statin drugs, although the usefulness of routine measurement is questionable (186). If there is evidence of hyperhomocysteinemia, then assessment of non-genetic factors should be performed (e.g., measurement of B vitamins, renal function, thyroid function, and review of medications) and B vitamin supplementation should be considered, titrating the amount of folic acid to the fasting homocysteine level (97–100). Homocysteine levels can become abnormal with niacin, fibric acid derivatives, and metformin (96), drugs that are often used in individuals at risk for CAD. Insulin resistance can be effectively treated with lifestyle changes or metformin (125).

SYNOPSIS

Individuals with genetic predisposition to atherosclerosis are at the greatest risk for developing CAD, especially at early ages. They may derive the greatest benefit from traditional preventive strategies as well as those targeting novel, emerging risk factors. Because CAD is a complex, multifactorial disorder, global risk assessment has been recognized as an effective approach in preventing CAD and its manifestations. However, genetic susceptibility to CAD is not adequately addressed by widely-used risk models such as the Framingham risk score, and underestimation of risk and missed opportunities for prevention can result for people who are genetically predisposed. The systematic collection and interpretation of family history information is currently the most appropriate screening approach to identify individuals with genetic susceptibility to CAD. Much of the familial aggregation of CAD might be explained by familial aggregation of established risk factors, such as elevated LDL cholesterol, low HDL cholesterol, and diabetes, and emerging CAD risk factors including hyperhomocysteinemia, CRP, elevated fibrin D-dimer, tissue plasminogen activator, fibrinogen, and insulin resistance. Tests to assess genetic risk for CAD are primarily biochemical analyses that measure the different pathways involved in development and progression of disease. Some of these can guide and

explain responses to treatment. Clinical applications for DNA-based testing of CAD susceptibility genes are minimal.

SUMMARY

Several lines of evidence support the contribution of genetic variations to the development and progression of CAD, and to response to risk factor modification and lifestyle choices. Genetically predisposed individuals generally have the highest risk for CAD and develop disease at an earlier age. Collection and interpretation of the family history is the best method to identify and stratify genetic risk for CAD. Additional information from the medical history, physical examination, biochemical, and DNA testing, interpreted in the context of the family history, can further refine the genetic risk assessment. Knowledge of genetic susceptibility to CAD has value in providing risk information and can influence lifestyle choices and management options. Genetically susceptible individuals might benefit the most from aggressive treatment of established CAD risk factors. In addition, many emerging risk factors are modifiable and targeting these risk factors with specific therapies may result in improved CAD prevention. Family-based prevention might be most effective for genetically predisposed individuals, since many established and emerging risk factors aggregate in families, and most are amenable to lifestyle changes. Early detection of CAD may be appropriate for genetically susceptible individuals to guide decision-making about risk factor modification. Studies are needed to generate evidence regarding the feasibility, validity, and utility of using familial risk assessment to inform CAD prevention strategies, as well as the ethical, legal, and social issues that may arise.

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9

Oxidants and Antioxidants

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Risk factors are generally first identified in epidemiological studies and the mechanisms through which they cause disease subsequently analyzed in cellular and animal models. Although this is partly true also for low levels of antioxidant vitamins as being a cardiovascular risk factor, the concept of increased oxidative stress in atherosclerosis originates mainly from experimental studies. The idea that oxidation of low density lipoprotein (LDL) in the arterial wall plays a key role in the development of atherosclerosis has gained impressive support from molecular studies in cell culture as well as from experiments performed in different animal models of atherosclerosis and subsequently also by extensive epidemiology (1,2). However, the outcome of both primary and secondary randomized intervention trials in which the effect of antioxidant vitamins on cardiovascular disease has been studied have so far largely been disappointing (3). Keeping in mind the complexity of cardiovascular disease, this may not be entirely surprising. We have for many years been well aware of the fact that smoking, diabetes, and hypertension are major risk factors for cardiovascular disease, but our knowledge of the molecular mechanism by which they cause disease remains incomplete and superficial. It may turn out to be equally difficult to identify proper treatments for specific disease mechanisms even if they are well characterized at the molecular level, such as in the case of lipid oxidation and atherosclerosis. This chapter will summarize the experimental studies on which the concept of LDL oxidation as a key mechanism in atherosclerosis is based as well as the epidemiological and intervention studies performed to assess the role of antioxidants in cardiovascular disease.

THE ANTIOXIDATIVE DEFENSE AND LIPID OXIDATION

The Antioxidative Defense

The controlled use of oxidation reactions is of course vital for all cells. The oxidation of molecular oxygen to produce energy is an extremely efficient way to produce energy but is at the same time quite hazardous as it depends on the generation and metabolism of a number of highly reactive oxygen intermediates. If these intermediates escape from the protected environment of the mitochondria they will react with and cause severe damage to cellular lipids, proteins, and DNA. A number of cellular antioxidative enzymes such as glutathione peroxidase and the cellular form of superoxide dismutase provide an effective defense against these molecules (4).

Reactive oxygen intermediates are generated also in the extracellular environment. One example is activated neutrophils that secrete oxygen radicals to kill infecting microorganisms. Other reactive radicals are produced by enzymatic reactions or through interactions with metal ions. A radical is a molecule that contains an unpaired electron. Unpaired electrons are highly reactive and will attract an electron from another molecule. The molecule that loses this electron is oxidized (oxidation is the same as loss of an electron) and becomes in turn a radical (a molecule containing an unpaired electron). If not stopped by an antioxidant this process results in an oxidative chain reaction. Lipids are particularly susceptible to oxidation. The extracellular antioxidative defense includes enzymes (such as extracellular superoxide dismutase) and vitamins. The most important antioxidative vitamins are vitamin A, C, and E and beta-carotene. Vitamin E is the main lipid-soluble antioxidant in the circulation and tissues and acts by converting the peroxyl-free radical to the less reactive hydro peroxide radical. Beta-carotene is a carotenoid that acts as a scavenger of radicals. When lipoproteins are exposed to oxidative stress, vitamin E functions as the first line of defense and beta-carotene as the second. Vitamin C is a water-soluble antioxidant that will interact with radicals primarily in non-lipid compartments. However, it also plays an important role in the regeneration of vitamin E.

Dietary Sources of Antioxidant Vitamins

The recommended daily intake of vitamin E is 10 mg. The major dietary sources of vitamin E are vegetable oils, such as olive and sunflower oil. Wheat germs and green vegetables are also good sources of vitamin E. The daily intake of vitamin E in olive oil-based Mediterranean diet is about 20 mg. Beta-carotene is present in yellow, red, and dark green vegetables such as carrots, tomatoes, yellow pumpkin, yellow chili, apricot, and beetroot. To reach a daily intake of 20 mg of beta-carotene (a supplement level commonly used in intervention trials), one should eat at least 290 g of carrots, 2496 g of tomatoes, or 2395 g of yellow chili. The

recommended intake of vitamin C is 50 mg. An orange or a grapefruit per day is sufficient to meet this requirement (3).

LDL Oxidation

Oxidation of LDL is initiated by an attack of a radical on an unsaturated fatty acid. As a result of this attack a hydrogen atom is extracted from a double bond, leaving behind an unpaired electron on the carbon atom. This carbon radical becomes stabilized by rearrangement into a conjugated diene, which rapidly reacts with oxygen to produce a hydroperoxy radical. The hydroperoxy radical will subsequently abstract hydrogen atoms from other lipid molecules resulting in a chain reaction of lipid peroxidation. The oxidative modification of LDL also results in a number of compositional changes, including increased electrophoretic mobility, increased fragmentation of apolipoprotein B, formation of reactive aldehydes such as malondealdehyde, formation of oxysterols, and derivatization of lysine amino groups in the LDL binding domain. The net result of these changes is that the LDL particle loses its ability to bind to the LDL receptor and becomes highly cytotoxic (5).

LIPID OXIDATION IN ATHEROSCLEROSIS

Lipid Oxidation Induces Vascular Inflammation

The initial step in the development of atherosclerosis is the accumulation of lipoproteins in the extracellular matrix of large and medium-sized arteries. Areas exposed to low shear stress, such as branching points, are particularly susceptible to lipoprotein penetration and accumulation. Lipoproteins are normally present in the extracellular fluid of arteries and represent an important source of cholesterol and other lipids for vascular cells. At lower levels, all lipoproteins are metabolized or removed from the vascular wall, but if present in higher concentrations they begin to attach to proteoglycans in the extracellular matrix and aggregate. These lipoproteins also become oxidized. The mechanisms responsible for inducing this oxidation remains to be fully elucidated but appear to include enzymatic modifications and reactive oxygen intermediates (Fig. 1).

In hypercholesterolemic animals, accumulation of lipoproteins in the arterial wall is associated with activation of a local inflammation (6). The process is initially manifested as an endothelial expression of adhesion molecules, including VCAM, ICAM-1, and E-selectin. These adhesion molecules recruit circulating leukocytes, primarily monocytes, and T cells (Fig. 1) that migrate through the endothelium into the intima. The function of this process is probably to remove the oxidized lipoproteins that may otherwise disturb vascular function and even cause cell death. In the vessel wall, monocytes differentiate into macrophages that express scavenger receptors. These receptors effectively bind and ingest oxidized lipoproteins. Scavenger receptors are not only specific for oxidized LDL but also recognize membrane phospholipids on dying cells. Hence,

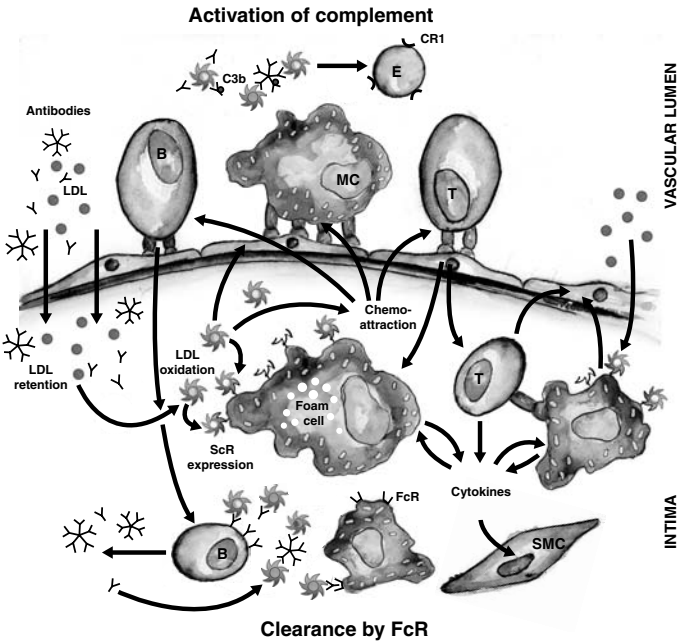

Figure 1 *The role of LDL oxidation in the initiation of atherosclerosis*. LDL particles penetrate the endothelial lining and become trapped in the extracellular matrix of the arterial wall. Some of these LDL particles become aggregated and oxidized, resulting in the release of a number of proinflammatory and toxic substances that activates endothelial expression of adhesion molecules. Monocytes (MC), T cells (T), and occasional B cells (B) attach to the endothelial surface and infiltrate the arterial intima. Monocytes differentiate into scavenger receptor (ScR), expressing macrophages that ingest oxidized LDL and become foam cells. Activation of macrophages and T cells result in release of cytokines that further enhance inflammation and stimulate smooth muscle cell (SMC) proliferation. Antibodies produced against oxidized LDL released from activated B cells may help to clear oxidized LDL from the extracellular space and the circulation.

it is likely to be part of a more general system used by the body to remove degenerated lipids and phospholipids that otherwise may cause severe damage to the surrounding tissue. When looked upon in this way, inflammation activated by oxidized lipoproteins serves a good function by limiting further injury to the vessel. However, if the accumulation of oxidized lipoprotein in vessel continues to occur at a rate exceeding their removal by macrophages, the inflammatory reaction may instead contribute to the tissue injury caused by oxidized lipids.

Macrophages that have ingested large amounts of oxidized lipids have a very characteristic morphology and are referred to as foam cells. Although they have been able to remove oxidized LDL from the extracellular matrix, they appear

to have a greater difficulty in removing the oxidized lipids from the vessel itself. Instead, large numbers of foam cells accumulate and form fatty streaks that represent the earliest stage of visible atherosclerotic lesions. The reason for the inability of the macrophage foam cell to leave the vascular wall remains to be clarified, but may involve disturbances in basic cellular functions by the large amounts of ingested oxidized lipids.

In most animal models, treatment with antioxidants such as probucol, BHT, and vitamin E leads to a reduced expression of adhesion molecules and to inhibition of fatty streak formation. Notably, most animal studies using antioxidants to inhibit atherosclerosis are relatively short-term and have focused on the role of early lesion growth. More recently, the notion that oxidation of LDL is involved in atherogenesis has also been supported by experiments carried out in knockout mice. For example, a targeted disruption of the gene for 12/15 lipoxygenase, a potent inducer of LDL oxidation, profoundly inhibits atherogenesis in apoE-deficient mice. Disruption of the gene for CD 36, one of the members of the scavenger receptor family, also results in significant reduction of atherosclerosis in apoE-deficient mice.

Immune Reactions Against Oxidized LDL

In 1989 Palinski and coworkers (7) were able to produce antibodies that specifically recognized epitopes in oxidized LDL. Using these antibodies, they could demonstrate that human atherosclerotic plaques contained oxidized LDL. However, they also found that circulating antibodies against oxidized LDL was a quite common phenomenon in man. Indeed, exactly as was found to be the case for the scavenger receptors, these antibodies cross-reacted with phospholipids occurring on dying cells. Both the scavenger receptors and the oxidized LDL antibodies are thus likely to be part of an immune response involved in identifying and removing debris from dying cells, oxidatively damaged lipoproteins, and other potentially damaging phospholipid-associated degradation products (Fig. 1).

These observations suggested the interesting possibility that atherosclerosis may be caused by autoimmune reactions against our own lipoproteins once they had been oxidized in the vascular wall. Some studies also reported association between high levels of oxidized LDL antibodies and a more aggressive progression of atherosclerosis. One possibility to test this hypothesis is to immunize experimental animals with oxidized LDL and to study if this results in development of a more aggressive disease. However, somewhat surprisingly, such studies demonstrated that immune responses against oxidized LDL have a protective effect (8–11). Activation of these immune responses represents a new and promising target for prevention and treatment of cardiovascular disease (12).

Lipid Oxidation and Development of Advanced Lesions

As the process of atherosclerosis continues, fatty streaks develop into fibromuscular plaques. The initial step in this transition is the phenotypic

modulation of contractile smooth muscle cells of the media into synthetically active repair cells. The mechanisms involved in activation of this modulation remain to be fully understood, but they appear to involve degradation of the extracellular matrix surrounding the smooth muscle cells by proteases released from inflammatory cells. Accordingly, inflammation has a key role in activation of the vascular fibroproliferative process. This is not surprising since inflammation is the general signal for repair after any tissue injury. Again, lipid oxidation may influence this process primarily by causing injury and inflammation.

At lower concentrations, products generated during lipoprotein oxidation may also act directly as growth factors for smooth muscle cells. However, at higher concentrations, these products generally become cytotoxic and limit the proliferative capacity of the smooth muscle cells. The latter situation may indeed be more relevant for the clinical situation. Fibromuscular plaques that cover more than 75% of the lumen may significantly compromise blood flow and give rise to angina, but they rarely cause infarction. Most infarcts are attributed to degenerative changes in plaques leading to plaque rupture and formation of occlusive thrombosis.

A necrotic core covered by a thin fibrous cap characterizes vulnerable plaques. The fibrous cap consists of smooth muscle cells, collagen, and other extracellular matrix. This matrix holds the plaque together, but it is constantly under the risk of being degraded by collagenase and other matrix proteases released by macrophages surrounding the necrotic core. If the fibrous cap contains viable smooth muscle cells, these may replace the degraded extracellular matrix. However, if the smooth muscle cells, are severely injured or killed by toxic lipid oxidation metabolites, the fibrous cap will become weakened and rupture in response to shear stress caused by the blood flow (13). In man, treatment with statins have been shown to decrease the amount of oxidize lipids and inflammatory cells and to increase collagen content and smooth muscle cell viability in atherosclerotic plaques (14).

EPIDEMIOLOGICAL STUDIES OF ANTIOXIDANTS AND CARDIOVASCULAR DISEASE

A number of prospective observational studies have addressed whether intake of vitamin C, vitamin E, or beta-carotene affects cardiovascular risk after adjustments for known cardiovascular risk factors. Both dietary intake, as well as vitamin supplementation, have been assessed in several of these studies. The study designs used include cross-sectional studies comparing population in different countries, large epidemiological cohort studies, and case-control studies. In general, the results of these studies provide support for a protective effect of antioxidant vitamins on cardiovascular disease.

The most consistent and solid support for a protective effect of antioxidants has been obtained for vitamin E. In the Nurse's Health Study, 87,000 women were followed for an average of 8 years. Those in the highest quintile of vitamin E

intake had a 34% reduction in risk of cardiovascular events as compared with the lowest quintile after adjustment for known risk factors and the intake of other antioxidant vitamins (15). Similar analyses in the Health Professional's Study (40,000 adult men followed for an average of 4 years) demonstrated a 39% reduction in coronary events for those with the highest intake of vitamin E as compared to those with the lowest intake (16). In the Iowa Women's Health Study, a study performed on almost 35,000 post-menopausal women followed for an average of 7 years, a 62% reduction in cardiovascular deaths was demonstrated in those with the highest intake of vitamin E from food (17). A meta-analysis based on studies that together included 166,774 subjects found statistically significant inverse relations between intake of vitamin E and risk of developing cardiovascular disease (OR 0.64; 95% CI 0.56–0.73) (3). Most studies suggest that a cardiovascular protection by vitamin E requires a longer period of increased intake (>2 years).

Cross-sectional studies have shown that median plasma levels of vitamin E is significantly higher in countries with a low cardiovascular mortality, such as the Mediterranean countries, than in countries with a high cardiovascular mortality, such as Finland and Scotland.

Some epidemiological studies have also reported that a high intake of beta-carotene is associated with a reduced risk for cardiovascular disease. The Health Professional's Study reported a 29% reduction (95% CI—14 to -47%) of coronary heart disease in the highest quintile of beta-carotene intake as compared to the lowest (16). However, in contrast, no beneficial effect of beta-carotene was found in the Nurse's Health Study (15) and the Iowa Women's Health Study (17). These three large studies also failed to demonstrate any association between intake of vitamin C and cardiovascular mortality.

Randomized Clinical Trials

The effects of antioxidant vitamins on cardiovascular disease have now been evaluated in several double blind, placebo-controlled, randomized clinical trials. With the exception of the Cambridge Heart Antioxidative Study (CHAOS) (18), all of these studies have failed to demonstrate any beneficial cardiovascular effect of antioxidants. In the CHAOS, 2,002 patients with angiographically documented coronary heart disease were randomized to vitamin E (400–800 IU daily) for an average of 18 months. Cardiovascular events were reduced by 53% (95% CI -17 to -66%), but there was no significant effect on cardiovascular or total mortality.

The Alpha-Tocopherol Beta Carotene Cancer Prevention Study tested daily supplementation with 20 mg of beta-carotene and 50 mg of alpha-tocopherol in 1,862 men with previous myocardial infarction (19). The study was a randomized, double blind, placebo-controlled trial with a median follow-up of 5.3 years. Beta-carotene supplementation led to a significant increase in total mortality ($+9\%$; 95% CI $+2$ to $+17\%$) and lung cancer ($+18\%$; 95%CI $+3$ to $+36\%$), and a

non-significant increase in mortality from cardiovascular disease (+11%; 95% CI -1 to +23%).

In the Beta-Carotene and Retinol Efficacy Trial, the effect of 30 mg beta-carotene and 25,000 IU of vitamin A daily on lung cancer and cardiovascular disease was tested in multicenter, randomized, double-blind, placebo-controlled, primary prevention trial involving 18,314 smokers, former smokers, and workers exposed to asbestos followed for up to 4 years (20). Treatment was associated with a higher risk of lung cancer (+28%; 95% CI +4 to +57%) and had no protective effect on cardiovascular disease.

In the Physicians' Health Study, 22,071 healthy, adult U.S. male physicians were randomized to 50 mg of beta-carotene on alternate days or placebo (21). There was no effect on total mortality, cancer mortality, or cardiovascular mortality after an average follow-up of 12 years.

The Heart Outcomes Prevention Evaluation (HOPE) study used a two-by-two factorial design to test the effect of 10 mg of the angiotensin-converting enzyme inhibitor ramipril and 400 IU of vitamin E daily on cardiovascular disease (the primary outcome was a composite of myocardial infarction, stroke, or death from cardiovascular causes) in 9297 high-risk patients (22). The average age of the patients was 66 years; a little over half had had a previous myocardial infarction and about one-quarter had unstable angina. Whereas ramipril significantly reduced death, myocardial infarction, and stroke, vitamin E was without effect.

The GISSI (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico)-Prevenzione trial also used a two-by-two factorial design to study the effect of 300 mg of vitamin E, 1 g of n-3polyunsaturated fatty acids (PUFA) or the combination of both in 11,324 patients surviving a recent (<3 months) myocardial infarction (23). Treatment with n-3 PUFA significantly reduced the risk of death by 14% (95% CI 3 to 24%) and cardiovascular death by 17% (95% CI 3 to 29%). The effect of combined treatment was the same as that for n-3 PUFA alone and vitamin E was without effect.

The Heart Protection Study analyzed the effects of 40 mg simvastatin and/or antioxidant vitamins (600 mg E, 250 mg C and 20 mg beta-carotene daily) in 20,563 individuals with or without prior cardiovascular disease. Whereas treatment with simvastatin was found to reduce risk of major cardiovascular events by more than 30%, there was no effect of treatment with antioxidant vitamins.

In the Women's Health Study conducted between 1992 and 2004, 39,876 apparently healthy U.S. women aged at least 45 years were randomly assigned to receive vitamin E (600 IU) or placebo and aspirin or placebo, using a 2×2 factorial design, and were followed up for an average of 10.1 years. During follow-up, there were 482 major cardiovascular events in the vitamin E group and 517 in the placebo group, a nonsignificant 7% risk reduction. There were no significant effects on the incidences of myocardial infarction or stroke, as well as ischemic or hemorrhagic stroke. For cardiovascular death, there was a significant

24% reduction (P = .03). There was no significant effect of vitamin E on total mortality (24).

Antioxidants have also been tested for a potential therapeutic role in the reduction of restenosis after angioplasty. In the Multivitamin and Probucol study, the effect of a combination of vitamins (1 g of vitamin C, 1,400 IU of vitamin E and 100 mg of beta-carotene) and probucol given separately or together on the rate of restenosis after angioplasty was investigated (25). Probucol was found to significantly reduce restenosis, whereas antioxidant vitamins were without effect. Also the Probucol Angioplasty Restenosis Trial reported a significant reduction in restenosis in response to probucol treatment (26).

In 2004, the American Heart Association Science Advisory concludes that at present the existing scientific database does not justify routine use of antioxidants for prevention and treatment of cardiovascular disease, but it recommends that research continue to clarify the discrepancy between the randomized trials and the population studies (27).

Why have so many Antioxidant Trials been Negative?

Against the background of all experimental and epidemiological data suggesting that lipid oxidation plays an important role in atherogenesis, the outcome of large intervention trials such as HOPE and GISSI are clearly disappointing. The reasons for this lack of effect have been discussed by Daniel Steinberg (28):

1. Vitamin E, while able to inhibit the early stages in atherosclerosis in animal models, has little or no effect on advanced lesions or on plaque rupture and thrombosis
2. Vitamin E is not a sufficiently potent antioxidant in humans or does not have appropriate pharmacokinetic properties
3. There is a true species difference between humans and animal models such that antioxidants will simply never have an effect on atherogenesis in humans.

If any of the first two explanations are true, we have not yet seen the results of a trial that tests the lipid oxidation hypothesis under proper conditions.

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