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Cardiovascular Disease

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Introduction

Cardiovascular epidemiology has made use of biomarkers over several decades of research and can, therefore, be viewed as a resource for methods in molecular epidemiology that can be applied to other diseases. This chapter selects examples from the vast literature in this area to illustrate some of the challenges of etiologic research involving biomarkers and, at the same time, provides examples of some of the exciting advances being made in cardiovascular epidemiology.

Cardiovascular diseases span a broad range of pathologic processes, including coronary heart disease (CHD), hypertension, cardiomyopathies, valvular abnormalities, and arrhythmias. This chapter focuses predominantly on the application of lipid and lipoprotein biomarkers to CHD (Table 13.1).

Historically, cardiovascular studies began with the measurement of very broad categories of lipids, such as total cholesterol (Figure 13.1), and progressed to more specific lipid-related biomarkers, such as very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), low density lipoprotein subclass phenotypes A and B, and plasma levels of apo AI and B (Keys and Parlin, 1966; Kannel *et al.*, 1971b; Brunzell *et al.*, 1984; Austin *et al.*, 1988,1990b). Genetic markers such as restriction fragment length polymorphisms (RFLPs) for apo B, apo E, and the LDL receptor genes have been used to evaluate directly the inherited contribution to CHD (Humphries, 1987; Friedl *et al.*, 1990b; Dallongeville *et al.*, 1991) (Table 13.2). Thus, a range of lipid markers and their environmental and genetic determinants have been used in epidemiologic studies to evaluate risk of developing coronary heart disease.

Molecular Epidemiology: Principles and Practices

TABLE 13.1 Biologic Markers of Lipid Metabolism Related to Cardiovascular Disease Risk

Marker type	Example(s)
Plasma lipid levels	Total cholesterol, triglyceride, LDL-C, HDL-C
Plasma apolipoprotein levels	ApoB, ApoAI
Enzyme activity	Lipoprotein lipase
Lipoprotein mass, lipid and protein components	Lp(a)
Subclasses of LDL, subpopulations of HDL	Subclass A and B phenotypes
DNA markers for candidate genes	ApoB, AI–CIII–AIV complex
RFLP VNTR[a] markers	LDL receptor
Genetic protein markers	ApoE isoforms; Apo(a) phenotypes

[a]Variable number of tandem repeats.

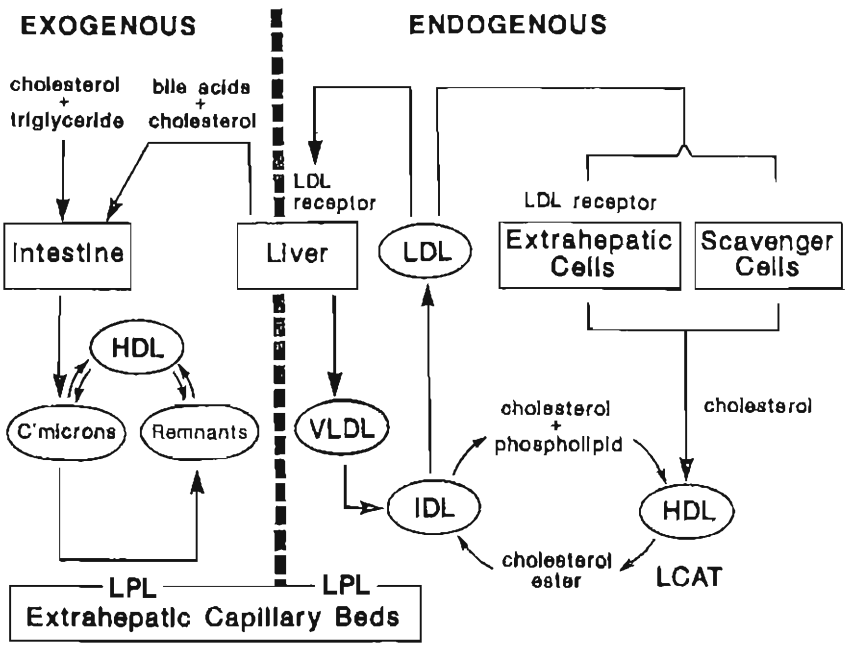

FIGURE 13.1 Dietary cholesterol is packaged into large triglyceride-rich chylomicron particles in the intestinal enterocytes and secreted into the blood stream via the mesenteric lymphatic chain. Lipoprotein lipase in adipose tissue and striated muscle hydrolyses the triglyceride core of the chylomicron, shedding redundant surface phospholipid and protein into HDL. The resulting remnant is assimilated by a hepatic receptor. Hepatic lipids are secreted as triglyceride-rich VLDL that enter a delipidation cascade that reduces their triglyceride content and results in the production of cholesterol-enriched remnants or intermediate density lipoprotein (IDL). The majority of the latter is further remodeled to LDL. This, in turn, is catabolized primarily by receptors present on liver and peripheral tissues. Cholesterol balance at these peripheral sites is maintained by a system of reverse cholesterol transport involving HDL. (Reprinted with permission from Shepherd *et al.*, 1991. Copyright © 1991 by John Wiley & Sons.)

TABLE 13.2 Some Candidate Genes Involved in Lipid Metabolism

Class	Gene
Apolipoproteins	ApoAI
	ApoAII
	ApoB
	ApoCI
	ApoCII
	ApoCIII
	ApoE
Enzymes	HMG CoA reductase
	Lecithin cholesterol acyl transferase
	Fatty acyl-CoA cholesterol acyl transferase
	Endothelial lipoprotein lipase
	Hepatic triglyceride lipase
	Fatty acid synthetase
	Phosphatidic acid phosphohydrolase
	Cholesterol ester hydrolase
	Cholesterol 7-α hydrolase
Transfer proteins	Lipid transfer proteins
Receptors	Low density lipoprotein
	High density lipoprotein

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Plasma Lipids

Markers of Biologically Effective Dose

Characterization of the role of plasma lipids in CHD can be traced to 1847 when Vogel reported that atherosclerotic plaques contained relatively large amounts of cholesterol (Vogel, 1847). In 1873, Fagge reported a post-mortem examination of a patient with xanthomas (yellow colored nodules or plaques made of lipid that affect the eyelids and other sites) and atheromatous degeneration of the arteries and cardiac disease. Thus, one of the first clues to the importance of plasma lipids in the pathogenesis of CHD literally stared physicians in the face! Familial aggregation of individuals with tendon xanthomas, hypercholesterolemia, and CHD was demonstrated first by Thannhauser (1938) and Muller (1938) (Brunzell *et al.*, 1984) and subsequently has been studied by numerous investigators (Goldstein *et al.*, 1973). The Framingham study was one of the first studies to generalize the finding of hypercholesterolemia as a risk factor in families to the concept that it was also a risk factor for the general population (Kannel *et al.*, 1971b). This observation has been demonstrated repeatedly in a large number of subsequent programs, including the Multiple Risk Factor Intervention screening, the largest to date, which demonstrated a strong dose-dependent association of

baseline serum cholesterol levels with subsequent risk of CHD mortality in 356,222 men (Stamler *et al.*, 1986).

The relationship between cholesterol and CHD has been evaluated in case-control, prospective, nested case-control, intervention, twin, and family studies. Of these various study designs, case-control studies are somewhat limited in their ability to study CHD since a substantial number of individuals with myocardial infarctions experience sudden death outside the hospital. Further, the demonstration that cholesterol levels decline in the period immediately following a myocardial infarction has resulted in the need to study cases several months after infarction, further increasing the selection bias of case-control studies that can evaluate only survivors (Tibblin and Cramer, 1963). With the advent of widespread use of coronary angiography, however, it has been possible to perform case-control studies in individuals with and without coronary artery disease (Dahlen *et al.*, 1986). This option has provided an opportunity to evaluate a range of biomarkers associated with the severity of atherogenesis. Overall, prospective observational studies have been perhaps the most important study designs for assessing lipid risk factors for CHD because of their ability to show the predictive value of lipid biomarkers. In addition, angiographic evaluation has strengthened further any knowledge about causal associations between cholesterol level and risk of CHD derived from observational studies (Blankenhorn *et al.*, 1987; Brown *et al.*, 1990).

Thus, using a variety of epidemiologic study designs, total serum cholesterol has been demonstrated to be the first validated biomarker associated with increased risk of CHD. Serum cholesterol conforms to the definition of a marker of biologically effective dose, for it represents a measure of potentially atherogenic lipids that can be delivered to the target organ (atheromas in coronary arteries). It integrates exogenous influences, such as dietary intake of cholesterol and its precursors, and other factors such as exercise, smoking, and medication (e.g., estrogen treatment in women) (Austin *et al.*, 1987; Rose, 1990; Castelli *et al.*, 1990; Nora *et al.*, 1991), and endogenous effects, such as hepatic cholesterol synthesis and genetic influences. Although total plasma cholesterol measures the cholesterol carried by several types of lipoprotein particles that are associated with increased risk (LDL-C) and decreased risk (HDL-C), this overall measurement still remains a relevant risk factor for most individuals in the general population.

Low density lipoprotein cholesterol (LDL-C) is an important risk factor for the development of CHD. Prospective epidemiologic studies (Kannel *et al.*, 1971a; Pekkanen *et al.*, 1990) and intervention trials [Lipid Research Clinics Program (LRCP), 1984; Ross, 1986; Blankenhorn *et al.*, 1987; Manninen *et al.*, 1988] have demonstrated that plasma levels of LDLs, the primary carriers of cholesterol in the bloodstream, are directly related to disease risk.

Several studies have demonstrated decreased risk of CHD with high levels of HDL cholesterol (Stampfer *et al.*, 1985; Miller, 1987; Gordon *et al.*,

els of HDL cholesterol (Stampfer *et al.*, 1985; Miller, 1987; Gordon *et al.*, 1989). The ratio of total cholesterol to HDL has been demonstrated to be one of the strongest risk factors for developing CHD, particularly in individuals with total cholesterol levels under 200 (Stampfer *et al.*, 1985). The evidence for a causal association between elevated HDL levels and decreased risk for CHD has been strengthened by intervention studies such as the Helsinki Heart Study, which demonstrated that raising HDL cholesterol levels through drug treatment of dyslipidemic men resulted in a decline in CHD (Manninen *et al.*, 1988).

Markers of Susceptibility

Extensive evidence is available that CHD is mediated by many genetic loci (MacCleur and Kammerer, 1991) (Figure 13.2). Nora and colleagues (1991) have reviewed the environmental and genetic determinants of several of the most important lipids and apoproteins. Nora *et al.* (1991) have concluded that although monogenic hyperlipidemias account for at least 20% of myocardial infarctions prior to age 60, other "genetic dyslipidemias" must account for a portion of excess risk for early onset CHD in the general population, based on data from family studies. Some of the more important potential susceptibility markers follow.

Apoliprotein E Polymorphism

Apoliprotein (apo) E polymorphism was among the first reported genetic polymorphisms that explained part of the normal variation in cholesterol concentrations in humans (Sing and Davignon, 1985). Because apo E is present on VLDL particles and is a ligand for the LDL receptor, it is important in the catabolism of cholesterol. The three common alleles or isoforms, E2, E3, and E4, reflect specific amino acid substitutions in the apo E protein (Davignon and Gregg, 1988). Relative to the most common E3 allele, E2 has been associated consistently with lower levels of plasma cholesterol whereas E4 is associated with increases in LDL cholesterol in a variety of ethnic groups (Sing and Davignon, 1985). Allele variation at the apo E gene was compared in 182 subjects with endogenous hypertriglyceridemia, 98 subjects with familial hypercholesterolemia due to a 10-kb deletion in their LDL receptor gene, and 424 normolipidemic controls from the same environmental background (Dallongeville *et al.*, 1991). The study showed that lipid and lipoprotein concentration variability within the dyslipoproteinemic groups is related to apo E phenotype. The apo E polymorphism has been reported to explain 5–8% of the variance in serum cholesterol levels in populations from several countries (Humphries *et al.*, 1991). Although reports of direct associations between these alleles and atherosclerotic diseases are less consistent (Davignon and Gregg, 1988), a report based on young males who died from accidental causes demonstrated that apo E genotypes were associated with

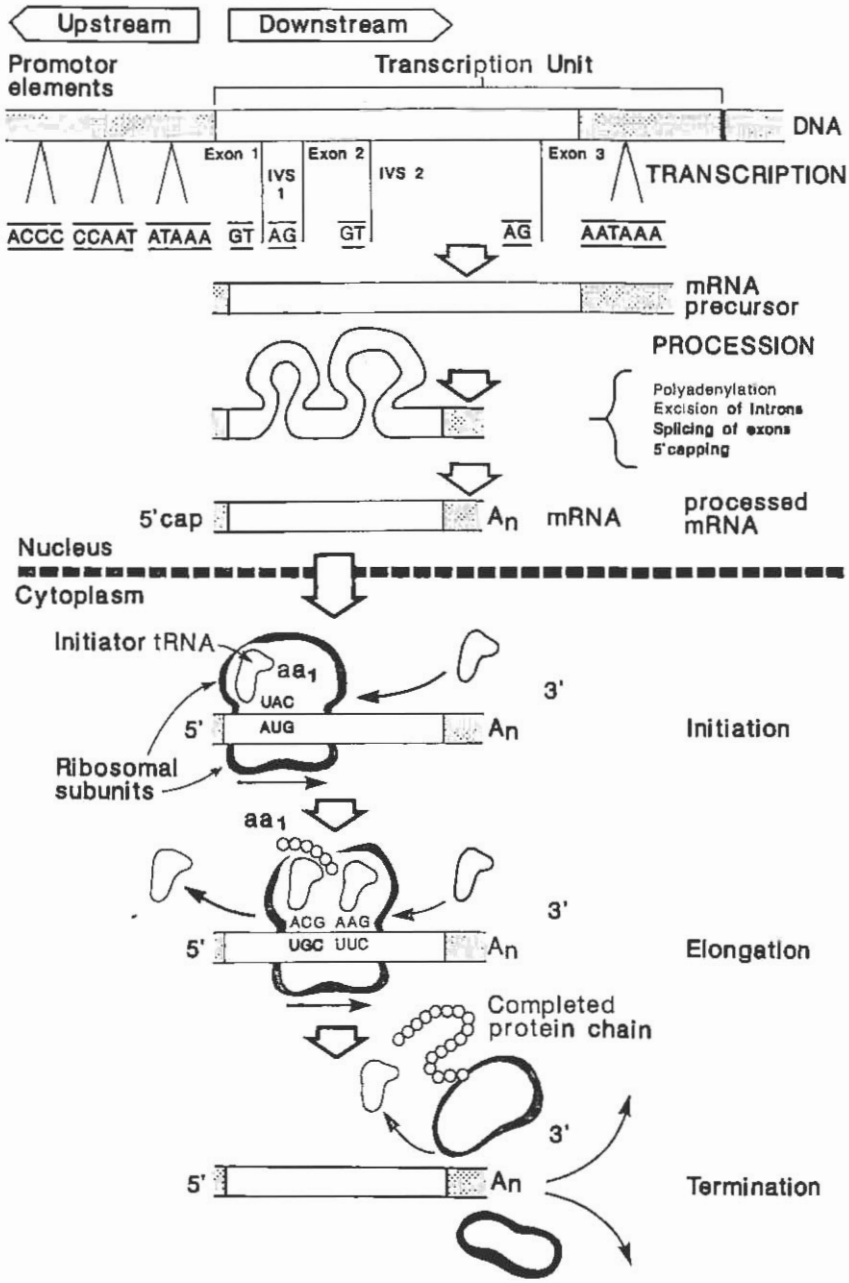

FIGURE 13.2 Different levels at which the phenotypic effects of the mutations are mediated. (Reprinted with permission from Weatherall, *et al.*, 1991.)

ciation persisted after adjustment for total cholesterol, suggesting that apo E alleles may have a direct effect on atherosclerosis.

Lipoprotein(a)

Lipoprotein(a) [Lp(a)] is a unique lipoprotein consisting of two components: a particle similar to LDL, including apo B, and the apo(a) protein linked by a disulfide bond to the apo B molecule (Utermann, 1989). The apo(a) protein is the distinguishing characteristic of Lp(a) and results in a lipoprotein particle that is larger and more dense than LDL. The gene for the apo(a) protein has been mapped to the tip of the long arm of chromosome 6, closely linked to the gene for plasminogen (Frank *et al.*, 1988). These two genes show a high degree of homology, including the protease domain and several sequences that code protein kringle domains (McLean *et al.*, 1987).

Lp(a) has been the focus of intensive research interest since its original discovery by Berg (1963). It is now well established that increased plasma levels of Lp(a) constitute an independent risk factor for CHD that also may be involved in thrombogenesis (Utermann *et al.*, 1987; Scanu, 1991). In 1987, Utermann reported size isoforms of apo(a) that were inherited in families and were associated with Lp(a) concentrations in plasma (Utermann *et al.*, 1987). Mean levels of plasma Lp(a) consistently have been associated inversely with the apo(a) size polymorphism in subsequent studies as well (Gaubatz *et al.*, 1990). Boerwinkle *et al.* (1989) have reported that 41.6% of the variance in Lp(a) levels can be attributed to apo(a) phenotypes in Caucasians, compared with as much as 70% of the variation in Asians (Sandholzer *et al.*, 1991). Lp(a) levels also are known to vary dramatically among ethnic groups (Sandholzer *et al.*, 1991). Lackner *et al.* (1991) have identified 19 alleles at the apo(a) locus using pulsed-field gel electrophoresis. These alleles reflect the number of kringle IV repeats, each of which is approximately 5.5 kb in length; nearly all individuals are heterozygous using this system. Thus, the apo(a) locus is even more polymorphic than can be recognized by apo(a) size isoforms. Further studies leading to an understanding of the genetics of Lp(a) undoubtedly will provide important insights into the role of Lp(a) in atherosclerosis risk among families.

Apolipoprotein B

Apo B is the primary protein component of LDL particles. Elevated plasma apo B levels are another important risk factor for CHD. Although a prospective study of apolipoproteins found only a borderline association between apo B levels and myocardial infarction (Stampfer *et al.*, 1991), case-control studies have shown significant associations (Sniderman *et al.*, 1980; Brunzell *et al.*, 1984). Regression of coronary lesions was associated with reductions in apo B levels as a result of intensive lipid-lowering intervention (Brown *et al.*, 1990). A disorder denoted hyperapobetalipoproteinemia was identified in a study that found a subset of subjects with CHD who had normal LDL cholesterol levels but increased apo B levels (Sniderman *et al.*,

1980). A number of large-scale family studies have examined genetic models for the inheritance of apo B levels. Three of these, using complex segregation analysis, found evidence for major gene effects (Amos *et al.*, 1987; Hasstedt *et al.*, 1987; Pairitz *et al.*, 1988), whereas another found significant polygenic effects (Beaty *et al.*, 1986). An analysis of familial combined hyperlipidemia, the most common of the familial hyperlipoproteinemias, demonstrated that apo B levels were distributed bimodally among family members with LDL subclass phenotype B (Austin *et al.*, 1992), providing additional evidence for genetic influences on apo B in this form of familial hyperlipidemia. Although two specific kinds of relatively rare mutations in the apo B gene on chromosome 2 (familial defective apo B100 and truncated apo B isoforms) are known to alter apo B plasma levels (Assman *et al.*, 1991), associations have been more difficult to establish in larger population-based studies (Galton, 1990).

Apolipoprotein AI

Similar to HDL cholesterol, apo AI, the major protein component of HDL particles, is associated inversely with risk of CHD (Brunzell *et al.*, 1984; Stampfer *et al.*, 1991). Based on a large population-based sample of families, evidence for the influence of a single major gene was found in a subset of the pedigrees (Moll *et al.*, 1989). Lower levels of apo AI also have been reported in children whose fathers had histories of myocardial infarction than in children with no such parental history (Freedman *et al.*, 1986). At least one study of RFLPs in the region of the AI-CIII-AIV gene complex on chromosome 11 has reported an association between apo AI plasma levels and genetic variation at these RFLP sites (Kessling *et al.*, 1988). Thus, decreased apo AI levels are likely to be another genetically influenced factor related to CHD.

LDL-Receptor Gene

The mechanism underlying familial hypercholesterolemia (FH) is the best understood genetic defect of LDL metabolism known to cause atherosclerosis (Brown and Goldstein, 1986). In FH, defects in the LDL receptor gene on chromosome 19 lead to an accumulation of cholesterol in plasma and to premature CHD. Clinically, the presence of tendon xanthomas is a hallmark of this disease. The homozygous form is very rare and very severe: cholesterol levels are often 10 times above normal, and CHD occurs in the teens or twenties (Brown and Goldstein, 1986). However, FH is a relatively rare disorder; the prevalence of heterozygotes and homozygotes for this disease is estimated to be 1 in 500 and 1 in a million, respectively (Goldstein *et al.*, 1973). It is now known that there are few true homozygotes, since a variety of different mutations in the LDL receptor gene exist. With the exception of the French Canadian population (Moorjani *et al.*, 1989), most apparent homozygotes are actually compound heterozygotes (Brown and Goldstein, 1986). The heterozygous form of FH, although less severe, re-

sults in cholesterol elevations 2–3 times greater than normal and premature coronary disease. The metabolism and feedback system for receptor-mediated cholesterol homeostasis is now well understood and provides some of the most compelling evidence that elevated LDL cholesterol levels cause atherosclerosis.

Using four RFLPs of the human LDL receptor (LDL-R) gene, Humphries *et al.* (1991) studied 289 normolipidemic individuals in Italy and found that the *Pvu*II RFLP explained 9.6% of the variance in LDL cholesterol levels. This RFLP was associated with lower levels of total and LDL cholesterol. The data suggest it may be associated with increased survival. The 3′ half of the LDL-R gene, showing localization of restriction sites and polymorphic sites, is shown in Figure 13.3A. Autoradiographs of Southern blot hybridization showing variable bands of the LDL-R gene observed in individuals with different genotypes for four polymorphisms are shown in Figure 13.3B.

Markers of Thrombosis

In addition to markers related to atherogenesis, another contributor to CHD is a tendency toward arterial thrombosis. Studies have suggested that variability in factor VII coagulant activity and plasma fibrinogen (Meade *et al.*, 1986), platelet count and platelet aggregation (Thaulow *et al.*, 1991), fast acting plasminogen activator inhibitor (an indicator of reduced fibrinolytic capacity; Hamsten *et al.*, 1987), and aspirin intake [Steering Committee of the Physician's Health Study Research Group (1989)] may affect risk of CHD. This area of CHD research promises to continue to be active.

Assessing Genetic and Environmental Interactions for Coronary Heart Disease Risk

Multivariate analyses have been used extensively to combine biomarker and nonbiomarker data to assess the effects of environmental exposures (such as the role of smoking and diet), blood pressure, and genetic risk factors for risk of CHD and their potential interactions.

MacCleur and Kammerer (1991) summarized the progression of thinking about genetic and environmental interactions:

> In 1954, Lerner (1954) proposed that genes influence not only the mean levels of quantitative traits but also the variation around the mean. He suggested that differences in intragenotypic variances may be due to an inability by individuals of a specific genotype to "buffer" their phenotypes against other genetic and environmental factors. Murphy (1979) extended these concepts, with particular reference to human populations. . . . Berg (1988, 1990; also see Berg *et al.*, 1989) has investigated the effects of "variability genes" on CHD risk factors. For such genes, the mean value of two genotypes may be similar, but the intragenotypic variances differ. Increased phe-

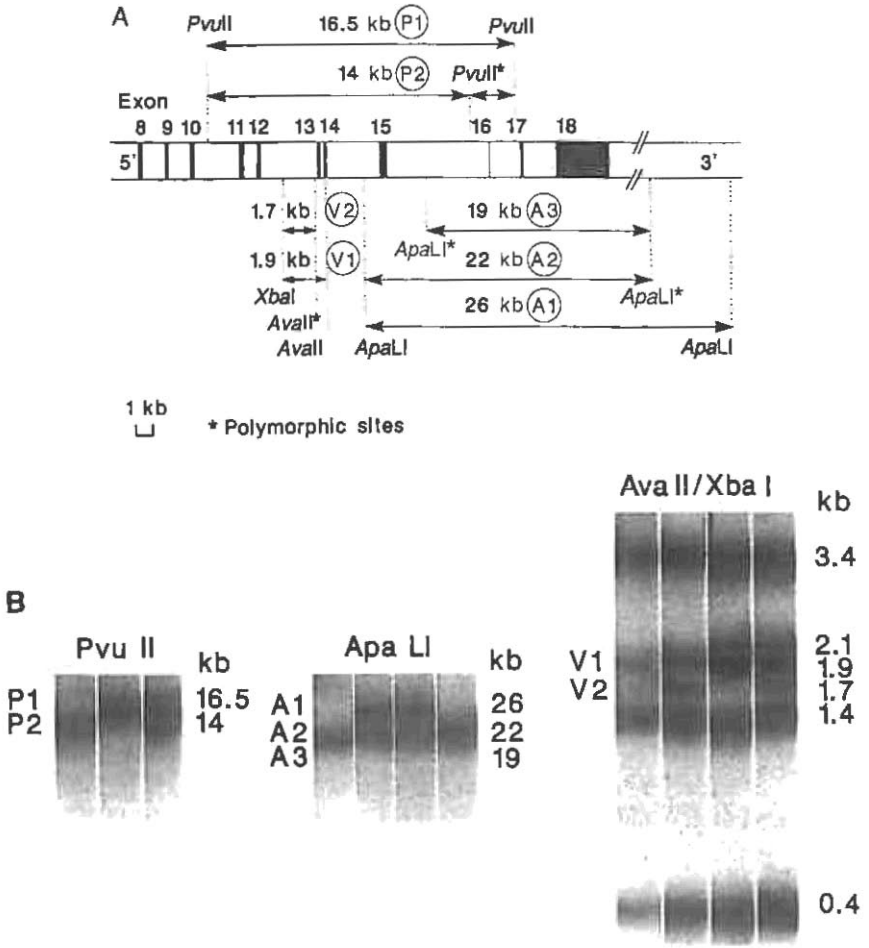

FIGURE 13.3 (A) Map of the 3′ half of the low density lipoprotein receptor (LDL-R) gene showing localization of *Pvu*II, *Apa*LI, and *Ava*II restriction sites and polymorphic sites. Also shown are alleles P1, P2, V1, V2, A1, A2, and A3. (B) Autoradiograms of Southern blot hybridization showing variable bands of the LDL-R gene, observed in individuals with different genotypes for four polymorphisms. (Reprinted with permission from Humphries *et al.*, 1991).

notypic variance within a genotype may be due either to genotype × environment or genotype × genotype interaction or to linkage disequilibrium.

Markers of Effect

Few validated biological markers of effect are available for CHD, with the exception of short-term markers (cardiolytic enzymes) associated with acute myocardial infarction. Many of the lipid and lipoprotein markers indicate

risk of coronary artery disease, but do not necessarily indicate the presence or degree of atherosclerotic disease. Although often the diagnosis of coronary artery disease can be made from the history and by noninvasive techniques, angiography provides a definitive indication of the disease (Pasternak and Reis, 1991). Angiographic findings represent a biologic marker different than the serum or plasma molecular genetic markers considered elsewhere in this chapter. Nonetheless, angiographic imaging is a biomarker of disease. It is valid because it is a direct representation of arterial stenosis. It allows for an examination of the coronary tree and details of the coronary anatomy, assessment of individual variations in arterial distribution, anatomic or functional pathology (atherosclerosis, thrombosis, congenital anomaly, or focal coronary spasm), and the presence of inter- and intracoronary collateral connections (Baim and Grossman, 1991). Angiographic assessments of disease severity have been used particularly effectively in intervention trials (Blankenhorn *et al.*, 1987; Brown *et al.*, 1990).

Angiography historically involved nonselective injections of contrast medium into the aortic root, with simultaneous specification of both the left and right coronary arteries, and recording of the angiographic images on a conventional sheet of film. Although this approach is still in use, it is being replaced by selective coronary injections using specially designed catheters advanced from the brachial or femoral approach. The outcomes of angiography are images that can be used to quantitate coronary stenosis. The degree of stenosis usually is quantitated by visual evaluation of the percentage of diametric reduction relative to the caliber of the adjacent normal segments (Baim and Grossman, 1991). This assessment usually gives a fairly accurate representation in very mild or very severe stenoses, although overestimation of stenosis severity is common. There is substantial interobserver variability (frequently ± 20% in the visual quantitation of moderate stenoses between 40 and 82%). This range of stenosis is particularly important because a 50% diameter stenosis (75% cross-sectional area) is barely "hemodynamically significant" at peak coronary flows, but a 70% diameter stenosis (90% cross-sectional area) is restrictive at these same peak flows (Wilson *et al.*, 1987; Baim and Grossman, 1991). Angiography studies can suffer from incomplete, uninterpretable, or misinterpreted findings due to the inexperience of the operator who uses faulty techniques or cannot recognize misleading images. In addition, observations are not always independent, since multiple lesions and arteries may be evaluated within one individual.

Angiographic results are being used more frequently to define "cases" in case-control studies (Maciejko *et al.*, 1983; Dahlen *et al.*, 1986). In addition to traditional cinegraphic angiography are a number of imaging techniques, such as magnetic resonance angiography, Doppler sonography, B mode ultrasonography, and single photon emission computed tomography (Underwood *et al.*, 1990; van der Wall *et al.*, 1991; Polak *et al.*, 1992) that may become useful in epidemiologic research. As Rose (1990) notes:

> The future lies with a new style of cardiovascular epidemiology, much more closely linked with clinical and laboratory disciplines. . . . It will seek to integrate epidemiology with the study of intermediary outcomes; and in its measurement of disease, it will avail itself of advanced techniques such as noninvasive imaging, which can so powerfully supplement clinical outcomes as a measure of the occurrence and progress of disease.

Issues in the Analysis of Biomarkers

The biologic markers discussed thus far are part of a web of causes and effects. Clarification of their roles and relationships may be enhanced by multivariate analyses. There are potential difficulties in the simultaneous analysis of multiple markers; markers may exhibit different degrees of laboratory precision in their measurement, different degrees of true intraindividual variations, and some degree of correlation. These problems are not new to epidemiologists, but are illustrated well in cardiovascular epidemiology.

Intraindividual Variation in Biomarker Measurements

Most large-scale prospective studies biologically monitor study participants at only one point in time. Substantial intraindividual variation in biomarker levels may result in marked misclassification of the true mean level of the marker. If the misclassification bias is nondifferential (i.e., does not differ for diseased and control groups), this bias may attenuate measures of association between a given marker and disease (Kelsey *et al.*, 1986).

Investigators have attempted to understand and control some of the determinants of the daily fluctuation of serum cholesterol levels by standardizing phlebotomy conditions (Cooper *et al.*, 1992). Given the multifactorial determinants of serum cholesterol, however, some degree of intraindividual variation is inevitable and may be accounted for in data analysis if it is known. For example, using data from the Western Electric Study, which determined the intraclass correlation for repeat cholesterol measurements at a 2-year interval (Shekelle *et al.*, 1981), Willett (1990) demonstrated how that variability could be accounted for in risk estimates.

Not all lipid measurements show the same degree of intraindividual variability. For example, Figure 13.4 shows that the intraindividual variation in triglyceride values is considerably higher than the variation in plasma cholesterol values (Jacobs and Barrett-Connor, 1982). This difference can be determined by sampling the same individual at two different time points and calculating the retest reliability (equivalent to the standard deviation of the paired values) (Austin, 1991) (Figures 13.4 and 13.5). Based on Lipid Research Clinics data with an average of 2.5 months between measurements, the retest reliability of triglyceride was consistently lower than the retest reliability for cholesterol over a range of values of 100 to more than

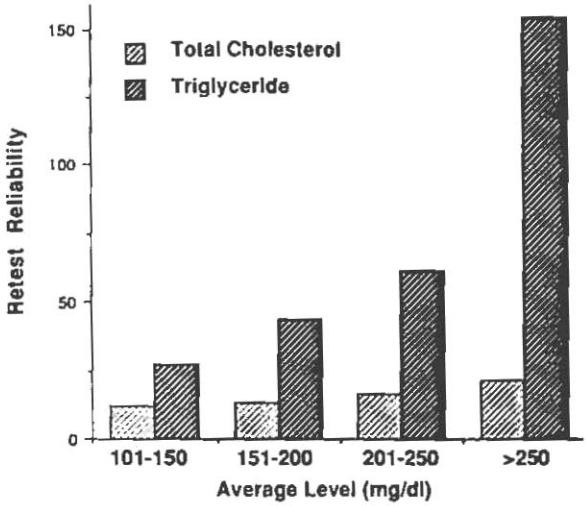

FIGURE 13.4 Intraindividual variation in total cholesterol and triglyceride (mg/dl), based on two measurements from the same individuals. Retest reliability (*y* axis) is equivalent to the SD of paired individual values. Larger values reflect greater intraindividual variation. (Reprinted with permission from Austin, 1991.)

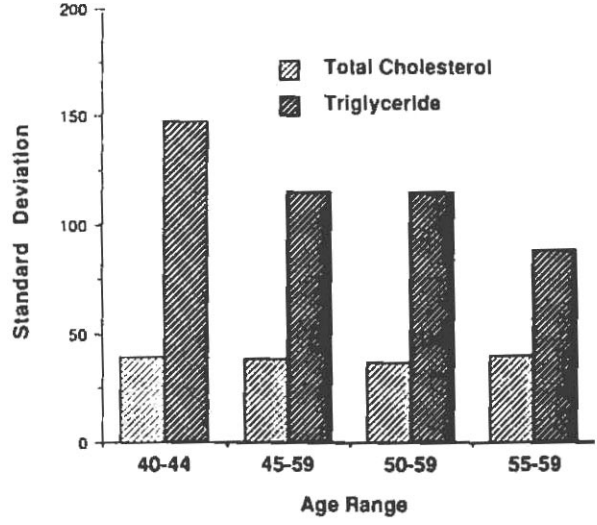

FIGURE 13.5 Interindividual variation in plasma cholesterol and triglyceride, based on sample of middle-aged men. (Reprinted with permission from Austin, 1991.)

250 mg/dl. This difference is likely to result in greater attenuation of the association between triglyceride levels and risk of CHD than of cholesterol levels and CHD. Laboratory measurement error also may attenuate the association between a given biomarker and risk of CHD. Markers measured with less precision will reduce further the ability to detect associations with CHD, especially in comparison with other less variable lipid markers.

Correlations among Lipid Biomarkers

Many studies report significant correlations between triglyceride and other lipid and lipoprotein levels. Such a correlation is illustrated by the relationship between plasma triglyceride and HDL-C levels. Four studies reporting such correlations are summarized in Table 13.3 (Rhoads *et al.*, 1976; Gordon *et al.*, 1977; Albrink *et al.*, 1980; Davis *et al.*, 1980). Consistent, strong, inverse correlations are seen between triglyceride and HDL-C, that is, increased triglyceride levels are associated with decreased HDL-C levels. As seen in the table, the correlations range from -0.2 to -0.7 (Austin, 1991). These statistical correlations no doubt reflect metabolic interrelations of lipoprotein particles involving exchanges in core lipids. In fact, it has been noted that "the close coupling of HDL-C to plasma triglyceride transport makes it difficult indeed to evaluate the distinct roles of hypertriglyceridemia and HDL-C" (Havel, 1988).

A subgroup analysis of the Helsinki Heart Study results has shown that "joint" lipid risk factors can be important for CHD risk as well (Manninen *et al.*, 1992). The results revealed a high-risk subgroup of subjects with LDL-C-to-HDL-C ratio greater than 5.0 and triglyceride of more than 2.3 mmol/liter, that constituted approximately 12% of the study sample. In the placebo group, the relative risk in this subgroup over the 5 years of the study was 3.8 (95% confidence interval, 2.2–6.6), compared with the subgroup of subjects who had a ratio less than or equal to 5.0 and triglyceride less than or equal to 2.3 mmol/liter. Relative risks were close to 1 in the

TABLE 13.3 **Correlation of Triglyceride and High Density Lipoprotein Cholesterol in Several Epidemiological Studies**

Study/year	Subjects	Correlation coefficient
Honolulu Heart Study, 1976	Men	−0.36
Framingham study, 1977	Men	−0.35
	Women	−0.43
Modesto, California, 1980	Men and women	−0.32
Lipid Research Clinics Prevalence Study, 1980	Men	−0.23 to −0.55
	Women	−0.21 to −0.65

Source: Reprinted with permission from Austin, 1991.

remaining two placebo groups, that is, those with only a high ratio or only high triglyceride. Among the treated subjects, the corresponding high-risk subgroup also received by far the most benefit from gemfibrozil treatment, with a remarkable 71% decrease in incidence of CHD. Much less benefit was seen in other groups. The authors conclude that "serum triglyceride concentration has prognostic value in combination with LDL-C and HDL-C levels" (Austin, 1992).

Determining the relative contribution of lipid subcategories is critical, since specific interventions may raise some and lower other lipid categories. This task can be challenging when biomarkers being evaluated have different degrees of true intraindividual variation, have different degrees of precision in their measurement, and are partially correlated. A modeling exercise of the effects of triglyceride and HDL-C for risk of heart disease suggested that a true association between triglyceride levels and risk of CHD might be masked in the presence of HDL-C due to less intraindividual variation in HDL-C and its partial correlation with triglycerides (Davis and Kim, 1990).

Future Contributions

The key contribution of molecular epidemiologic approaches to the future of cardiovascular research will be in the area of (1) identifying the sources of variation for various blood lipids and lipoproteins, (2) targeting people for specific interventions, and (3) assessing the impact of interventions.

Identifying Sources of Biomarker Variation

Molecular epidemiologic research will assist in disentangling the genetic and environmental factors that account for variation in biomarkers shown to be predictive of increased CHD risk (Berg, 1989). The research will include metabolic ward studies (e.g., Weinberg *et al.*, 1990), international comparison studies (e.g., International Collaborative Study Group, 1986), family studies (e.g., Bodurtha *et al.*, 1991), and population-based studies. These studies will assist in developing prevention strategies that will combine risk factor modification and appropriate screening.

An important consideration in all such studies is the standardization of laboratory measurements. A report from the International Federation of Clinical Chemistry Committee on Apolipoproteins addresses many of the issues involved (Marcovina and Albers, 1991).

Targeting People for Specific Interventions

Cardiovascular research has pioneered the concept of using biologic markers in calculating the probability that an individual with a given combination of risk factors will develop disease (Truett *et al.*, 1967). Truett and colleagues

(1967) applied multivariate statistical procedures to determine the association of cholesterol and other risk factors for coronary heart disease in the Framingham study, which followed 2187 men and 2669 women aged 30–62, free of CHD at entry, for 12 years. Using the logistic function, the researchers calculated the probability of an individual developing coronary heart disease given the baseline serum cholesterol level, age, systolic blood pressure, relative weight, hemoglobin, cigarettes smoked per day, and ECG (normal vs. abnormal). This study represents one of the first attempts to determine the probability of an individual developing disease based on a premorbid biomarker measurement. Using the logistic function, Truett *et al.*, (1967) showed that each person in the sample from the Framingham study had an estimated probability of the event (i.e., CHD) that could be expressed as:

$$y(x) = [1 + e^{-(a + bx)}] - 1$$

Thus, if x is the specific serum cholesterol at baseline, y is the probability that the person will develop CHD during a specified duration of follow-up, given that he or she has the high risk serum level. This model may be expanded to involve a number of risk factors, some characterized by markers and others that are not. The multivariate model is

$$y(x) = [1 + e^{-(a + \Sigma b_i x_i)}] - 1$$

With an efficient set of variables, it is possible to use these "risk functions" to identify "high risk" individuals (that is, individuals whose conditional probabilities are higher than average). The model will inevitably be enhanced as markers of susceptibility [such as the apo E polymorphisms and Lp(a)] are understood further and applied.

Assessing the Impact of Intervention

Observational studies first demonstrated associations between several lipid biomarkers and risk of CHD. These studies, as well as a host of observational and controlled studies of the determinants of these lipid measurements, have given rise to several generations of intervention trials aimed at determining if the lowering of total cholesterol and other relevant lipid levels with diet or medication will decrease individual risk for developing disease.

The Lipid Research Clinics Program conducted an intervention trial in 12 centers in North America. Subjects were assigned randomly to groups receiving a cholesterol-lowering drug or a placebo. A dose–response relationship was observed between the amount of drug and reduction of cholesterol and LDL-C cholesterol. Metabolic and molecular markers applied to intervention trials in the future may allow for a shorter time for such trials, since some markers may reflect intermediate conditions. If these markers have been validated with respect to the prediction of disease, they can be used as dependent variables. Another approach described by Brown *et al.* (1990)

used the percentage change in apolipoprotein B and HDL, among other independent variables, in an assessment of the effect of therapies for hyperlipidemia. The effect of intensive lipid lowering therapy on coronary arteriosclerosis among men at high risk for cardiovascular events was assessed by quantitative arteriography (Brown *et al.*, 1990). Multivariate analysis indicated that a reduction in the level of apolipoprotein B and systolic blood pressure and an increase in HDL cholesterol correlated independently with regression of coronary lesions (see Figure 13.6).

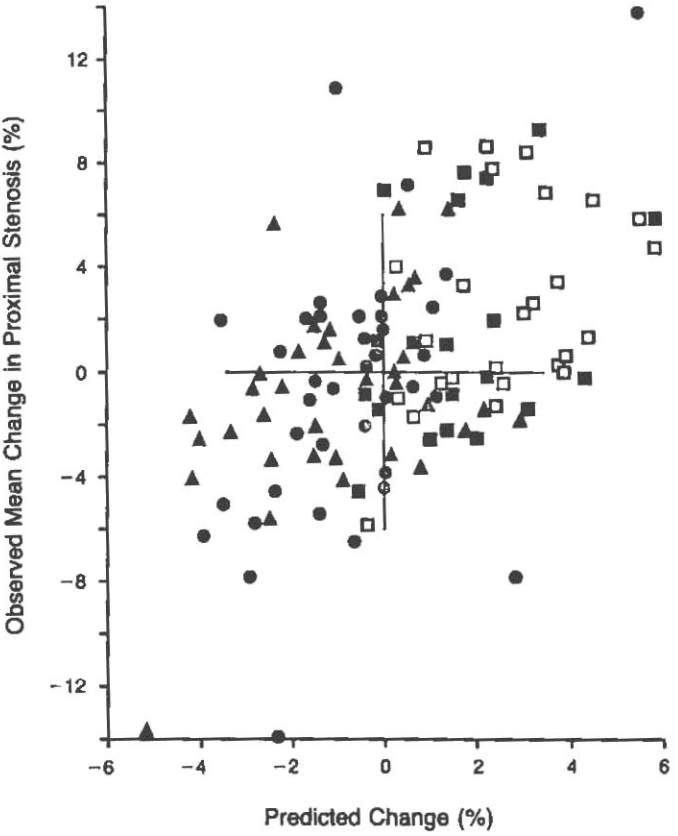

FIGURE 13.6 Results of multivariate statistical analysis. For each patient, the observed mean change in the severity of proximal stenosis ($\Delta\%S_{prox}$) is plotted against a value estimated with the following predictive expression: $\%S_{prox} = 0.07\%\ \Delta apoB - 0.032\%\ \Delta HDL_c + 0.14\%\ \Delta BP_{sys} - 0.7\ \Delta ST + 1.5$. The expression uses the maximal ST-segment depression (ΔST) during the baseline treadmill exercise test and the percentage change in the apolipoprotein B level ($\%\Delta apoB$), in the HDL cholesterol level ($\%\Delta HDL_c$), and in systolic blood pressure ($\%\Delta BP_{sys}$) during treatment to provide the most accurate estimate of observed change ($r = 0.51$, $P<0.0001$). Open squares indicate that the patient received only placebos, solid squares represent placebo and colestipol, solid triangles represent niacin and colestipol, and solid circles represent lovastatin and colestipol. (Reprinted by permission of the *New England Journal of Medicine* from Brown *et al.*, 1990.)

Researchers are developing computer models to assess the impact of cholesterol and other risk factor modifications on reducing CHD morbidity and mortality (Grover *et al* 1992). Grover noted that the wide variation surrounding estimates of the impact of lowering cholesterol levels for men and women in different age groups demonstrates that future research will need to define better which groups of individuals will gain the most from cholesterol modification (Grover *et al.*, 1992).

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Molecular Epidemiology

Principles and Practices

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