Epidemiology of Blood Lipids and Lipoproteins

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Introduction

Research has shown that total cholesterol levels in the blood are highly associated with greater coronary heart disease (CHD) risk in middle-aged American adults, especially in men. Triglyceride levels are also associated with greater risk for cardiovascular disease (CVD) events, but the results were less consistent [1]. Associations between total cholesterol levels and CVD risk, which includes stroke as an outcome, have been less convincing in older adults. This chapter focuses on findings related to observational studies that investigated cholesterol levels and vascular disease risk, the determinants of low-density (LDL-C) and high-density lipoprotein cholesterol (HDL-C) levels, associations with blood triglyceride levels, information related to apolipoproteins and lipoprotein (a), data related to lipid levels in the setting of the metabolic syndrome and obesity, trends in lipoprotein cholesterol levels for the USA and around the world, and current population strategies to screen children and adults at high risk for CVD or hypercholesterolemia.

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Blood Lipids and Cardiovascular Risk

Higher concentrations of blood cholesterol are associated with greater risk for CHD death. The largest project that addressed this issue included screenees from the Multiple Risk Factor Intervention Trial (MRFIT), and more than 350,000 middle-aged men aged 35–57 years at baseline who were followed for more than a decade, as shown in Fig. 2.1 [2, 3]. Higher cholesterol levels and greater risk for CHD death and risk were synergistically associated with cigarette smoking, blood pressure levels, and diabetes mellitus [4].

Initial assessments of lipid levels in cardiovascular population studies such as Framingham, Chicago, MRFIT screenees, and the Seven Countries Study focused on total cholesterol levels [5–7]. Complementary to the MRFIT findings, the Seven Countries investigators analyzed the role of serum cholesterol levels as predictors of CHD death around the world and Fig. 2.2 shows the results according to cholesterol quartiles for sites in Japan, Southern Europe, Serbia, USA, Southern Europe coastal region, and Northern Europe. The relation between cholesterol and risk of CHD death was relatively flat at low cholesterol levels. On the other hand, cholesterol levels were uniformly much higher in Northern Europe and the relation between cholesterol and CHD death was relatively steep in that region [8].

Research has generally concentrated on the associations of risk factors and the development of CVD events over 5–15 years of follow-up, but

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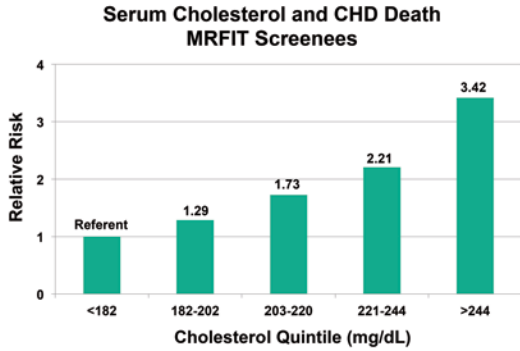

Fig. 2.1 Multiple Risk Factor Intervention Trial (MRFIT) screenees and relative risk for CHD death according to blood cholesterol in men aged 35–57 years at baseline [2, 3]

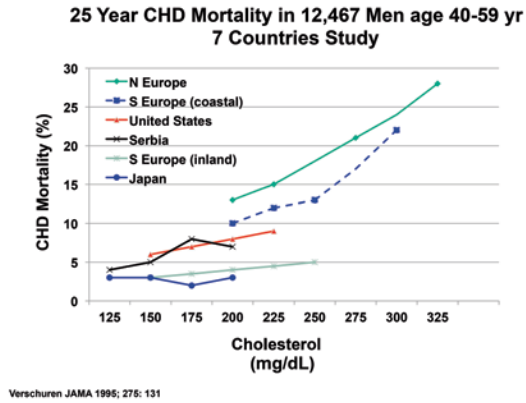

Fig. 2.2 CHD mortality over 25 years of follow-up in men aged 40–59 years at baseline in the Seven Countries Study [8]. *CHD* coronary heart disease

newer analytical methods have led to the development of estimates over a longer time frame and now it is possible to estimate risk for vascular disease over a person's lifetime. As shown in Fig. 2.3, both age and blood cholesterol levels are highly associated with a greater lifetime risk of CVD in both sexes for Framingham participants at all ages [9]. More recently, this approach has been widened to include data and estimates from a broad range of population groups [10].

Lipoprotein quantification was developed at the National Heart, Lung and Blood Institute (NHLBI) in the 1970s and the methods employed ultracentrifugation [11]. Subsequently, the Lipid Research Clinics (LRC) Program expanded the use of lipoprotein cholesterol quantification using ultracentrifugation and precipitation techniques that allowed estimation of LDL, HDL, and very-low-density lipoprotein (VLDL) cholesterol. The NHLBI subsequently sponsored a large LRC program that featured the use of these newer lipoprotein measurements. Quality control and standardization of the measurements were coordinated through the NHLBI and the Centers for Disease Control in several NHLBI observational studies and clinical trials that followed [12, 13].

The advent of lipoprotein cholesterol measurement led to epidemiologic analyses that considered the potential effects of the various particles on CVD risk. Reports from the late 1970s by Gordon, Miller, and other investigators using Framingham and other population data showed that both total cholesterol and HDL-C were highly associated with greater CVD risk, the effects were statistically independent, and the results persisted in multivariable risk formulations [14–17]. As an example of these findings, Figs. 2.4 and 2.5 show the risks for myocardial infarction in Framingham men and women over 12 years of follow-up after baseline measurement of lipids [18]. The heights of the vertical bars display the 12-year risk for myocardial infarction according to sex-specific quartiles of total cholesterol and HDL-C. Higher levels of total cholesterol were associated with greater risk of myocardial infarction and higher HDL-C appears to be cardioprotective in both sexes. Even in the lowest quartile of total cholesterol, the individuals with low HDL-C experienced greater risk for developing myocardial infarction.

The determinants of LDL-C are shown in Table 2.1. Dietary intake of fat is the most important determinant and research by Hegsted, Keys, and others showed that LDL-C levels vary according to the dietary composition [19]. Greater intake of dietary saturated fat and cholesterol increases blood cholesterol levels and greater intake of polyunsaturated fat decreases LDL-C [20]. Differences in blood cholesterol levels and vascular disease risk in populations around the world are believed to be greatly attributable to such dietary differences as shown in Verschuren's

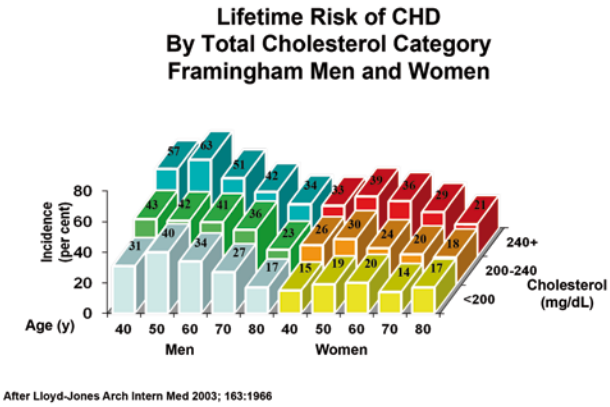

Fig. 2.3 Lifetime risk of coronary heart disease showed according to total cholesterol level groupings for men and women at various ages. (After Lloyd-Jones et al. [9])

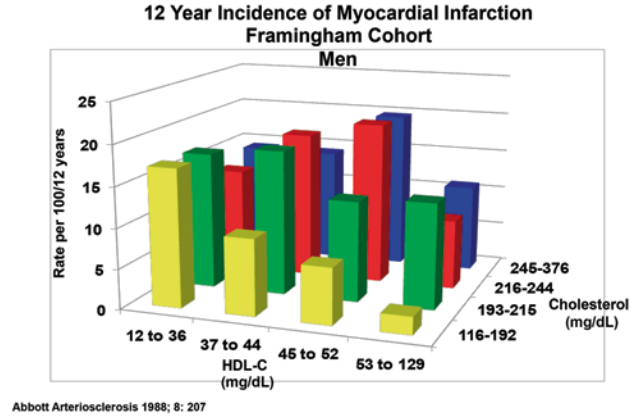

Fig. 2.4 Twelve-year risk of myocardial infarction shown for Framingham men according to quartiles of HDL-C and total cholesterol. (Adapted from Abbott et al. [18]). *HDL-C* high-density lipoprotein cholesterol

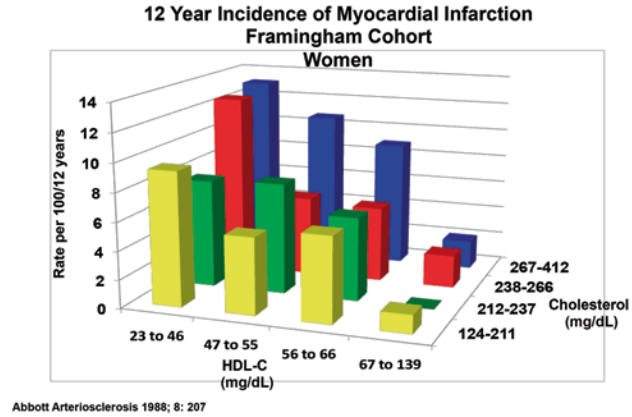

Fig. 2.5 Twelve-year risk of myocardial infarction shown for Framingham women according to quartiles of HDL-C and total cholesterol. (Adapted from Abbott et al. [18]). *HDL-C* high-density lipoprotein cholesterol

Table 2.1 Determinants of LDL-cholesterol

Lower	Higher
Low dietary saturated fat	High dietary saturated fat
Low dietary cholesterol	High dietary cholesterol
High dietary polyunsaturated fat	Low dietary polyunsaturated fat
Estrogen	
Genetic	Genetic

LDL low-density lipoprotein

Table 2.2 Determinants of HDL-cholesterol

Lower	Higher
Male	Female
Androgens, progestins	Estrogen
Adiposity	Leanness
Cigarette use	No cigarettes
Low alcohol intake	High alcohol intake
Low dietary saturated fat	High dietary saturated fat
Genetic	Genetic

HDL high-density lipoprotein

Table 2.3 Prevalence* of dyslipidemia according to BMI levels in nonsmoker Framingham offspring study. (Adapted from Lamon-Fava et al. [69])

		Body Mass Index Level (kg/m^2)					
		<21	≥21 to <23	≥23 to <25	≥25 to <27.5	≥27.5 to <30	≥30.0
Men (n)		(27)	(72)	(188)	(347)	(253)	(240)
	Triglycerides (>200 mg/dL)	0	6.9	8.0	14.4	20.9	27.1
	Elevated LDL-C (>160 mg/dL)	7.4	11.1	18.6	24.5	26.9	25.0
	Low HDL-C (<35 mg/dL)	7.4	8.3	9.0	13.8	19.0	24.2
Women (n)		(163)	(264)	(207)	(194)	(119)	(194)
	Triglycerides (>200 mg/dL)	0.0	1.9%	3.9%	9.3	15.9	14.9
	Elevated LDL-C (>160 mg/dL)	8.6	15.2	15.5	28.4	28.6	28.9
	Low HDL-C (<35 mg/dL)	0.6	1.1	0.5	2.6	2.5	7.7

BMI body mass index, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol All trends across BMI level $P<0.001$
Entries in table are percents

report from the Seven Countries Study [8]. In addition to the dietary influences, there are important genetic determinants for high LDL-C that underlie familial hypercholesterolemia and for low LDL-C in association with hypobetalipoproteinemia [21]. The prevalence of heterozygous familial hypercholesterolemia is approximately 1 in 500 persons, but the condition is more common in South Africa, presumably because of a founder effect [22]. Finally, LDL-C levels are lower in adult women prior to menopause, lack of naturally occurring estrogen in post-menopausal women is associated with higher LDL-C, and exogenous products containing estrogens such as oral contraceptives and post-menopausal estrogens may reduce LDL-C [23, 24].

Table 2.2 summarizes the population-based determinants of HDL-C. The key lifestyle factors associated with higher HDL cholesterol levels are reduced adiposity, absence of cigarette smoking, greater exercise, and greater alcohol intake. For example, Garrison reported that relative weight was highly associated with HDL-C and there were weaker correlations between measures of obesity and VLDL-C or LDL-C [25]. There were very few lean individuals in some of the age groups, which prevented making firm conclusions concerning associations between lipoprotein cholesterol levels and adiposity in some men. Other associations between adiposity and lipoprotein cholesterol levels are shown in Table 2.3, as reported by Lamon-Fava. Greater body mass index was associated with hypertriglyceridemia, similar relationships tended to be observed for elevated LDL-C, and the opposite effect was observed for HDL-C

Table 2.4 Means for lipid levels according to self-reported weekly vigorous physical activity level Framingham offspring study. (Adapted from Dannenberg et al. [29])

Factor	Men		Women	
	<1 h	≥1 h	<1 h	≥1 h
HDL-C (mg/dL)	42.0	47.8*	53.5	61.1*
LDL-C (mg/dL)	133.5	135.0	126.3	131.6
VLDL-C (mg/dL)	29.3	20.5*	19.6	17.8

HDL-C high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *VLDL-C* very low-density lipoprotein cholesterol
*$P<0.001$

[26]. Longitudinal analyses were undertaken concerning weight change and lipid levels. Over an 8-year study interval in adults who were aged 25–34 years at baseline, their weight increased, HDL-C decreased, and both LDL-C and VLDL-C increased in both sexes [27].

Estrogen levels and treatments have been shown to have strong associations with HDL-C and LDL-C levels. As women go through menopause, their LDL-C levels typically increase, HDL-C declines or does not change, and LDL particles shift toward smaller sizes [24, 28]. Estrogen replacement therapy was associated with a shift toward higher HDL-C concentrations, lower LDL-C levels, and oral progestins tended to have unfavorable effects on the lipoprotein cholesterol levels [24].

Greater physical activity is highly associated with higher HDL-C levels. As shown in Table 2.4, among Framingham participants, an hour or more of vigorous physical activity was associated with HDL levels that were approximately 5.8 mg/dL greater in men and 7.7 mg/dL greater in women [29]. Research in runners and other competitive athletes has consistently shown much greater HDL-C levels in athletes and the differences are attributable to the training level, lack of adiposity, and lack of smoking in such individuals [30–32].

The determinants of triglyceride levels are shown in Table 2.5. For many of the factors, the associations are in the opposite direction from HDL-C. Obese type 2 diabetic patients who consume a diet that is high in saturated fat are especially prone to have elevated triglycerides. Greater alcohol intake and estrogen use have been associated with higher triglyceride levels, and

Table 2.5 Determinants of triglycerides

Lower	Higher
High intake of omega-3 fatty acids	Obesity
	Greater saturated fat intake
	Diabetes mellitus
	Greater alcohol intake
	Estrogens

persons with very high triglycerides are treated with diet and medications to lower triglyceride levels. Metabolic conditions such as chronic kidney disease, the nephrotic syndrome, pancreatitis, and diabetic ketoacidosis may all lead to higher concentrations of triglycerides in the blood [33]. Additionally, genetic variants associated with deficient or abnormal regulation of lipoprotein lipase are associated with increased concentration of triglycerides [34].

Greater prevalence of very atherogenic lipoprotein cholesterol levels was observed in Framingham offspring participants with diabetes mellitus, and these results are shown in Fig. 2.6 for men and women. The diabetic patients were much more likely than nondiabetic participants to have low HDL-C, elevated triglycerides, and combinations of lipid abnormalities. Interestingly, the diabetic patients did not tend to have elevated LDL-C levels [35].

On average, cigarette smoking has been associated with HDL-C levels that are approximately 4 mg/dL lower in men and 6 mg/dL lower in women compared to nonsmokers. On the other hand, greater alcohol consumption was highly associated with higher levels of HDL-C in the Framingham offspring studies [36, 37].

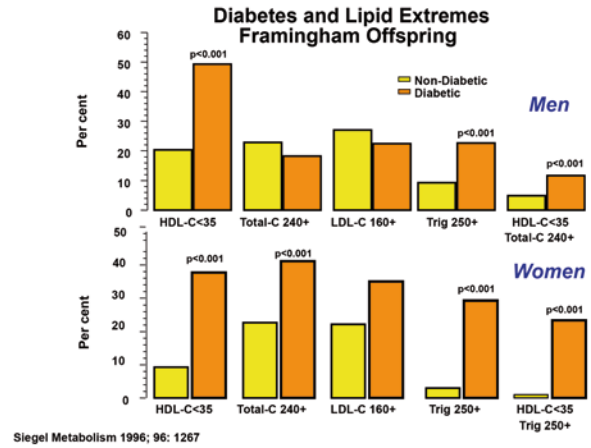

Fig. 2.6 Prevalence of lipid extremes in diabetic and nondiabetic participants is shown for Framingham offspring participant. (Adapted from Siegel et al. [35]). *HDL-C* High-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, Trig triglyceride

Table 2.6 Baseline lipoprotein risk factors and 14-year CVD incidence Framingham offspring study. (Adapted from Cromwell et al. [41])

Factor	Men			Women		
	No CVD	Yes CVD	*P* value	No CVD	Yes CVD	*P* value
HDL-C (mg/dL)	45	42	0.001	57	51	<0.0001
LDL-C (mg/dL)	134	138	0.09	126	143	<0.0001
Non-HDL-C (mg/dL)	158	168	0.0002	146	170	<0.0001
LDL Particle Number (nmol/L)	1509	1641	<0.0001	1344	1628	<0.0001

CVD cardiovascular disease, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol

Lipoprotein cholesterol, biomarker, and genetic investigations have increased greatly in the last two decades. Relevant to lipid research, these collaborations included measurement of insulin, apolipoproteins, lipoprotein particle number, and determination of gene variants such as apolipoprotein E that have been shown to be associated with lipid levels [38–40]. Lower HDL-C, higher LDL-C, higher non-HDL-C, and greater LDL particle number are all associated with greater risk of developing cardiovascular risk, as shown in Framingham analyses as well as others (Table 2.6) [41].

Metabolic Syndrome and Insulin Resistance

Since the late 1990s, it has been recognized that many individuals who develop CVD or diabetes mellitus tend to have greater adiposity, elevated triglycerides, low HDL cholesterol, elevated blood pressure, or impaired fasting glucose. Presence of three or more of these five traits was given the name metabolic syndrome, and it was felt that the syndrome was highly related to insulin resistance. As displayed in Fig. 2.7, principal components analysis showed that the metabolic syndrome traits clustered. The presence of three or more of the traits typically led to a doubling or tripling of risk for CVD, and more than a 20-fold greater risk for diabetes mellitus [42, 43]. A variety of other plasma biomarkers were subsequently used to study these phenomena, including laboratory biomarkers, traditional lipoprotein cholesterol levels, smaller LDL particles, and greater LDL particle number [28, 44–48].

Apolipoproteins

Lipoprotein particles include apolipoproteins, cholesterol, triglycerides, and phospholipid moieties. Protein assays became more prevalent

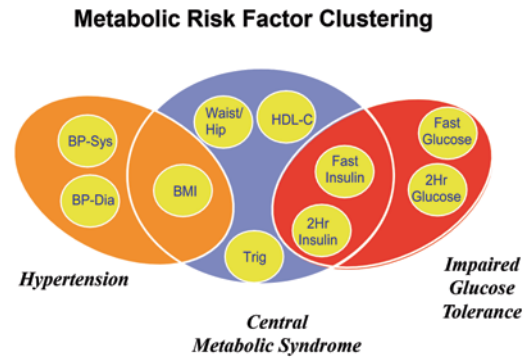

Fig. 2.7 Metabolic risk factor clustering is shown for domains related to hypertension, central metabolic syndrome, and impaired glucose tolerance. Models were developed from the Framingham offspring using principal components analysis. (Adapted from Meigs et al. [70]). *BMI* body mass index, *HDL-C* high-density lipoprotein cholesterol, Trig triglyceride

starting in the 1990s and associations with CVD were evaluated. For example, lipoprotein(a) originally tested using paper electrophoresis in Framingham was moderately associated with greater risk of heart disease and the effect was independent of LDL-C and HDL-C [49, 50].

Automated protein immunoassays were developed and apolipoprotein B was shown to be highly associated with LDL-C and greater CVD risk, especially in European studies [51, 52]. Concentrations of apolipoprotein A1 were highly associated with HDL-C and higher levels of each appeared to be cardioprotective. In analyses that compared prediction models with LDL-C and HDL-C versus models with apolipoprotein B and apolipoprotein A1, the overall ability to discriminate was similar. The results were interpreted as showing that measurement of apolipoproteins did not improve estimation beyond the traditional analytic approach with total cholesterol and HDL-C to estimate risk for initial CVD events [38].

Blood levels of an unusual lipid particle, lipoprotein (a), are very heritable and higher levels are very common in persons of African ancestry. An elevated level has been shown to be a risk factor for premature CVD in white populations and also in African American population groups [49, 53, 54].

Apolipoprotein E is of special interest because inherited deficiency is associated with increased atherosclerosis in animal models, and genetic variants have been associated with abnormal lipids, CVD, and dementia. Within the Framingham population cohorts, it was reported that higher concentrations of LDL-C were related to the presence and number of apolipoprotein E4 alleles present and lower levels of LDL-C were seen in persons with the E2 allele [40]. Results for triglycerides were slightly different, and both the E2 and E4 alleles were associated with higher triglyceride concentrations. The E4 allele was found to be present in approximately 24% of the Framingham participants and, on a population basis, it was estimated that approximately 10–15% of CVD could be attributed to the presence of the E4 allele. Separate analyses showed that the E4 allele was highly associated with greater risk for Alzheimer's disease and relative protection from dementia was found for persons with the E2 allele [55, 56].

Genetic research related to lipids led to a variety of collaborations with other laboratory scientists and other large population cohorts. Initially, these efforts included analyses with a limited number of genetic markers. Analyses were extended to include a large number of single-nucleotide polymorphisms and genome-wide association studies [57–60]. Genetic screening for gene variants associated with familial hypercholesterolemia has been used in tandem with screening blood cholesterol levels in families that include persons with very elevated cholesterol levels. Researchers in Europe have used these cased screening strategies to help identify persons with familial hypercholesterolemia at an early age in an effort to institute lipid lowering in the pediatric and young adult age groups [61].

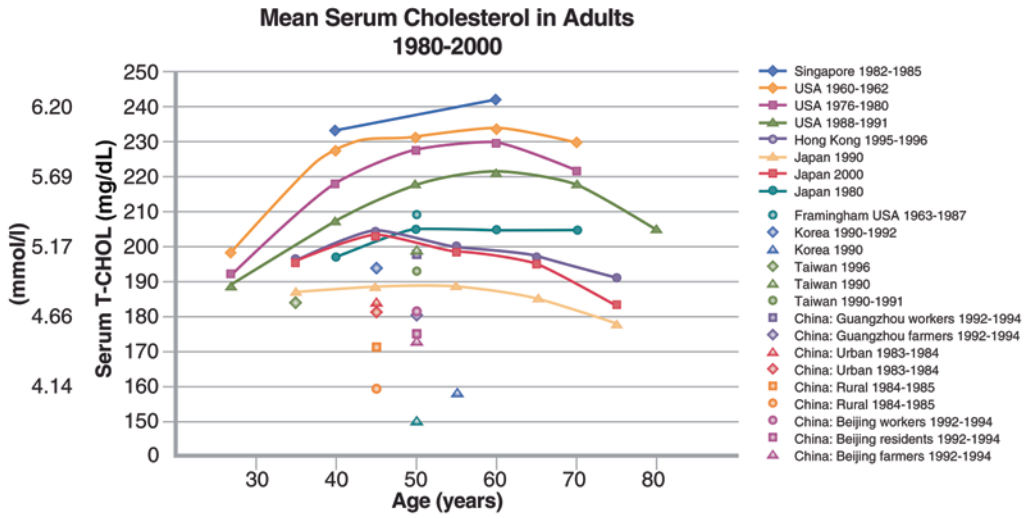

Fig. 2.8 Trends for mean serum cholesterol in adults from 1980 to 2000

Estimating Risk for CVD Outcomes

It was shown in the late 1980s that CVD risk could be predicted with reasonable accuracy using information obtained at the time of an outpatient clinical visit [62]. The variables used were age, sex, total cholesterol, HDL-C, systolic blood pressure, blood pressure treatment, diabetes mellitus, and cigarette smoking [63, 64]. A variety of lipid measures were assessed for potential use to estimate CHD and CVD risk. Concentrations of total cholesterol, HDL-C, LDL-C, non-HDL-C, and LDL particle number were shown to be highly associated with greater risk for CVD in the Framingham offspring [41]. Each of these measures has been used in modeling risk for initial CVD events, and specimens were most often obtained from healthy volunteers who were not taking lipid-lowering medications.

Debate has surrounded the utility of various lipoprotein cholesterol measurements and how they may be used in prediction equations. For example, the total/HDL-C ratio could be employed as a single lipid risk factor instead of using the total cholesterol and HDL-C as separate measures to estimate CVD risk. Alternatively, LDL-C and HDL-C could be used to estimate risk, but that approach did not appear to provide any advantage over simply using total cholesterol and HDL-C in the multivariable risk estimations [62]. As mentioned in the apolipoprotein section, use of the lipid measured apolipoprotein B and apolipoprotein A1 did not provide greater discrimination in estimation for risk of initial CVD events in comparisons with total cholesterol and HDL-C in multivariable models [38].

Mean Levels of Cholesterol Around the World

As seen in Fig. 2.8, cholesterol levels tend to rise in adulthood, peak between ages 50 and 60 years, and decline in older persons for a variety of population groups around the world. The review by Ueshima and coworkers shows that cholesterol levels that have historically been lower in Asia appear to be increasing in the past few decades [65]. Mean levels of total cholesterol in the control subjects from the INTERHEART participants who did not have a myocardial infarction are shown for men and women in Table 2.7 [66]. Among the male participants, the highest mean cholesterol levels (>200 mg/dL) were observed in Europeans and other Asians, intermediate levels (180–190 mg/dL)

Table 2.7 Means* and 95% confidence intervals for blood cholesterol levels INTERHEART controls. (Adapted from McQueen et al. [66])

Region	Men	Women
	Mean (95% confidence Interval)	Mean (95% confidence Interval)
European	203 (201–205)	216 (212–220)
Chinese	182 (181–184)	191 (188–194)
South Asian	184 (182–186)	193 (187–198)
Other Asian	208 (204–211)	222 (215–229)
Latin American	188 (188–190)	210 (205–215)
Arab/Persian	190 (187–192)	199 (194–205)
Black African	158 (153–164)	182 (175–190)
Colored African	190 (185–197)	211 (202–220)

*Means expressed in mg/dL units

were observed for most of the regions, and the lowest means (< 160 mg/dL) were seen in Black Africans. Similar patterns, with some notable differences, were observed for the female participants. Lower blood cholesterol in older persons partly explains why cholesterol levels in the elderly have not been highly associated with carotid artery disease or with stroke risk [67]. A Framingham analysis showed that cumulative exposures of cholesterol, blood pressure, and smoking were highly associated with greater carotid stenosis in person who underwent carotid ultrasound measurements at a mean age of 75 years [68].

Summary

This chapter has summarized many of the key findings related to lipid levels, risk factor levels, and vascular disease outcomes. At the outset of the study, the primary focus was simple measures such as total blood cholesterol and triglycerides and, over time, the scope expanded to include lipoprotein cholesterol quantification, apolipoproteins, genetics, lipid particles, and use of these measures in multivariable equations to estimate risk for the development of initial CVD outcomes. Research in lipids within populations continues to expand, and now we are beginning to trend over time effects of the treatments and the potential to assess CVD risk using on-treatment lipid measures in the future.

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