Lipids and Vascular Disease: A Framingham Perspective

Peter W. F. Wilson
Atlanta, GA, USA

SUMMARY

Research related to lipid levels, correlates of lipid levels, and how lipid levels are related to vascular disease outcomes in the Framingham cohorts are summarized for data obtained from 1948 to the present day. Initial lipid data in Framingham participants were largely confined to cholesterol and triglycerides. Technology evolved to later include lipoprotein cholesterol quantification using ultracentrifugation, apolipoproteins, genetics, lipid particle size and number, and use of lipid information in multivariable equations to estimate risk for the development of initial cardiovascular disease outcomes. The information is presented chronologically to highlight the developments related to the lipids and heart disease over the past 50 years.

This paper summarizes the experience of the Framingham Heart Study concerning lipid measurements, their associations with common risk factors, and how they are related to coronary heart disease and cardiovascular disease (CVD) risk. The presentation generally follows the time line of available lipid measures from 1950 to the present. At the initial Framingham Heart Study examinations, only simple laboratory measurements, such as total cholesterol and triglycerides, were available, and methodologies progressed to lipoprotein cholesterol analyses, apolipoprotein determinations, special lipoprotein particle considerations, lifetime risk estimates, and CVD risk estimation.

INITIAL LABORATORY MEASUREMENTS OF LIPIDS

Research in the early 1900s had generally shown that higher blood cholesterol levels were associated with greater atherosclerosis at the time of death. Both animal and human investigations supported the hypothesis that a greater concentration of total cholesterol in the blood would lead to an increased risk for heart attacks and CVD death. The Framingham Heart Study was initially planned in the late 1940s, and it was felt that blood cholesterol level should be evaluated as an antecedent factor potentially associated with CVD risk in middle-aged adults who would be followed for 20 years or more.

Blood cholesterol was measured regularly in the Framingham original cohort participants, and the determinations were repeated at most of the early biennial examinations. Triglyceride levels were also assayed at many of the examinations, and specimens were often obtained from nonfasting study participants. The methods involved simple chemical determinations, and a chemist oversaw the laboratory measurements. Several milliliters of plasma were typically required and glass pipettes were used to transfer specimens and reagents to carry out the measurements. Compared with today's techniques, these methods are antiquated. The same chemical techniques were used to measure cholesterol and triglycerides from 1948 to 1970 in the Framingham laboratory [1,2]. Maintenance of reliable methods over time did improve accuracy and reduce imprecision. Laboratory results from this era showed that total cholesterol was highly associated with greater cardiovascular risk in middle-aged adults, especially in men; cholesterol levels were less predictive of CVD risk after 50 years of age. Triglyceride levels were also associated with greater risk for CVD events, but the results were less consistent [3].

In the early 1950s, Gofman and colleagues from the Lawrence Livermore Laboratory in Berkeley, California collaborated with Framingham investigators. Specimens were shipped from Massachusetts to California and ultracentrifugation of plasma was undertaken with determination of Svedberg fraction lipids. These Svedberg unit data provided the first assessment of atherogenic lipid particles in a population setting, and the levels were more highly associated with CVD events than total cholesterol in middle-aged adults was [4,5]. The major focus at the Framingham Heart Study at the time was simple measures of risk factors, and in a 1961 publication, the presence of blood cholesterol >260 mg/dl was identified by Kannel et al. [6] as one of the "factors of risk" for CVD along with elevated blood pressure and left ventricular hypertrophy on the electrocardiogram. As shown in Figure 1, these 3 factors acted synergistically to increase risk of developing coronary heart disease over a 6-year follow-up for Framingham participants.

A sentinel publication in 1967 by National Institutes of Health scientists Fredrickson, Levy, and Lees [7] included determination of cholesterol levels in lipoprotein particles after ultracentrifugation of plasma. Figure 2 portrays the different lipid measurements that were developed. Ultracentrifugation used a sucrose gradient at density 1.006 and separated very low-density lipoprotein (VLDL) particles in the top fraction from the low-density lipoproteins (LDL), intermediate-density lipoproteins, and high-density lipoproteins (HDL) located in the bottom fraction [7]. A second ultracentrifugation of plasma with a different density gradient was required to measure the particles at

From Atlanta Veterans Affairs Medical Center, and Emory Clinical Cardiovascular Research Institute, Atlanta, GA, USA. Correspondence: P. W. F. Wilson (pwwilso@emory.edu).

GLOBAL HEART
Published by Elsevier Ltd. on behalf of World Heart Federation (Geneva).
VOL. 8, NO. 1, 2013
ISSN 2211-8160/$36.00.
http://dx.doi.org/10.1016/j.gheart.2012.12.009

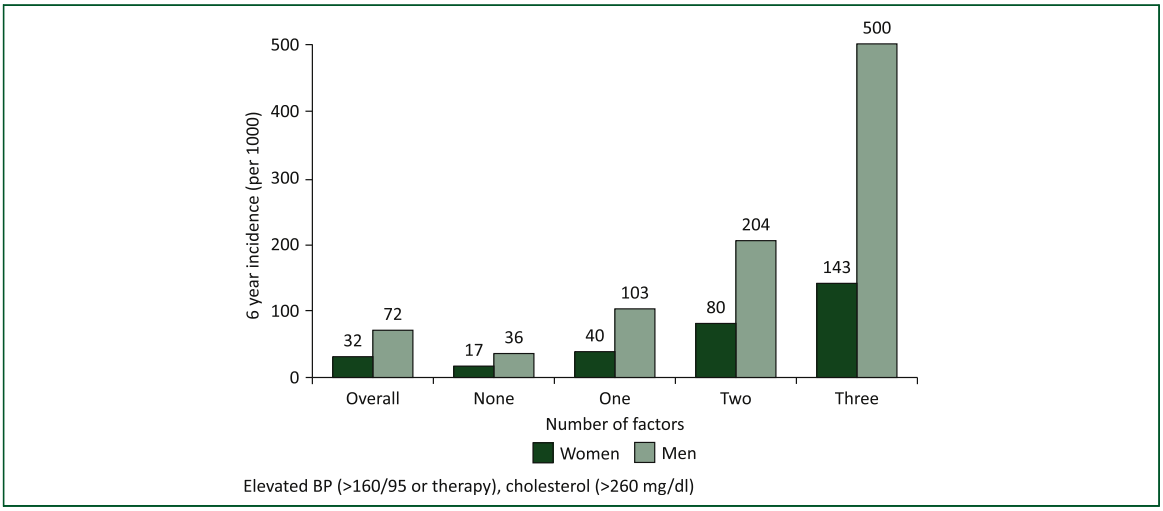

FIGURE 1. Risk of coronary heart disease (CHD) according to elevated blood pressure (BP), elevated cholesterol, and left ventricular hypertrophy (LVH) in the original Framingham cohort—6 years of follow-up for men and women. Adapted, with permission, from Kannel et al. [6].

density less than 1.063, and this procedure was time-consuming. Fortunately, alternative methods were developed to precipitate LDL and VLDL particles from the plasma, allowing the measurement of high-density lipoprotein cholesterol (HDL-C) in the supernatant [8,9].

The National Heart, Lung, and Blood Institute sponsored a large Lipid Research Clinic (LRC) program that featured using these newer lipoprotein measurements. Quality control and standardization of the measurements was coordinated through the National Heart, Lung, and Blood Institute and the Centers for Disease Control in several National Heart, Lung, and Blood Institute observational studies and clinical trials that followed [10,11]. The Framingham Heart Study adopted the LRC methods for lipid measurement, using ultracentrifugation and an Auto Analyzer II (Technicon, Tarrytown, NY) to make LRC lipid determinations in both the original cohort and the offspring from 1970 onward [8]. Additionally, an unusual lipid particle called "sinking pre-beta lipoprotein," later shown to be lipoprotein(a), was measured in the early 1970s using paper electrophoresis methods. In long-term follow-up studies, the sinking pre-beta levels were shown to be associated with CVD risk [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 et al. and Miller et al. [14,15] using Framingham and other data showed a positive association with total cholesterol and an inverse association with HDL-C and CVD risk. The effects were statistically independent, and the results persisted in multivariable risk formulations [14,15]. As an example of these findings, Figures 3 and 4 show results for Framingham men and women over 12 years of follow-up for myocardial infarction after baseline measurement of lipids. The heights of the vertical bars display the 12-year risk for myocardial infarct 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. These results were published at a time that the National Cholesterol Education Program did not include HDL-C screening, and these findings helped to foster incorporation of HDL-C measurements into the initial screening for coronary

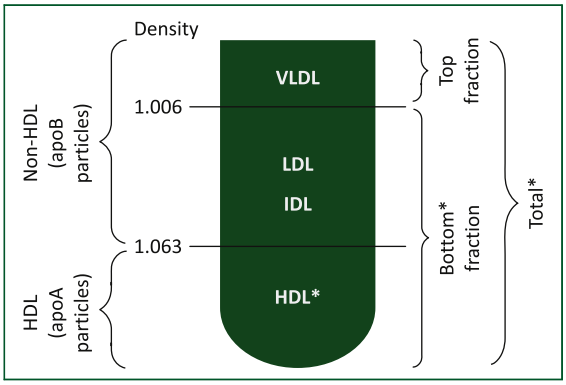

FIGURE 2. Lipid measurements are shown according to lipoprotein cholesterol measurements and apolipoproteins as well as density gradients. apoA, apolipoprotein A; apoB, apolipoprotein B; HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

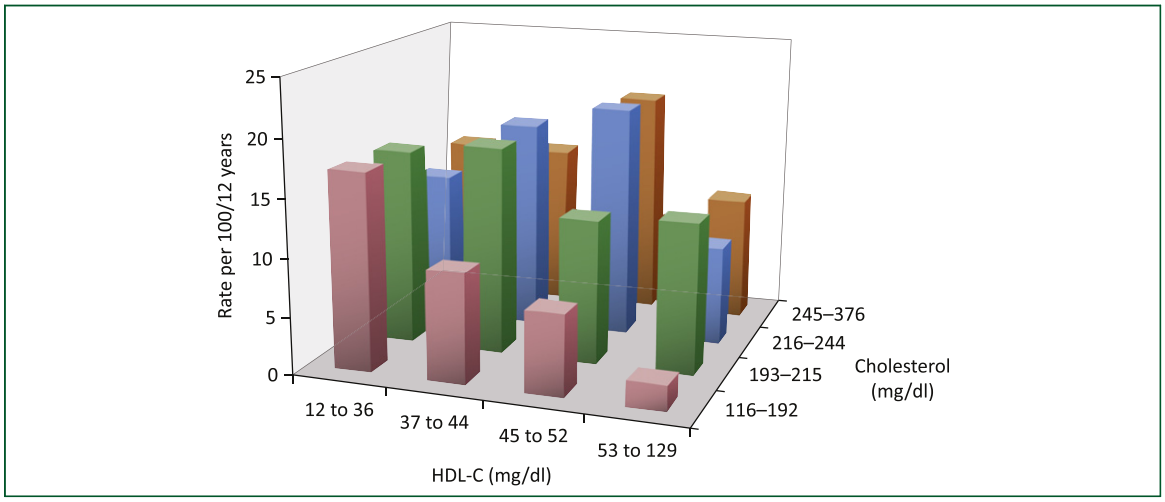

FIGURE 3. Twelve-year risk of myocardial infarction shown for Framingham cohort men according to quartiles of high-density lipoprotein cholesterol (HDL-C) and total cholesterol. Adapted, with permission, from Abbott et al. [55].

disease risk when the next National Cholesterol Education Program recommendations were published [16,17].

Population-based determinants of HDL-C were reported in a variety of publications based on the experience of the Framingham offspring. The key lifestyle factors associated with higher HDL-C levels were 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 low-density lipoprotein cholesterol (LDL-C) [18]. 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 1, as reported by Lamon-Fava et al. [19]. 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 [19]. Longitudinal analyses were undertaken concerning weight change and lipid levels. Over an 8-year study interval in adults who were 25 to 34 years of age at baseline, their weight increased, HDL-C decreased, and LDL-C and very low-density lipoprotein cholesterol (VLDL-C) increased in both sexes [20].

Estrogen levels and treatments were shown to have strong associations with cholesterol in the HDL and LDL

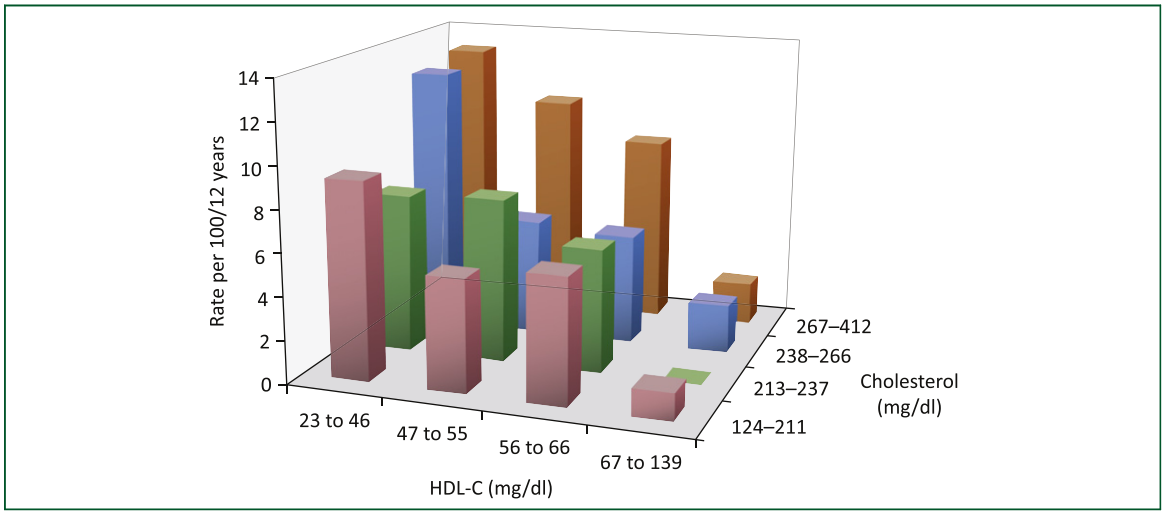

FIGURE 4. Twelve-year risk of myocardial infarction shown for Framingham cohort women according to quartiles of high-density lipoprotein cholesterol (HDL-C) and total cholesterol. Adapted, with permission, from Abbott et al. [55].

TABLE 1. Prevalence and means for risk factors according to BMI level: the Framingham Offspring Study

	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						
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						
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

All trends across BMI level: $p < 0.001$.
BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Adapted, with permission, from Lamon-Fava et al. [54].

fractions. As women went through menopause, their LDL-C levels increased, HDL-C declined or did not change, and LDL particles shifted toward smaller sizes [21,22]. Estrogen replacement therapy was associated with a shift toward higher HDL-C concentrations and lower LDL-C levels, and oral progestins tended to have unfavorable effects on the lipoprotein cholesterol levels [22].

A greater prevalence of very atherogenic lipoprotein cholesterol levels was observed in Framingham offspring participants with diabetes mellitus, and these results are shown in Figure 5 for men and Figure 6 for women. Almost all of the diabetic patients in the original and offspring cohorts had type 2 diabetes, and these individuals were much more likely than nondiabetic participants were 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 [23].

Leisure time physical activity was reported to be associated with increased HDL-C in Framingham offspring, and the findings were evident for both men and women [24]. Activities associated with greater aerobic conditioning were especially associated with higher HDL-C levels, including less cigarette smoking, lower body mass index, and a lower resting heart rate, as shown in Table 2. On average, compared with nonsmokers, cigarette smoking was associated with HDL-C levels that were approximately 4 mg/dl lower in men and 6 mg/dl lower in women. On the other hand, greater alcohol consumption was highly associated with higher levels of HDL-C and plasma in the Framingham offspring [25,26].

By the early 1980s, lipid measurements at the Framingham Heart Study were fully automated with robotic pipetting, and it was possible to measure total cholesterol, LDL-C, HDL-C, and triglycerides with very small volumes of specimen [27]. New laboratory instrumentation

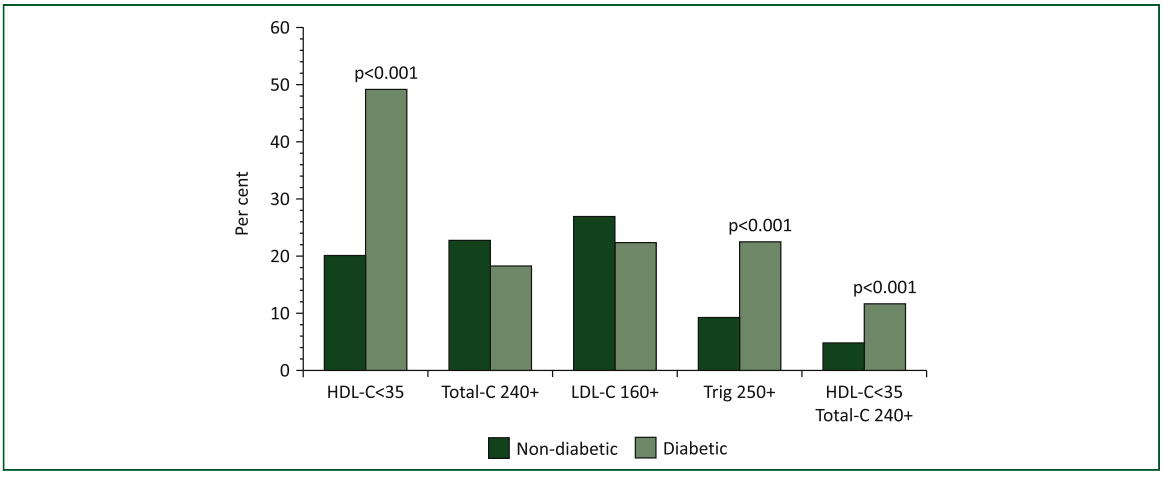

FIGURE 5. Prevalence of lipid extremes in diabetic and nondiabetic participants is shown for Framingham offspring men. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; total-C, total cholesterol; Trig, triglycerides. Adapted, with permission, from Siegel et al. [23].

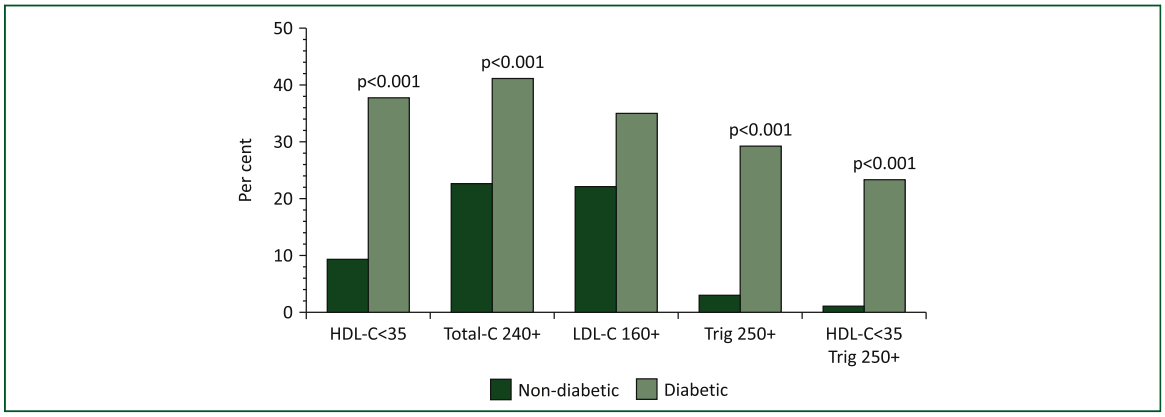

FIGURE 6. Prevalence of lipid extremes in diabetic and nondiabetic participants is shown for Framingham offspring women. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; total-C, total cholesterol; Trig, triglycerides. Adapted, with permission, from Siegel et al. [23].

ensured greater accuracy and precision of the laboratory determinations. Lipoprotein cholesterol, biomarker, and genetic investigations increased greatly and involved scientists at many other institutions. Relevant to lipid research, these collaborations included measurement of insulin, apolipoproteins, lipoprotein particle number, and determination of gene variants such as apolipoprotein E that were known to be associated with lipid levels [28–30].

METABOLIC SYNDROME AND INSULIN RESISTANCE

In the 1990s, it was recognized that many individuals who went on to develop CVD or diabetes mellitus tended to have greater adiposity, elevated triglycerides, low HDL-C, elevated blood pressure, or impaired fasting glucose. Presence of 3 or more of these 5 traits was given the name metabolic syndrome, and it was felt that the syndrome was highly related to insulin resistance. As displayed in Figure 7, Framingham analyses used factor analysis and showed that the metabolic syndrome traits clustered, and the presence of 3 or more of the traits typically led to a doubling or tripling of risk for CVD and more than a 20-fold greater risk of diabetes mellitus [31,32]. 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 [21,33–37]. The metabolic syndrome was identified as a practical way to identify persons at high risk to develop vascular disease in the National Cholesterol Education Program's adult treatment guidelines that were published in 2001 [38].

TABLE 2. Means for risk factors according to self-reported weekly vigorous physical activity level: the Framingham Offspring Study

	Men		Women	
Factor	<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
Cigarettes/day	10.5	4.1	7.7	4.9
Body mass index, kg/m^2	26.7	25.4*	24.4	23.7
Heart rate, per min	71.9	67.0*	77.5	72.5†

*$p < 0.001$.
†$p < 0.01$.
Adapted, with permission, from Dannenberg et al. [24].

APOLIPOPROTEINS

Lipoprotein particles include apolipoproteins, cholesterol, triglycerides, and phospholipid moieties. Protein assays became more prevalent 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 [39].

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 [40,41]. Concentrations of apolipoprotein A-1 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 A-1, 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 [28].

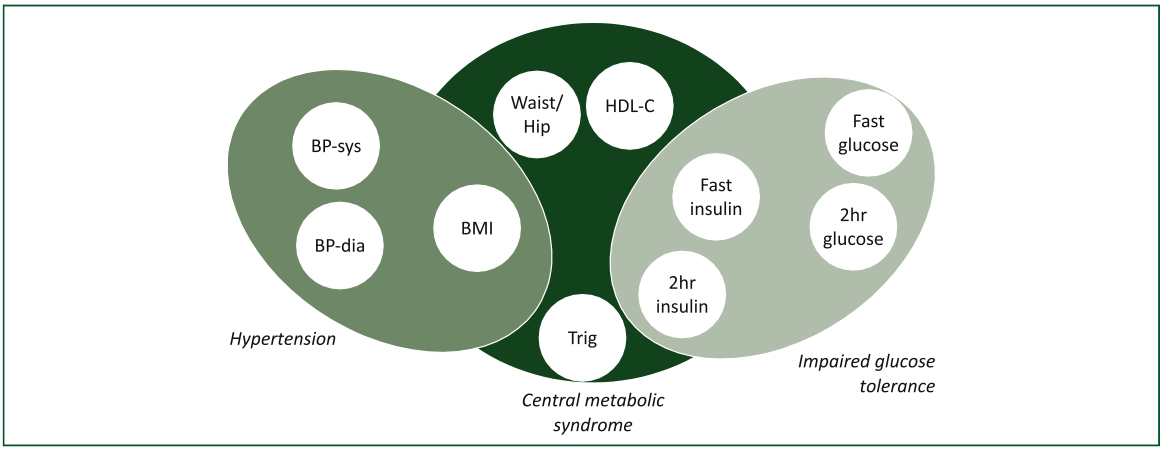

FIGURE 7. Metabolic risk factor clustering is shown for domains related to hypertension, central metabolic syndrome, and impaired glucose tolerance. Models were developed from the Framingham offspring using principal components analysis. BMI, body mass index; BP-Dia, diastolic blood pressure; BP-Sys, systolic blood pressure; HDL-C, high-density lipoprotein cholesterol; Trig, triglycerides. Adapted, with permission, from Meigs et al. [56].

Apolipoprotein E is an apoprotein of special interest because deficiency is associated with increased atherosclerosis in animal models, and genetic variants have been associated with abnormal lipids, cardiovascular disease, 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 E-4 alleles present and lower levels of LDL-C were seen in persons with the E-2 allele [30]. Results for triglycerides were slightly different, and both the E-2 and E-4 alleles were associated with higher triglyceride concentrations. The E-4 allele was found to be present in approximately 24% of the Framingham participants, and on a population basis, it was estimated that approximately 10% to 15% of CVD could be attributed to the presence of the E-4 allele. Separate analyses showed that the E-4 allele was highly associated with a greater risk for Alzheimer's disease and relative protection from dementia was found for persons with the E-2 allele [42,43].

Genetic research in Framingham 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 [44—47]. Enumerating the specific polymorphisms is beyond the scope of this review, and the reader should consult the consortium manuscripts referenced.

ESTIMATING RISK FOR CVD OUTCOMES

It was shown in the late 1980s that CVD risk could be predicted with reasonable accuracy using variables that had been measured in the Framingham periodic examinations [48]. The traditional variables included age, sex, total cholesterol, HDL-C, systolic blood pressure, blood pressure treatment, diabetes mellitus, and cigarette smoking [49,50]. A variety of lipid measures was 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

TABLE 3. Baseline lipoprotein risk factors and 14-year CVD incidence: the Framingham Offspring Study

	Men			Women		
Factor	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	1,509	1,641	<0.0001	1,344	1,628	<0.0001

CVD, cardiovascular disease; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
Adapted, with permission, from Cromwell et al. [51].

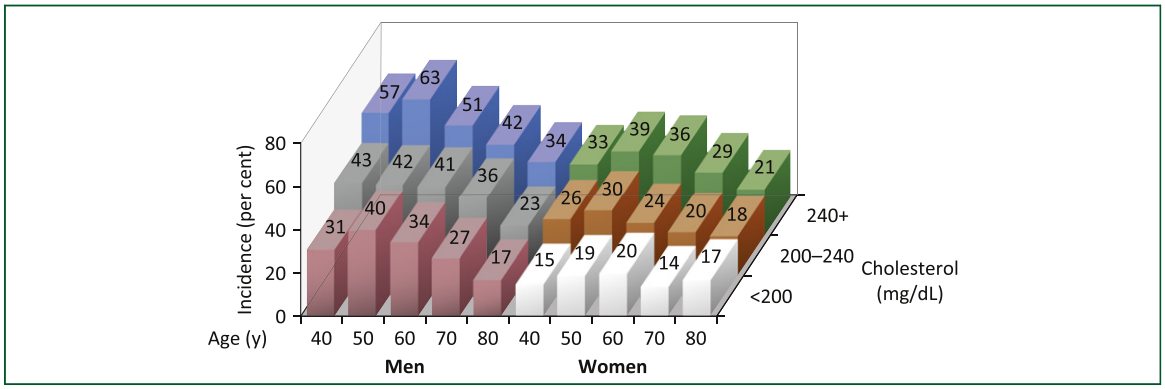

FIGURE 8. Lifetime risk of coronary heart disease (CHD) shown according to total cholesterol level groupings for men and women at various ages. Modeled after Lloyd-Jones et al. [52], with permission.

Framingham offspring [51] (Table 3). 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 [48]. As mentioned in the apolipoprotein discussion, using the lipid measures apolipoprotein B and apolipoprotein A-1 did not provide greater discrimination in estimation for risk of initial CVD events in comparisons with total and HDL-C in multivariable models [28].

Considerable interest in lifetime risk of CVD has developed over the past 20 years; both age and blood cholesterol levels are highly associated with greater lifetime risk of CVD in both sexes. As shown in Figure 8, higher cholesterol levels increased risk for CVD events and have the greatest effect on lifetime risk for persons at younger ages [52]. Cholesterol levels tend to rise in adulthood, peak between ages 50 and 60 years, and decline in older persons. These trends and the varying association of total cholesterol level with CVD risk were considered in the development of risk estimation equations, and the latter include age × cholesterol interaction terms that attempt to account for these effects [48]. Lower blood cholesterol in older persons partly explains why cholesterol levels in the elderly have not been highly associated with carotid artery disease. A Framingham analysis showed that cumulative exposures of cholesterol, blood pressure, and smoking were highly associated with greater carotid stenosis in persons who underwent carotid ultrasound measurements at a mean age of 75 years [53].

SUMMARY

This paper has summarized many of the key findings related to lipid levels, risk factor levels, and vascular disease outcomes in the Framingham cohorts. At the outset of the study, the primary focus was simple measures such as total blood cholesterol and triglycerides; over time, the scope expanded to include lipoprotein cholesterol quantification, apolipoproteins, genetics, lipid particles, and using 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 see trends over time and the effects of the treatments. Also, there is the potential to assess CVD risk using on-treatment lipid measures in the future.

REFERENCES

1. Abell LL, Levy BB, Brodie BB, Kendall FE. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. J Biol Chem 1952;195:357–66.
2. Kessler G, Lederer H. Automation in Analytical Chemistry, Technicon Symposia 1965. 1st edition. New York, NY: Mediad Inc.; 1965.
3. Kannel WB, McGee DL. Diabetes and cardiovascular risk factors: the Framingham study. Circulation 1979;59:8–13.
4. Evaluation of serum lipoprotein and cholesterol mesurements as predictors of clinical complication of atherosclerosis: report of a cooperative study of lipoproteins and atherosclerosis. Circulation 1956;14:691–742.
5. Kannel WB, Castelli WP, Gordon T, McNamara PM. Serum cholesterol, lipoproteins, and the risk of coronary heart disease: the Framingham study. Ann Intern Med 1971;74:1–12.
6. Kannel WB, Dawber TR, Kagan A, Revotskie N, Stokes J 3rd. Factors of risk in the development of coronary heart disease—six year follow-up experience: the Framingham study. Ann Intern Med 1961;55:33–50.
7. Fredrickson DS, Levy RI, Lees RS. Fat transport in lipoproteins—an integrated approach to mechanisms and disorders. N Engl J Med 1967;276:94–103.
8. Lipid Research Clinics Program. Manual of Laboratory Operation. 1st edition. Bethesda, MD: National Institutes of Health; 1974.
9. Gidez LI, Miller GJ, Burstein M, Slagle S, Eder HA. Separation and quantitation of subclasses of human plasma high

densitylipoproteins by a simple precipitation procedure. J Lipid Res 1982;23:1206–33.
10. Heiss G, Tamir I, Davis CE, et al. Lipoprotein-cholesterol distributions in selected North American populations: the Lipid Research Clinics Program Prevalence Study. Circulation 1980;61:302–15.
11. Brensike JF, Levy RI, Kelsey SF, et al. Effects of therapy with cholestyramine on progression of coronary arteriosclerosis: results of the NHLBI Type II Coronary Intervention Study. Circulation 1984;69:313–24.
12. Bostom AG, Gagnon DR, Cupples LA, et al. A prospective investigation of elevated lipoprotein (a) detected by electrophoresis and cardiovascular disease in women: the Framingham Heart Study. Circulation 1994;90:1688–95.
13. Scanu AM, Scandiani L. Lipoprotein(a): structure, biology, and clinical relevance. Adv Intern Med 1991;36:249–70.
14. Gordon T, Castelli WP, Hjortland MC, Kannel WB, Dawber TR. High density lipoprotein as a protective factor against coronary heart disease: the Framingham study. Am J Med 1977;62:707–14.
15. Miller GJ, Miller NE. Plasma-high-density-lipoprotein concentration and development of ischemic heart disease. Lancet 1975;1:16–9.
16. Summary of the second report of the National Cholesterol Education Panel on Detection. Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel II). JAMA 1993;269: 3015–23.
17. Report of the National Cholesterol Education Program Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Arch Intern Med 1988;34:193–201.
18. Garrison RJ, Wilson PW, Castelli WP, Feinleib M, Kannel WB, McNamara PM. Obesity and lipoprotein cholesterol in the Framingham offspring study. Metabolism 1980;29:1053–60.
19. Lamon-Fava S, Jimenez D, Christian J, et al. The NHLBI Twin Study: heritability of apolipoprotein A-I, B, and low density lipoprotein subclasses and condordance for lipoprotein (a). Atherosclerosis 1991; 91:97–106.
20. Anderson KM, Wilson PW, Garrison RJ, Castelli WP. Longitudinal and secular trends in lipoprotein cholesterol measurements in a general population sample: the Framingham Offspring Study. Atherosclerosis 1987;68:59–66.
21. Campos H, Wilson PWF, Jimenez D, McNamara JR, Ordovas J, Schaefer EJ. Differences in apolipoproteins and low-density lipoprotein subfractions in postmenopausal women on and off estrogen therapy: results from the Framingham Offspring Study. Metabolism 1990;39:1033–8.
22. Vaziri SM, Evans JC, Larson MG, Wilson PW. The impact of female hormone usage on the lipid profile: the Framingham Offspring Study. Arch Intern Med 1993;153:2200–6.
23. Siegel RD, Cupples A, Schaefer EJ, Wilson PW. Lipoproteins, apolipoproteins, and low-density lipoprotein size among diabetics in the Framingham Offspring Study. Metabolism 1996;45:1267–72.
24. Dannenberg AL, Keller JB, Wilson PW, Castelli WP. Leisure time physical activity in the Framingham Offspring Study: description, seasonal variation, and risk factor correlates. Am J Epidemiol 1989; 129:76–88.
25. Garrison RJ, Kannel WB, Feinleib M, Castelli WP, McNamara PM, Padgett SJ. Cigarette smoking and HDL cholesterol: the Framingham Offspring Study. Atherosclerosis 1978;30:17–25.
26. Mukamal KJ, Jadhav PP, D'Agostino RB, et al. Alcohol consumption and hemostatic factors: analysis of the Framingham Offspring cohort. Circulation 2001;104:1367–73.
27. Wilson PW. Lipoprotein measurements—setting priorities. Am J Med 2001;110:71–2.
28. Ingelsson E, Schaefer EJ, Contois JH, et al. Clinical utility of different lipid measures for prediction of coronary heart disease in men and women. JAMA 2007;298:776–85.
29. Freedman DS, Otvos JD, Jeyarajah EJ, et al. Sex and age differences in lipoprotein subclasses measured by nuclear magnetic resonance spectroscopy: the Framingham study. Clin Chem 2004;50:1189–200.
30. Wilson PW, Myers RH, Larson MG, Ordovas JM, Wolf PA, Schaefer EJ. Apolipoprotein E alleles, dyslipidemia, and coronary heart disease: the Framingham Offspring Study. JAMA 1994;272:1666–71.
31. Wilson PW, D'Agostino RB, Parise H, Sullivan L, Meigs JB. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. Circulation 2005;112:3066–72.
32. Meigs JB, Rutter MK, Sullivan LM, Fox CS, D'Agostino RB Sr, Wilson PW. Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. Diabetes Care 2007;30:1219–25.
33. McNamara JR, Campos H, Ordovas JM, Peterson J, Wilson PW, Schaefer EJ. Effect of gender, age, and lipid status on low density lipoprotein subfraction distribution: results from the Framingham Offspring Study. Arteriosclerosis 1987;7:483–90.
34. Campos H, McNamara JR, Wilson PW, Ordovas JM, Schaefer EJ. Differences in low density lipoprotein subfractions and apolipoproteins in premenopausal and postmenopausal women. J Clin Endo Metab 1988;67:30–5.
35. Meigs JB, Jacques PF, Selhub J, et al. Fasting plasma homocysteine levels in the insulin resistance syndrome: the Framingham offspring study. Diabetes Care 2001;24:1403–10.
36. Meigs JB, Nathan DM, Wilson PW, Cupples LA, Singer DE. Metabolic risk factors worsen continuously across the spectrum of nondiabetic glucose tolerance: the Framingham Offspring Study. Ann Intern Med 1998;128:524–33.
37. Hivert MF, Sullivan LM, Fox CS, et al. Associations of adiponectin, resistin, and tumor necrosis factor-alpha with insulin resistance. J Clin Endocrinol Metab 2008;93:3165–72.
38. Expert Panel on Detection. Evaluation, and Treatment of High Blood Cholesterol In Adults. 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). JAMA 2001;285:2486–97.
39. Bostom AG, Cupples LA, Jenner JL, et al. Elevated plasma lipoprotein (a) and coronary heart disease in men aged 55 years and younger: a prospective study. JAMA 1996;276:544–8.
40. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W, Steiner E. High apolipoprotein B, low apolipoprotein A-I, and improvement in the prediction of fatal myocardial infarction (AMORIS study): a prospective study. Lancet 2001;358:2026–33.
41. Barter PJ, Ballantyne CM, Carmena R, et al. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. J Intern Med 2006;259: 247–58.
42. Myers RH, Schaefer EJ, Wilson PW, et al. Apolipoprotein E allele 4 is associated with dementia in the Framingham Study. In: Iqbal K, Mortimer JA, Winblad B, Wisniewski HM, editors. Research Advances in Alzheimer's Disease and Related Disorders. 1st edition. New York, NY: Wiley; 1995. 63—70.
43. Myers RH, Schaefer EJ, Wilson PW, et al. Apolipoprotein E epsilon4 asociation with dementia in a population-based study: the Framingham study. Neurology 1996;46:673–7.
44. Kathiresan S, Melander O, Anevski D, et al. Polymorphisms associated with cholesterol and risk of cardiovascular events. N Engl J Med 2008; 358:1240–9.
45. Kathiresan S, Musunuru K, Orho-Melander M. Defining the spectrum of alleles that contribute to blood lipid concentrations in humans. Curr Opin Lipidol 2008;19:122–7.
46. Kathiresan S, Melander O, Guiducci C, et al. Six new loci associated with blood low-density lipoprotein cholesterol, high-density lipoprotein cholesterol or triglycerides in humans. Nat Genet 2008;40: 189–97.
47. Cupples LA, Arruda HT, Benjamin EJ, et al. The Framingham Heart Study 100K SNP genome-wide association study resource: overview of 17 phenotype working group reports. BMC Med Genet 2007;8-(Suppl 1):S1.
48. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837–47.
49. Anderson KM, Wilson PW, Odell PM, Kannel WB. An updated coronary risk profile: a statement for health professionals. Circulation 1991;83:356–62.

50. Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. Am Heart J 1991;121:293–8.
51. Cromwell WC, Otvos JD, Keyes MJ, et al. LDL particle number and risk of future cardiovascular disease in the Framingham Offspring Study—implications for LDL management. J Clin Lipidol 2007;1:583–92.
52. Lloyd-Jones DM, Wilson PW, Larson MG, et al. Lifetime risk of coronary heart disease by cholesterol levels at selected ages. Arch Intern Med 2003;163:1966–72.
53. Wilson PW, Hoeg JM, D'Agostino RB, et al. Cumulative effects of high cholesterol levels, high blood pressure, and cigarette smoking on carotid stenosis. N Engl J Med 1997;337:516–22.
54. Lamon-Fava S, Wilson PW, Schaefer EJ. Impact of body mass index on coronary heart disease risk factors in men and women: the Framingham Offspring Study. Arterioscler Thromb Vasc Biol 1996;16:1509–15.
55. Abbott RD, Wilson PW, Kannel WB, Castelli WP. High density lipoprotein cholesterol, total cholesterol screening, and myocardial infarction: the Framingham study. Arteriosclerosis 1988;8:207–11.
56. Meigs JB, D'Agostino RB Sr, Wilson PW, Cupples LA, Nathan DM, Singer DE. Risk variable clustering in the insulin resistance syndrome: the Framingham Offspring Study. Diabetes 1997;46:1594–600.