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 LIPOSES

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.ghheart.2012.12.009
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 disease.

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].

![Figure 1](image1.png)

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.
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 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].

---

**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].
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 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

<table>
<thead>
<tr>
<th>Body Mass Index Level, kg/m²</th>
<th>&lt;21</th>
<th>≥21 to &lt;23</th>
<th>≥23 to &lt;25</th>
<th>≥25 to &lt;27.5</th>
<th>≥27.5 to &lt;30</th>
<th>≥30.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, ≥200 mg/dl</td>
<td>7.4</td>
<td>11.1</td>
<td>18.6</td>
<td>24.5</td>
<td>26.9</td>
<td>25.0</td>
</tr>
<tr>
<td>Elevated LDL-C, ≥160 mg/dl</td>
<td>7.4</td>
<td>11.1</td>
<td>18.6</td>
<td>24.5</td>
<td>26.9</td>
<td>25.0</td>
</tr>
<tr>
<td>Low HDL-C, &lt;35 mg/dl</td>
<td>7.4</td>
<td>11.1</td>
<td>18.6</td>
<td>24.5</td>
<td>26.9</td>
<td>25.0</td>
</tr>
<tr>
<td>Women</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides, ≥200 mg/dl</td>
<td>0.0</td>
<td>1.9</td>
<td>3.9</td>
<td>9.3</td>
<td>15.9</td>
<td>14.9</td>
</tr>
<tr>
<td>Elevated LDL-C, ≥160 mg/dl</td>
<td>8.6</td>
<td>15.2</td>
<td>15.5</td>
<td>28.4</td>
<td>28.6</td>
<td>28.9</td>
</tr>
<tr>
<td>Low HDL-C, &lt;35 mg/dl</td>
<td>0.6</td>
<td>1.1</td>
<td>0.5</td>
<td>2.6</td>
<td>2.5</td>
<td>7.7</td>
</tr>
</tbody>
</table>

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].

**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].
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].

**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-I 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-I, 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].

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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;1 h</td>
<td>≥1 h</td>
</tr>
<tr>
<td>HDL-C, mg/dl</td>
<td>42.0</td>
<td>47.8*</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>133.5</td>
<td>135.0</td>
</tr>
<tr>
<td>VLDL-C, mg/dl</td>
<td>29.3</td>
<td>20.5*</td>
</tr>
<tr>
<td>Cigarettes/day</td>
<td>10.5</td>
<td>4.1</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.7</td>
<td>25.4*</td>
</tr>
<tr>
<td>Heart rate, per min</td>
<td>71.9</td>
<td>67.0*</td>
</tr>
</tbody>
</table>

*p < 0.001.

Adapted, with permission, from Dannenberg et al. [24].

**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].
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 [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

<table>
<thead>
<tr>
<th>Factor</th>
<th>Men No CVD</th>
<th>Men Yes CVD</th>
<th>p Value</th>
<th>Women No CVD</th>
<th>Women Yes CVD</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL-C, mg/dl</td>
<td>45</td>
<td>42</td>
<td>&lt;0.001</td>
<td>57</td>
<td>51</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL-C, mg/dl</td>
<td>134</td>
<td>138</td>
<td>0.09</td>
<td>126</td>
<td>143</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Non-HDL-C, mg/dl</td>
<td>158</td>
<td>168</td>
<td>0.0002</td>
<td>146</td>
<td>170</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LDL particle number, nmol/l</td>
<td>1,509</td>
<td>1,641</td>
<td>&lt;0.0001</td>
<td>1,344</td>
<td>1,628</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

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

![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].](image)
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


