Low-Density Lipoproteins Containing Apolipoprotein C-III and the Risk of Coronary Heart Disease

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Background—Low-density lipoprotein (LDL) that contains apolipoprotein (apo) C-III makes up only 10% to 20% of plasma LDL but has a markedly altered metabolism and proatherogenic effects on vascular cells.

Methods and Results—We examined the association between plasma LDL with apoC-III and coronary heart disease in 320 women and 419 men initially free of cardiovascular disease who developed a fatal or nonfatal myocardial infarction during 10 to 14 years of follow-up and matched controls who remained free of coronary heart disease. Concentrations of LDL with apoC-III (measured as apoB in this fraction) were associated with risk of coronary heart disease in multivariable analysis that included the ratio of total cholesterol to high-density lipoprotein cholesterol, LDL cholesterol, apoB, triglycerides, or high-density lipoprotein cholesterol and other risk factors. In all models, the relative risks for the top versus bottom quintile of LDL with apoC-III were greater than those for LDL without apoC-III. When included in the same multivariable-adjusted model, the risk associated with LDL with apoC-III (relative risk for top versus bottom quintile, 2.38; 95% confidence interval, 1.54–3.68; P for trend <0.001) was significantly greater than that associated with LDL without apoC-III (relative risk for top versus bottom quintile, 1.25; 95% confidence interval, 0.76–2.05; P for trend=0.97; P for interaction <0.001). This divergence in association with coronary heart disease persisted even after adjustment for plasma triglycerides.

Conclusions—The risk of coronary heart disease contributed by LDL appeared to result to a large extent from LDL that contains apoC-III. (Circulation. 2011;124:2065-2072.)

Key Words: apolipoproteins • cholesterol • metabolism • myocardial infarction • risk factors

Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) are heterogeneous lipoprotein classes that vary in size and content of lipid and protein. Apolipoprotein (apo) B is the required structural apolipoprotein of VLDL and LDL. Each VLDL and LDL has only 1 molecule of apoB but may have no or many molecules of apoC-III attached to its surface. About 40% to 60% of VLDL and 10% to 20% of LDL have apoC-III. ApoC-III has deleterious effects on the metabolism of VLDL and LDL and on functions of cells that participate in atherosclerosis. ApoC-III interferes with the binding of VLDL to receptors in the liver, which inhibits the removal of VLDL from plasma. Nearly all VLDL that has apoC-III undergoes intravascular lipolysis of its triglyceride content, producing LDL with apoC-III, and large LDL with apoC-III is metabolized to small LDL.

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ApoC-III-containing VLDL and LDL prepared from human plasma activate monocytes that circulate in blood to adhere to vascular endothelial cells, an early step in atherosclerosis. ApoC-III also activates vascular endothelial cells to produce adhesion molecules and induces insulin resistance in these cells, reducing their secretion of nitric oxide. These actions are not shared by VLDL or LDL that does not have apoC-III. The presence of apoC-III on LDL is also associated with compositional changes that favor LDL adhesion to the subendothelial extracellular matrix. Thus, apoC-III may trap its associated lipoproteins in the arterial wall and bring in blood monocytes, crucial steps in the initiation and progression of atherosclerosis. ApoC-III concentrations in VLDL and LDL are positively associated with the progression of atherosclerosis or risk of coronary heart disease (CHD), and we had reported that LDL with apoC-III is associated with recurrent cardiovascular disease among patients with a prior myocardial infarction and type 2 diabetes mellitus.

To evaluate whether plasma concentrations of VLDL and LDL that have apoC-III, as measured by apoB in each of their LDL fractions, are associated with the risk of coronary heart disease, we examined the association between LDL with apoC-III and coronary heart disease in 320 women and 419 men initially free of cardiovascular disease who developed a fatal or nonfatal myocardial infarction during 10 to 14 years of follow-up and matched controls who remained free of coronary heart disease. Concentrations of LDL with apoC-III were associated with risk of coronary heart disease in multivariable analysis that included the ratio of total cholesterol to high-density lipoprotein cholesterol, LDL cholesterol, apoB, triglycerides, or high-density lipoprotein cholesterol and other risk factors. In all models, the relative risks for the top versus bottom quintile of LDL with apoC-III were greater than those for LDL without apoC-III. When included in the same multivariable-adjusted model, the risk associated with LDL with apoC-III (relative risk for top versus bottom quintile, 2.38; 95% confidence interval, 1.54–3.68; P for trend <0.001) was significantly greater than that associated with LDL without apoC-III (relative risk for top versus bottom quintile, 1.25; 95% confidence interval, 0.76–2.05; P for trend=0.97; P for interaction <0.001). This divergence in association with coronary heart disease persisted even after adjustment for plasma triglycerides.

Conclusions—The risk of coronary heart disease contributed by LDL appeared to result to a large extent from LDL that contains apoC-III.
them, are more strongly associated with CHD risk than those of the same lipoproteins without apoC-III, we prospectively studied 2 US populations initially free of CHD: the Nurses’ Health Study (NHS; women) and the Health Professionals Follow-up Study (HPFS; men).

Methods

Study Population

The NHS and the HPFS are prospective cohort investigations involving 121,700 female US registered nurses who were 30 to 55 years of age at baseline in 1976 and 51,529 US male health professionals who were 40 to 75 years of age at baseline in 1986, respectively. Information about health and disease is assessed biennially.18–21

From 1989 through 1990, a blood sample was requested from all participants in the NHS, which 32,826 women provided. Similarly, between 1993 and 1995, a blood sample was provided as requested by 18,225 men in the HPFS. Participants who provided blood samples were similar to those who did not. In the NHS, among women without cardiovascular disease or cancer before 1990, we identified 350 women who had a nonfatal myocardial infarction or fatal CHD between the date of the blood draw and June 2004. In the HPFS, we identified 425 men initially free of cardiovascular disease who had a nonfatal myocardial infarction or fatal CHD between the date of the blood draw and the return of the 2004 questionnaire. Using risk-set sampling (a technique in which, in a prospective study, control subjects are selected among the group of individuals exposed to the studied factor at the time the event occurs in the case22), we randomly selected controls in a 1:1 ratio who were matched for age (±1 year), smoking status (never, past, or current), and date of blood sampling (±2 months) from the participants who were free of cardiovascular disease at the time CHD was diagnosed in the case patients. Within the NHS cohort, an additional matching criterion was fasting status at the time of blood sampling.

Assessment of CHD

Study physicians who were unaware of the participant’s exposure status confirmed the diagnosis of myocardial infarction on the basis of the criteria of the World Health Organization. Deaths were identified from state vital records and the National Death Index or reported by the participant’s next of kin or the postal system. Fatal CHD was confirmed by an examination of hospital or autopsy records, by the listing of CHD as the cause of death on the death certificate, if CHD was the underlying and most plausible cause, and if evidence of previous CHD was available.

Assessment of Other Factors

Medical history, lifestyle, and demographic information was derived from the questionnaire administered in 1990 to women and 1994 to men, with missing information substituted from previous questionnaires. Physical activity was expressed in terms of metabolic equivalent–hours. The questionnaires and the validity and reproducibility of measurements have been described previously.18–21

Measurement of Lipids and Apolipoproteins Levels

Blood samples from women were collected in heparin-treated tubes; samples from men, in EDTA-treated tubes. The tubes were placed on ice packs, shipped in Styrofoam containers by overnight courier, centrifuged, divided into aliquots, and stored in liquid-nitrogen freezers (−130°C or colder). One vial containing 0.6 mL (NHS) or 0.5 mL (HPFS) frozen plasma was shipped to the lipoprotein laboratory at the Harvard School of Public Health for analysis of lipoprotein types. Study samples were sent to the laboratory for analysis in randomly ordered batches, and the laboratory personnel were unaware of a sample’s case-control status. The entire plasma sample was thawed, filtered, and incubated overnight with affinity-purified polyclonal goat antihuman apoC-III antibodies (Academy Biomedical, Houston, TX) bound to Sepharose 4B resin, as previously described and validated.22–23 The unbound lipoproteins that did not contain apoC-III were collected by gravity flow, and the bound lipoproteins that contained apoC-III were eluted with 3 mol/L sodium thiocyanate. The efficiency of the apoC-III immunooaffinity separation (percentage of ligand removed from plasma by the resin) was 99%. Subsequently, apoC-III–bound and unbound fractions were ultracentrifuged to isolate the VLDL (d<1.006 g/mL), LDL (1.006<d<1.063 g/mL), and high-density lipoprotein (HDL; d>1.063 g/mL).24 ApoB and apoC-III were measured in whole plasma and in VLDL and LDL with and without apoC-III by ELISA, and cholesterol and triglycerides were measured by enzymatic methods (Infinity Kit, Thermo Fischer Scientific, Waltham, MA). To test the repeatability of the assays, we ran blinded controls that were sent by the Channing Laboratory disguised as samples and included in every batch with their own identifications. These controls were included and run in every batch of real samples so that each control went through the whole process at least 8 times. The coefficients of variation for NHS were 9% for apoB in LDL without apoC-III and 17% for apoB in LDL with apoC-III; for HPFS, they were 17% for apoB in LDL without apoC-III and 13% for apoB in LDL with apoC-III.

The study protocol was approved by the institutional review board of the Brigham and Women’s Hospital and the Human Subjects Committee Review Board of the Harvard School of Public Health. After the exclusion of participants with insufficient sample or missing data for apoB in at least one of the LDL and VLDL fractions, the data set consisted of 1476 participants (320 female and 419 male cases, 320 female and 419 male controls). The cases were identified and the controls selected in 2-year cycles. Two men selected as controls in 1 cycle later developed CHD and were selected in a subsequent cycle, this time as cases. For this reason, the study sample contains 1476, not 1478, individuals. For these 2 participants, we used the same baseline measurements in the pair in which they were cases and in the pair in which they were controls.

Because there is only 1 apoB molecule per lipoprotein particle, the apoB concentration represents the concentration of lipoproteins of a given type. Our main exposure, specified in the protocol and according to our previous result,1 was the concentration of LDL with apoC-III, as reflected by the concentration of apoB in this fraction, ie, apoB in LDL with apoC-III.

Statistical Analysis

Data from the 2 cohorts were analyzed at the individual-participant level (as opposed to separate analysis in each cohort plus meta-analysis). Apolipoprotein measurements were divided into quintiles, from the lowest to highest levels, based on the sex-specific distributions among the controls. We analyzed the association between apolipoprotein levels and the risk of CHD using conditional logistic regression. With risk-set sampling, the odds ratio derived from the logistic regression directly estimates the hazard ratio and thus the relative risk.22 We analyzed our exposures in 4 logistic models: model 1 conditioned on matching factors only; model 2 with additional adjustment for parental history of CHD before 60 years of age, alcohol intake, and personal history of hypertension at baseline; model 3 with adjustment for all the variables in model 2 plus body mass index and personal history of diabetes mellitus; and model 4 with the addition of triglyceride concentration at baseline. Baseline was defined as the year blood was drawn. To test for linear trend, we used the median levels of apolipoprotein measurements in the control categories as a continuous variable.

To test for an interaction between LDL types, we ran a model that included LDL quintile, presence or absence of apoC-III, and an interaction term.

All P values are 2 tailed, and ρ values <0.05 were considered to indicate statistical significance. All analyses were performed with the use of SAS software, version 9.1 (SAS Institute).

Results

LDL with apoC-III represented on average 12% of total LDL. Participants of both sexes who developed CHD during
follow-up had significantly higher concentrations of LDL with apoC-III. Male but not female cases had higher concentrations of LDL without apoC-III than controls (Table 1). Compared with participants in the lowest quintile, participants in the highest quintile of LDL with apoC-III had a significantly increased risk of CHD (relative risk for highest versus lowest quintile, 2.58; 95% confidence interval, 1.78–3.74; \( P \) for trend <0.001), conditioning in matching factors (Table 2). LDL without apoC-III was associated with CHD but with a lower relative risk than LDL with apoC-III (1.72; 95% confidence interval, 1.14–2.61). Adjustment for other risk factors in models 2 through 4, including personal history of diabetes mellitus and triglyceride concentration, had little effect on the relative risks (Table 2). The risk associated with LDL with apoC-III was similar in NHS and HPFS participants, with no significant sex interaction, but the risk associated with LDL without apoC-III was higher in HPFS (Table 1 in the online-only Data Supplement). It was not possible to perform multivariate logistic analyses in the diabetic subgroup (n = 126) because of its small size (model did not converge), but among nondiabetic participants (n = 1350), the multivariate-adjusted results were consistent with those for the complete study sample (relative risk for top versus bottom quintile of LDL with apoC-III, 2.39; 95% confidence interval, 1.56–3.67).

### Table 1. Baseline Characteristics of the Study Sample

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women*</th>
<th></th>
<th></th>
<th>Men*</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases (n=320)</td>
<td>Controls (n=320)</td>
<td>( P^\dagger )</td>
<td>Cases (n=419)</td>
<td>Controls (n=419)</td>
<td>( P^\dagger )</td>
</tr>
<tr>
<td>Age, y</td>
<td>60±6</td>
<td>60±6</td>
<td>. .</td>
<td>64±8</td>
<td>64±8</td>
<td>. .</td>
</tr>
<tr>
<td>Current smoker, %</td>
<td>25</td>
<td>25</td>
<td>. .</td>
<td>9</td>
<td>8</td>
<td>. .</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>26.8±5.6</td>
<td>25.4±4.2</td>
<td>&lt;0.001</td>
<td>26.1±3</td>
<td>25.5±3</td>
<td>0.017</td>
</tr>
<tr>
<td>Parental history of CHD before 60 y of age, %</td>
<td>20</td>
<td>14</td>
<td>&lt;0.001</td>
<td>15</td>
<td>12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Postmenopausal, %</td>
<td>91</td>
<td>88</td>
<td>0.001</td>
<td>. .</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>Postmenopausal hormone therapy among postmenopausal women, %</td>
<td>33</td>
<td>35</td>
<td>0.001</td>
<td>. .</td>
<td>. .</td>
<td>. .</td>
</tr>
<tr>
<td>Aspirin use, † %</td>
<td>26</td>
<td>30</td>
<td>&lt;0.001</td>
<td>40</td>
<td>33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of hypertension, %</td>
<td>53</td>
<td>31</td>
<td>0.013</td>
<td>37</td>
<td>28</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>History of diabetes mellitus, %</td>
<td>17</td>
<td>6</td>
<td>&lt;0.001</td>
<td>9</td>
<td>3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Alcohol consumption, g/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.9</td>
<td>1.1</td>
<td>0.31</td>
<td>4.4</td>
<td>7.5</td>
<td>0.009</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>0–4.1</td>
<td>0–6.4</td>
<td>. .</td>
<td>0–15.0</td>
<td>10–18.2</td>
<td>. .</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.11±1.19</td>
<td>6.01±1.22</td>
<td>0.20</td>
<td>5.62±1.01</td>
<td>5.41±0.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>3.81±1.04</td>
<td>3.65±1.01</td>
<td>0.059</td>
<td>3.47±0.85</td>
<td>3.26±0.83</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.35±0.36</td>
<td>1.53±0.44</td>
<td>&lt;0.001</td>
<td>1.09±0.28</td>
<td>1.22±0.34</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Ratio of total to HDL cholesterol</td>
<td>3.7±1.5</td>
<td>3.4±1.6</td>
<td>0.014</td>
<td>3.7±1.1</td>
<td>3.4±1.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.59±1.11</td>
<td>1.23±0.65</td>
<td>&lt;0.001</td>
<td>1.63±0.92</td>
<td>1.34±0.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>C-reactive protein, mg/L</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>0.52</td>
<td>0.38</td>
<td>0.34</td>
<td>0.34</td>
<td>0.26</td>
<td>0.83</td>
</tr>
<tr>
<td>Q1-Q3</td>
<td>0.20–1.45</td>
<td>0.14–1.02</td>
<td>. .</td>
<td>0.13–1.07</td>
<td>0.08–0.87</td>
<td>. .</td>
</tr>
<tr>
<td>Total plasma apoB, g/L</td>
<td>0.92±0.28</td>
<td>0.88±0.31</td>
<td>0.07</td>
<td>0.97±0.28</td>
<td>0.9±0.26</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB in VLDL with apoC-III, g/L</td>
<td>0.012±0.011</td>
<td>0.009±0.008</td>
<td>&lt;0.001</td>
<td>0.023±0.016</td>
<td>0.02±0.015</td>
<td>0.003</td>
</tr>
<tr>
<td>ApoB in VLDL without apoC-III, g/L</td>
<td>0.036±0.028</td>
<td>0.03±0.024</td>
<td>0.001</td>
<td>0.038±0.027</td>
<td>0.031±0.024</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB in LDL with apoC-III, g/L</td>
<td>0.09±0.084</td>
<td>0.075±0.056</td>
<td>0.003</td>
<td>0.135±0.085</td>
<td>0.117±0.08</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ApoB in LDL without apoC-III, g/L</td>
<td>0.78±0.24</td>
<td>0.77±0.28</td>
<td>0.51</td>
<td>0.77±0.23</td>
<td>0.73±0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total plasma apoC-III, g/L</td>
<td>0.18±0.11</td>
<td>0.16±0.1</td>
<td>0.30</td>
<td>0.17±0.09</td>
<td>0.16±0.09</td>
<td>0.16</td>
</tr>
<tr>
<td>ApoC-III in VLDL, g/L</td>
<td>0.02±0.019</td>
<td>0.017±0.016</td>
<td>0.028</td>
<td>0.027±0.018</td>
<td>0.024±0.016</td>
<td>0.019</td>
</tr>
<tr>
<td>ApoC-III in LDL, g/L</td>
<td>0.034±0.03</td>
<td>0.028±0.022</td>
<td>0.001</td>
<td>0.032±0.023</td>
<td>0.028±0.019</td>
<td>0.003</td>
</tr>
<tr>
<td>Molar ratio of apoC-III to apoB in VLDL</td>
<td>131±177</td>
<td>161±240</td>
<td>0.18</td>
<td>80±8</td>
<td>85±62</td>
<td>0.12</td>
</tr>
<tr>
<td>Molar ratio of apoC-III to apoB in LDL</td>
<td>27±20</td>
<td>28±23</td>
<td>0.35</td>
<td>16±7</td>
<td>16±7</td>
<td>0.35</td>
</tr>
</tbody>
</table>

CHD denotes coronary heart disease; Q1, quartile 1; Q3, quartile 3; LDL, low-density lipoprotein; HDL, high-density lipoprotein; apo, apolipoprotein; and VLDL, very low-density lipoprotein. Plus-minus values are mean±SD. To convert values for cholesterol to milligrams per deciliter, multiply by 38.6. To convert values for triglycerides to milligrams per deciliter, multiply by 88.57. The body mass index is weight in kilograms divided by the square of the height in meters.

*Data on women are from the Nurses’ Health Study; data on men are from the Health Professionals Follow-up Study. Matching criteria were age, smoking status, and date of blood sampling; among women, additional matching criteria included fasting status at the time of blood sampling.

† \( P \) values for the difference between cases and controls (unadjusted) were determined by paired Student \( t \) test for variables expressed as mean±SD, by the signed-rank test for variables expressed as medians, and by the McNemar \( \chi^2 \) test for variables expressed as percentages.

‡Current aspirin use was defined as 1 to 4 d/wk for women and as ≥2 times per week for men.
Independence of Association of LDL With apoC-III From Other Lipid Risk Factors

We explored whether the association between LDL with apoC-III and CHD was independent of other established lipid risk factors. In the background of model 2, we added LDL cholesterol, HDL cholesterol, plasma apoB, plasma triglycerides, ratio of total cholesterol to HDL cholesterol, or non-HDL cholesterol, one by one; in all models, LDL with apoC-III remained significantly associated with CHD (Figure 1). In contrast, LDL without apoC-III was not significantly independent of LDL cholesterol, plasma triglycerides, ratio of total cholesterol to HDL cholesterol, or non-HDL cholesterol (Figure I in the online-only Data Supplement).

No Effect Modification by Hypertriglyceridemia

To address the strength of the relationship between LDL with apoC-III and CHD at different triglyceride levels, we first added a quintile interaction term of triglycerides×LDL with apoC-III to the model, and its coefficient was not significant ($P=0.46$). Second, we computed the relative risk for LDL with apoC-III in the participants who had normal or high triglycerides according to the standard cut point, 150 mg/dL. The point estimates of the relative risks were similar: 1.61 for participants with triglycerides <150 mg/dL and 1.77 for participants with triglycerides ≥150 mg/dL ($P$ for interaction $=0.63$). Thus, our finding regarding LDL with apoC-III as an independent predictor of CHD was not significantly modified by hypertriglyceridemia.

Comparison of LDL With and Without apoC-III

When quintiles of LDL with apoC-III and quintiles of LDL without apoC-III were included in the same multivariable-adjusted model (Figure 2A), only LDL with apoC-III was...
associated with CHD (relative risk, 2.38; 95% confidence interval, 1.54–3.68; versus relative risk, 1.25; 95% confidence interval, 0.76–2.05 for LDL without apoC-III; \( P \) for difference in slopes < 0.001). Given that apoC-III stimulates hepatic secretion of triglycerides, we performed additional analyses adding plasma triglycerides to this model (Figure 2B). The association with CHD was still significantly higher for LDL with apoC-III (\( P \) for trend < 0.001). We obtained similar results in a model adjusted for the same covariates in which cholesterol substituted for apoB. Cholesterol in LDL with apoC-III (relative risk for highest versus lowest quintile, 2.01; 95% confidence interval, 1.35–2.99), but not cholesterol in LDL without apoC-III (relative risk for highest versus lowest quintile, 1.30; 95% confidence interval, 0.86–1.95), was significantly associated with CHD (Figure II in the online-only Data Supplement).

Analyses for apoC-III Concentrations

The concentration of apoC-III in LDL was associated with CHD after adjustment for risk factors (Table 2), but the association was less consistent than for the apoB concentration of LDL with apoC-III (concentration of LDL particles with any apoC-III; Figure 3). Total plasma apoC-III was associated with increased risk of CHD (Table 2), but the association lost significance in model 3 or after addition of any of the major known lipid risk factors to model 2.

Analyses of VLDL

Concentrations of VLDL with apoC-III and VLDL without apoC-III were associated with CHD similarly and significantly. Adjustment for body mass index and diabetes mellitus in model 3 attenuated the relative risks for both VLDL types, but both were still significant (Table II in the online-only Data Supplement).

Relative Size of VLDL and LDL With and Without apoC-III

The mean molar ratio of triglycerides to apoB, an indicator of VLDL particle size, was higher for VLDL with apoC-III than for VLDL without apoC-III (11 724 versus 7988) in the study sample. The mean molar ratio of cholesterol to apoB, an indicator of LDL particle size, was higher for LDL with apoC-III than for LDL without apoC-III (2934 versus 2373).
Characteristics Associated With a High Concentration of LDL With apoC-III

Higher levels of LDL with apoC-III were associated with a markedly higher prevalence of diabetes mellitus, higher plasma triglycerides, lower HDL cholesterol levels, slightly higher body mass indexes, and a higher prevalence of hypertension (Table III in the online-only Data Supplement). Contrastingly, higher levels of LDL without apoC-III were not associated with any of these conditions but were associated with higher LDL cholesterol and plasma apoB as was LDL with apoC-III.

Analyses Excluding Statin Users

In analyses restricted to the 704 participants who never received cholesterol-lowering medications during follow-up and adjusted for all variables in model 2, the relative risk of CHD for the top versus bottom quintile of LDL with apoC-III was 2.6 (95% confidence interval, 0.87–7.83; *P* for trend=0.049).

Use of Postmenopausal Hormone Replacement Therapy and LDL Types

To explore whether hormone replacement therapy might confound the association between LDL with apoC-III and CHD, we compared levels of LDL with and without apoC-III among women who used or did not use hormone replacement. Levels of LDL with apoC-III did not differ between users and nonusers (0.85 mg/dL for users versus 0.81 mg/dL for nonusers; *P*=0.53). The same was observed for LDL without apoC-III (77.6 mg/dL for users versus 77.5 mg/dL for nonusers; *P*=0.96).

Discussion

In this prospective study of individuals initially free of cardiovascular disease, the concentration of LDL that contains apoC-III was robustly associated with CHD, significantly more so than LDL that does not contain apoC-III.

The association between LDL with apoC-III and CHD was linear and persisted after adjustment for diverse risk factors. In fact, the association was only slightly affected by adjustment for alcohol use, family history of CHD, diabetes mellitus, hypertension, or hormone use, although the study had limited power to address subtle modification. When concentrations of LDL with apoC-III and LDL without apoC-III were included in the same model, only LDL with apoC-III was associated with CHD, suggesting that a considerable proportion of the CHD risk commonly attributed to LDL concentrations is in fact due to one of its subfractions that contains apoC-III. This result extends our previous finding in patients with preexisting CHD and type 2 diabetes mellitus. The sample size of our study, 739 cases, was able to identify a minimum increased relative risk of 1.4 for either type of LDL. However, it is possible that a larger sample size would identify a mild relation between LDL without apoC-III and CHD.

Besides its effects that impair lipoprotein metabolism and stimulate monocyte adhesion to vascular endothelial cells, apoC-III may promote the inflammatory process that fuels atherosclerosis through activation of Toll-like receptor 2 in monocytes and through induction of insulin resistance and inflammatory signaling pathways governed by nuclear factor-κB in endothelial cells. Finally, heterozygote carriers of a nonsense mutation in the apoC-III gene have significantly less coronary artery calcification and CHD than noncarrier subjects from the same population. All this evidence provides biological plausibility for a direct involvement of LDL with apoC-III in atherosclerosis.

There are on average 10 to 20 molecules of apoC-III but only 1 molecule of apoB in each LDL with apoC-III. The apoB concentration of LDL with apoC-III was more strongly associated with CHD than the apoC-III concentration of the same LDL. Thus, the number of apoC-III molecules per LDL may be less important than the concentration of this type of LDL. This was not what we found for VLDL, in which particles with or without apoC-III were similarly associated with CHD risk. Thus, the biological effects of apoC-III may be lipoprotein specific. In this respect, enhancement of LDL adhesion to proteoglycans by apoC-III depends on a critical site in apoB. It is conceivable that this site may be exposed in LDL only after most triglycerides in VLDL have been lipolyzed.

Our results suggest only a modest association between total plasma levels of apoC-III and CHD that was attenuated after adjustment for other lipid risk factors. Plasma apoC-III is a relatively simple determination in a clinical laboratory, whereas the measurement of LDL with apoC-III is more complex and time-consuming but seems to provide more meaningful information.

The association between LDL with apoC-III and CHD was independent of LDL cholesterol, in agreement with previous evidence showing that plasma VLDL and LDL with apoC-III predict the progression of atherosclerosis even among patients whose LDL cholesterol is markedly reduced with lovastatin. Thus, LDL with apoC-III may explain part of the residual CHD risk among individuals without elevated LDL cholesterol.
Our analyses excluding users of cholesterol-lowering drugs at any point during follow-up showed that, among never users, the relative risk estimate for LDL with apoC-III was quite similar to that in the complete study sample, ruling out confounding by indication of statins. This result is in accordance with a published analysis of the impact of pravastatin on concentrations of lipoproteins with and without apoC-III in patients with diabetes mellitus from the Cholesterol and Recurrent Events (CARE) trial.\(^20\) Statin treatment reduced LDL with apoC-III by 29% and LDL without apoC-III by 30%. In addition, all traditional lipid risk factors were associated with CHD in our study sample (Table IV in the online-only Data Supplement), so the results for LDL with apoC-III do not seem to be the consequence of an unusual population.

Our indirect estimation of LDL size by the molar ratio of cholesterol to apoB showed that LDL with apoC-III was on average larger than LDL without apoC-III, so it seems unlikely that LDL with apoC-III is just acting as a proxy for small, dense LDL. ApoC-III has been reported to transfer from VLDL to HDL during freezing or storage.\(^30\) In this regard, we performed a small experiment in samples from 4 volunteers measuring apoC-III in VLDL, LDL, and HDL immediately after plasma separation and after 5 months of storage at \(-80^\circ\text{C}\). The distribution of apoC-III changed only very slightly and nonsignificantly (from 14% in VLDL, 16% in LDL, and 70% in HDL when analyzed fresh to 12% in VLDL, 15% in LDL, and 73% in HDL after freezing/storage/thawing), so we believe this effect is unlikely to have had an important influence on our results under our sample handling and preserving procedures. Another potential concern is whether a high concentration of LDL with apoC-III might just be identifying subjects with hypertriglyceridemia and mild insulin resistance. Although there was a significant correlation between LDL with apoC-III and plasma triglycerides (r=0.42), the association between CHD and LDL with apoC-III persisted after adjustment for triglycerides, suggesting that both characteristics may have common origins but are independently associated with CHD.

A limitation of our study is that we did not have baseline measurements of plasma glucose and insulin that would have permitted a better adjustment for carbohydrate metabolism, so we relied on self-reported diagnosis of diabetes mellitus. Another relevant limitation is that we performed multiple statistical tests to explore multiple factors of interest and multiple models per factor. This may have inflated the rate of statistical tests to explore multiple factors of interest and multiple models per factor. This may have inflated the rate of type I error above the nominal 5% used per test.

We cannot be certain whether apoC-III is causally responsible for CHD or a marker for other properties of certain LDL particles. Human LDL with apoC-III has been found to have a different lipid composition than LDL without apoC-III,\(^2,13\) that may influence the effect of LDL with apoC-III on atherosclerosis. Nevertheless, when our results are combined with the evidence of reduced atherosclerosis in humans with genetically reduced apoC-III\(^27\) and with the abundant documentation of direct proatherogenic effects of apoC-III,\(^10–13,25,26\) they encourage further research into apoC-III as a potentially causative factor in atherosclerosis and cardiovascular disease and as a target for therapy. Ultimately, even if the presence of apoC-III is just a label for a type of LDL very strongly associated with CHD, the existence of a marker for such particles can prove useful in the targeting and optimization of preventive interventions.

### Conclusions

Our results suggest that the concentration of LDL with apoC-III is independently associated with the risk of CHD and that much of the risk ordinarily attributed to LDL may in fact be due to LDL particles that contain apoC-III. These findings are consistent with prior in vitro, animal, and epidemiological evidence suggesting enhanced atherogenicity of lipoproteins containing apoC-III. More than contributing to the improvement of already excellent prediction models,\(^31–33\) our results may endorse LDL with apoC-III as a highly attractive target for the prevention or treatment of atherosclerotic diseases.

### Sources of Funding

This work was supported by National Institutes of Health/National Heart, Lung, and Blood Institute grant HL070159.

### Disclosures

Dr Sacks has received research funding from ISIS Pharmaceuticals to investigate the effect of a biotechnology product on apoC-III levels. The other authors report no conflicts.

### References

6. Mendivil et al LDL With apoC-III and CHD 2071

### Mendivil et al LDL With apoC-III and CHD


17. Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, Pfeffer MA, Braunwald E. VLDL, apolipoproteins B, CIII and E and in apo of LDL may in fact be due to LDL with apoC-III. The findings were robust LDL with apoC-III was consistently and significantly associated with risk, indicating that an important fraction of the cardiovascular risk traditionally adjudicated to LDL may in fact be due to LDL with apoC-III.


**CLINICAL PERSPECTIVE**

During the last few years, evidence has accumulated indicating that the protein composition of lipoproteins may be more relevant to atherogenesis and cardiovascular risk than the absolute concentrations of plasma lipids. One of these compositional factors is apolipoprotein (apo) C-III, a small interchangeable apolipoprotein that impairs hepatic uptake of circulating lipoproteins and has various direct proatherogenic effects on the arterial wall. In this study, we prospectively analyzed the association between plasma concentrations of low-density lipoprotein (LDL) that contains or does not contain apoC-III and the appearance of coronary heart disease (CHD) events in >1400 men and women. Our key finding was that in a multivariate-adjusted model that includes concentrations of both LDL with apoC-III and LDL without apoC-III, only LDL with apoC-III was consistently and significantly associated with risk, indicating that an important fraction of the cardiovascular risk traditionally adjudicated to LDL may in fact be due to LDL with apoC-III. The findings were robust to adjustment for levels of plasma lipids, demographic cardiovascular risk factors, and diabetes status. The identification of LDL with apoC-III as a lipoprotein type disproportionately associated with the development of CHD adds to the enlarging body of evidence suggesting the involvement of apoC-III in atherogenesis and metabolic derangements and should stimulate further research exploring apoC-III as an interventional target in the prevention of CHD.
Low-Density Lipoproteins Containing Apolipoprotein C-III and the Risk of Coronary Heart Disease
Carlos O. Mendivil, Eric B. Rimm, Jeremy Furtado, Stephanie E. Chiuve and Frank M. Sacks

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Low density lipoproteins containing apolipoprotein C-III and the risk of coronary heart disease

Mendivil CO, Rimm EB, Furtado J, Chiuve S, Sacks FM

Supplemental material
**Supplemental Table 1.**

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<th>Variable*</th>
<th>1 (n=143)</th>
<th>2 (n=156)</th>
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**Supplemental Table 1.** Relative risks of coronary heart disease during follow-up, according to the quintile of apolipoprotein concentrations at baseline, by sex. * Model 1 was conditioned on matching factors (age, smoking status, and the month of blood sampling). Among women, data were also conditioned on fasting status at the time of blood sampling. Model 2 was additionally adjusted for presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake and personal history of hypertension. Model 3 was adjusted for all the variables in model 2 plus body-mass index and personal history of diabetes. † P values for trend are based on the median apolipoprotein levels in quintiles of the controls.
Supplemental Table 2. Association between apolipoprotein concentrations in VLDL and coronary heart disease in the complete study sample and by sex. * Model 1 was conditioned on matching factors. Model 2 was additionally adjusted for presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake and personal history of hypertension. Model 3 was adjusted for everything in model 2 plus body-mass index and personal history of diabetes. † P values for trend are based on the median apolipoprotein levels in quintiles of the controls. ‡ Calculated as the P value for the interaction between sex and median apolipoprotein levels in quintiles of the controls.

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<thead>
<tr>
<th>Variable*</th>
<th>Total sample</th>
<th>Men</th>
<th>Women</th>
<th>P for trend †</th>
<th>P for trend †</th>
<th>P for difference between sexes ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ApoB in VLDL with apoC-III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range-(g/L)</td>
<td>Q1&lt;0.010, Q5&gt;0.028</td>
<td>Q1&lt;0.004, Q5&gt;0.014</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median-(g/L)</td>
<td>Q1: 0.006, Q5: 0.036</td>
<td>Q1: 0.002, Q5: 0.018</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>2.07 (1.45-2.95)</td>
<td>&lt;0.001</td>
<td>1.85 (1.17-2.94)</td>
<td>0.002</td>
<td>2.43 (1.39-4.26)</td>
<td>0.001</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.96 (1.34-2.88)</td>
<td>&lt;0.001</td>
<td>1.97 (1.12-3.47)</td>
<td>0.005</td>
<td>2.22 (1.05-4.68)</td>
<td>0.08</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.73 (1.17-2.57)</td>
<td>0.002</td>
<td>1.79 (1.00-3.20)</td>
<td>0.011</td>
<td>1.85 (0.83-4.11)</td>
<td>0.27</td>
</tr>
<tr>
<td><strong>ApoB in VLDL without apoC-III</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range-(g/L)</td>
<td>Q1&lt;0.011, Q5&gt;0.047</td>
<td>Q1&lt;0.011, Q5&gt;0.046</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>Q1: 0.006, Q5: 0.062</td>
<td>Q1: 0.007, Q5: 0.064</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>2.55 (1.72-3.79)</td>
<td>&lt;0.001</td>
<td>3.06 (1.78-5.26)</td>
<td>&lt;0.001</td>
<td>2.08 (1.15-3.73)</td>
<td>0.009</td>
</tr>
<tr>
<td>Model 2</td>
<td>2.26 (1.47-3.46)</td>
<td>&lt;0.001</td>
<td>2.9 (1.49-5.65)</td>
<td>0.001</td>
<td>1.78 (0.81-3.87)</td>
<td>0.17</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.98 (1.27-3.08)</td>
<td>0.006</td>
<td>2.56 (1.28-5.12)</td>
<td>0.006</td>
<td>1.55 (0.69-3.49)</td>
<td>0.26</td>
</tr>
<tr>
<td><strong>ApoC-III in VLDL</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range-(g/L)</td>
<td>Q1&lt;0.011, Q5&gt;0.035</td>
<td>Q1&lt;0.006, Q5&gt;0.025</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>Q1: 0.008, Q5: 0.043</td>
<td>Q1: 0.004, Q5: 0.035</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.44 (1.01-2.05)</td>
<td>0.015</td>
<td>1.25 (0.78-2.01)</td>
<td>0.25</td>
<td>1.79 (1.04-3.11)</td>
<td>0.012</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.47 (1.00-2.16)</td>
<td>0.03</td>
<td>1.64 (0.93-2.90)</td>
<td>0.08</td>
<td>1.26 (0.61-2.60)</td>
<td>0.32</td>
</tr>
<tr>
<td>Model 3</td>
<td>1.32 (0.88-1.96)</td>
<td>0.11</td>
<td>1.49 (0.83-2.68)</td>
<td>0.15</td>
<td>1.15 (0.54-2.46)</td>
<td>0.62</td>
</tr>
</tbody>
</table>
Supplemental Table 3. Association between baseline characteristics (complete study sample) and plasma levels of apoB in LDL. **A.** Baseline covariates across quintiles of apoB in LDL with apoC-III. **B.** Baseline covariates across quintiles of apoB in LDL without apoC-III. Data are means unless specified otherwise. * Quintiles were calculated using sex-specific apoB levels among controls, the reported medians correspond to a weighted average of values in men and women.

### A. Baseline covariates across quintiles of apoB in LDL with apoC-III

<table>
<thead>
<tr>
<th>Quintile of apoB in LDL with apoC-III (g/L) *</th>
<th>Q1 n=240</th>
<th>Q2 n=270</th>
<th>Q3 n=297</th>
<th>Q4 n=318</th>
<th>Q5 n=351</th>
<th>p-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median apoB in LDL with apoC-III (g/L) *</td>
<td>0.038</td>
<td>0.059</td>
<td>0.082</td>
<td>0.11</td>
<td>0.17</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.0</td>
<td>62.1</td>
<td>63.2</td>
<td>63.4</td>
<td>61.5</td>
<td>0.58</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>25.0</td>
<td>25.5</td>
<td>26.1</td>
<td>26.0</td>
<td>26.7</td>
<td>0.007</td>
</tr>
<tr>
<td>Alcohol intake (g/day)</td>
<td>10.2</td>
<td>7.7</td>
<td>7.4</td>
<td>8.3</td>
<td>10.1</td>
<td>0.96</td>
</tr>
<tr>
<td>Physical activity (met/hr/week)</td>
<td>28.1</td>
<td>30.2</td>
<td>27.9</td>
<td>30.1</td>
<td>29.1</td>
<td>0.68</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>0.97</td>
<td>1.12</td>
<td>1.26</td>
<td>1.44</td>
<td>2.02</td>
<td>0.017</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.24</td>
<td>5.45</td>
<td>5.54</td>
<td>5.95</td>
<td>6.35</td>
<td>0.006</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.11</td>
<td>3.26</td>
<td>3.40</td>
<td>3.74</td>
<td>3.92</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.41</td>
<td>1.35</td>
<td>1.27</td>
<td>1.25</td>
<td>1.18</td>
<td>0.001</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>0.70</td>
<td>0.81</td>
<td>0.87</td>
<td>0.99</td>
<td>1.12</td>
<td>0.001</td>
</tr>
<tr>
<td>CRP (median) (mg/L)</td>
<td>0.36</td>
<td>0.36</td>
<td>0.39</td>
<td>0.38</td>
<td>0.39</td>
<td>0.13</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>59.6</td>
<td>57.8</td>
<td>51.5</td>
<td>57.6</td>
<td>57.3</td>
<td>0.68</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>18.8</td>
<td>12.6</td>
<td>14.1</td>
<td>16.4</td>
<td>17.4</td>
<td>0.92</td>
</tr>
<tr>
<td>Fasting (%)</td>
<td>62.9</td>
<td>70.7</td>
<td>71.4</td>
<td>70.1</td>
<td>72.4</td>
<td>0.13</td>
</tr>
<tr>
<td>Postmenopausal women (%)</td>
<td>85.1</td>
<td>86.2</td>
<td>90.9</td>
<td>93.1</td>
<td>90.7</td>
<td>0.07</td>
</tr>
<tr>
<td>Parental history of CHD (%)</td>
<td>16.7</td>
<td>14.8</td>
<td>13.1</td>
<td>15.7</td>
<td>16.2</td>
<td>0.99</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>2.5</td>
<td>8.5</td>
<td>8.1</td>
<td>6.3</td>
<td>15.1</td>
<td>0.11</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>28.8</td>
<td>37.0</td>
<td>36.0</td>
<td>39.3</td>
<td>41.0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

### B. Baseline covariates across quintiles of apoB in LDL without apoC-III

<table>
<thead>
<tr>
<th>Quintile of apoB in LDL without apoC-III (g/L) *</th>
<th>Q1 n=265</th>
<th>Q2 n=295</th>
<th>Q3 n=317</th>
<th>Q4 n=288</th>
<th>Q5 n=311</th>
<th>p-trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median apoB in LDL without apoC-III (g/L) *</td>
<td>0.47</td>
<td>0.62</td>
<td>0.73</td>
<td>0.85</td>
<td>1.05</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>63.4</td>
<td>62.5</td>
<td>62.4</td>
<td>62.7</td>
<td>62.3</td>
<td>0.15</td>
</tr>
<tr>
<td>BMI (Kg/m2)</td>
<td>26.0</td>
<td>25.6</td>
<td>25.9</td>
<td>26.2</td>
<td>26.0</td>
<td>0.41</td>
</tr>
<tr>
<td>Alcohol intake (g/day)</td>
<td>8.1</td>
<td>9.2</td>
<td>7.8</td>
<td>9.4</td>
<td>9.3</td>
<td>0.37</td>
</tr>
<tr>
<td>Physical activity (met/hr/week)</td>
<td>27.7</td>
<td>31.5</td>
<td>24.2</td>
<td>32.0</td>
<td>30.3</td>
<td>0.64</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.42</td>
<td>1.36</td>
<td>1.37</td>
<td>1.41</td>
<td>1.47</td>
<td>0.29</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.08</td>
<td>5.51</td>
<td>5.70</td>
<td>5.99</td>
<td>6.40</td>
<td>0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>2.94</td>
<td>3.28</td>
<td>3.47</td>
<td>3.69</td>
<td>4.16</td>
<td>0.002</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.28</td>
<td>1.32</td>
<td>1.31</td>
<td>1.26</td>
<td>1.23</td>
<td>0.19</td>
</tr>
<tr>
<td>Apolipoprotein B (g/L)</td>
<td>0.58</td>
<td>0.76</td>
<td>0.88</td>
<td>1.02</td>
<td>1.30</td>
<td>0.002</td>
</tr>
<tr>
<td>CRP (median) (mg/L)</td>
<td>0.36</td>
<td>0.28</td>
<td>0.44</td>
<td>0.42</td>
<td>0.38</td>
<td>0.45</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>55.9</td>
<td>58.0</td>
<td>50.5</td>
<td>59.4</td>
<td>59.8</td>
<td>0.52</td>
</tr>
<tr>
<td>Current smokers (%)</td>
<td>15.1</td>
<td>16.3</td>
<td>17.7</td>
<td>13.2</td>
<td>16.7</td>
<td>0.98</td>
</tr>
<tr>
<td>Fasting (%)</td>
<td>61.9</td>
<td>69.2</td>
<td>71.3</td>
<td>74.3</td>
<td>71.7</td>
<td>0.08</td>
</tr>
<tr>
<td>Postmenopausal women (%)</td>
<td>92.2</td>
<td>81.6</td>
<td>87.5</td>
<td>94.6</td>
<td>92.7</td>
<td>0.47</td>
</tr>
<tr>
<td>Parental history of CHD (%)</td>
<td>10.9</td>
<td>15.9</td>
<td>16.4</td>
<td>16.7</td>
<td>16.1</td>
<td>0.17</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>9.8</td>
<td>9.2</td>
<td>9.8</td>
<td>8.0</td>
<td>6.1</td>
<td>0.06</td>
</tr>
<tr>
<td>Hypertension (%)</td>
<td>37.4</td>
<td>39.3</td>
<td>42.0</td>
<td>34.4</td>
<td>31.5</td>
<td>0.24</td>
</tr>
</tbody>
</table>
Supplemental Table 4. Association between traditional lipid risk factors and coronary heart disease in the complete study sample. Model was conditioned on matching factors, and additionally adjusted for the presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake and personal history of hypertension. * P values for trend are based on the median levels of each variable in quintiles of the controls.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Relative risk for quintile 5 compared to quintile 1</th>
<th>P for trend*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol</td>
<td>Relative risk 1.80 (1.22-2.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol</td>
<td>Relative risk 1.79 (1.23-2.64)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma triglycerides</td>
<td>Relative risk 2.17 (1.51-3.11)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasma apolipoprotein B</td>
<td>Relative risk 3.11 (1.95-4.97)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Non HDL cholesterol</td>
<td>Relative risk 2.53 (1.72-3.72)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HDL cholesterol</td>
<td>Relative risk 0.49 (0.29-0.82)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Supplemental Figure 1.

Risk of coronary heart disease in quintile 5 relative to quintile 1

- Plasma triglycerides
- ApoB in LDL without apoC-III
- LDL cholesterol

- ApoB in LDL without apoC-III
- Plasma apoB

- ApoB in LDL without apoC-III
- Plasma triglycerides

- ApoB in LDL without apoC-III
- Total to HDL cholesterol ratio

- ApoB in LDL without apoC-III
- HDL cholesterol

- ApoB in LDL without apoC-III
- Non-HDL cholesterol
Supplemental Figure 1. Relative risk of coronary heart disease during follow up in the complete study sample, according to levels of apoB in LDL without apoC-III, in models including other major lipid risk factors. Each panel represents a separate model in which apoB and another lipid risk factor were mutually adjusted. Solid bars represent relative risks for quintile 5 compared to quintile 1 of each risk factor, and error bars represent 95% confidence intervals. All models were conditioned on matching factors, and additionally adjusted for the presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake and personal history of hypertension. * To be interpreted with caution, because the linear correlation coefficient between the two variables was 0.92.
Supplemental Figure 2. Relative risk of CHD during follow-up in the complete study sample, mutually adjusting levels of cholesterol in LDL with and without apoC-III. Relative risks and 95% confidence intervals are given for quintile 5 compared to quintile 1. The model was conditioned on matching factors, and additionally adjusted for the presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake and personal history of hypertension.