



DIGITAL ACCESS TO  
SCHOLARSHIP AT HARVARD  
DASH.HARVARD.EDU



HARVARD LIBRARY  
Office for Scholarly Communication

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

The Harvard community has made this article openly available. [Please share](#) how this access benefits you. Your story matters

Citation	Mendivil, C. O., E. B. Rimm, J. Furtado, S. E. Chiuve, and F. M. Sacks. 2011. "Low-Density Lipoproteins Containing Apolipoprotein C-III and the Risk of Coronary Heart Disease." <i>Circulation</i> 124 (19) (October 10): 2065–2072. doi:10.1161/circulationaha.111.056986.
Published Version	doi:10.1371/journal.pone.0114859
Citable link	<a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:30147221">http://nrs.harvard.edu/urn-3:HUL.InstRepos:30147221</a>
Terms of Use	This article was downloaded from Harvard University's DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at <a href="http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA">http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA</a>



Published in final edited form as:

*Circulation*. 2011 November 8; 124(19): 2065–2072. doi:10.1161/CIRCULATIONAHA.111.056986.

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

Carlos O Mendivil, MD, DSc<sup>1,2</sup>, Eric B. Rimm, DSc<sup>1,3,4</sup>, Jeremy Furtado, DSc<sup>1</sup>, Stephanie Chiuve, DSc<sup>1,4</sup>, and Frank M Sacks, MD<sup>1</sup>

<sup>1</sup>Harvard School of Public Health, Boston, MA

<sup>2</sup>Universidad de los Andes Medical School, Bogotá, Colombia

<sup>3</sup>Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

<sup>4</sup>Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA

### Abstract

**Background**—LDL that contains apolipoprotein C-III (apoC-III) comprises 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 (CHD) in 320 women and 419 men initially free of cardiovascular disease who developed a fatal or non-fatal myocardial infarction during 10 to 14 years of follow-up, and matched controls who remained free of CHD.

Concentrations of LDL with apoC-III (measured as apoB in this fraction) were associated with risk of CHD in multivariable analysis that included the total cholesterol to HDL cholesterol ratio, LDL cholesterol, apolipoprotein B, triglycerides, or HDL 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 percent confidence interval, 1.54 to 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 percent confidence interval, 0.76 to 2.05; P for trend=0.97), P for interaction <0.001. This divergence in association with CHD persisted even after adjustment for plasma triglycerides.

**Conclusions**—The risk of CHD contributed by LDL appeared to result to a large extent from LDL that contains apoC-III.

### Keywords

apolipoproteins; risk factors; metabolism; cholesterol; myocardial infarction

---

**Address correspondence to:** Dr. Frank M. Sacks, Harvard School of Public Health, Department of Nutrition, 665 Huntington Avenue, Building 1, Room 202, Boston, MA, USA, 02115, Telephone number: (617) 4321420, fsacks@hsph.harvard.edu.

**Publisher's Disclaimer:** This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

VLDL and LDL each are heterogeneous lipoprotein classes that vary in size and content of lipid and protein (1). ApoB is the required structural apolipoprotein of VLDL and LDL. Each VLDL and LDL has only one molecule of apoB but may have none or many molecules of apoC-III attached to its surface (1–3). About 40 to 60% of VLDL and 10–20% of LDL have apoC-III (2,3). ApoC-III has deleterious effects on the metabolism of VLDL and LDL (4–6) and on functions of cells that participate in atherosclerosis (7). ApoC-III interferes with the binding of VLDL to receptors on the liver, and this inhibits the removal of VLDL from plasma (5,6,8). Nearly all VLDL that has apoC-III undergoes intravascular lipolysis of its triglyceride content producing LDL with apoC-III, and large-size LDL with apoC-III is metabolized to small LDL (5,6,9).

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 (10,11). ApoC-III also activates vascular endothelial cells to produce adhesion molecules and it induces insulin resistance in these cells reducing their secretion of nitric oxide (12). These actions are not shared by VLDL or LDL that do 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 (13). Thus, apoC-III may trap its associated lipoproteins in the arterial wall and bring in blood monocytes, crucial steps in 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 (14–17), and we had reported that LDL with apoC-III associates with recurrent cardiovascular disease among patients with a prior myocardial infarction and type 2 diabetes (3).

To evaluate whether plasma concentrations of VLDL and LDL that have apoC-III, as measured by apoB in each of them, are more strongly associated with coronary heart disease (CHD) risk than those of the same lipoproteins without apoC-III, we prospectively studied two U.S. populations initially free of coronary heart disease (CHD): the Nurses' Health Study [NHS] in women and the Health Professionals Follow-up Study [HPFS] in men.

## Methods

### Study population

The NHS and the HPFS are prospective cohort investigations respectively involving 121,700 female U.S. registered nurses who were 30 to 55 years old at baseline in 1976 and 51,529 U.S. male health professionals who were 40 to 75 years old at baseline in 1986. 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, and 32,826 women provided one. 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 blood drawing 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 blood drawing and the return of the 2004 questionnaire. Using risk-set sampling (a technique in which in a prospective cohort, controls are selected among the group of individuals exposed to the studied factor at the time the event occurs in the case [22]), we randomly selected controls in a 1:1 ratio who were matched for age ( $\pm 1$  year), smoking status (never, past or current), and date of blood sampling ( $\pm 2$  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 coronary heart disease

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 (MET)-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, and 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 ( $-130^{\circ}\text{C}$  or colder). One vial containing 0.6 mL (Nurses' Health Study) or 0.5 mL (Health Professionals Follow-up Study) of 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 anti-human apoC-III antibodies (Academy Biomedical, Houston, TX) bound to Sepharose 4B resin, as previously described and validated (2,23). 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 3M sodium thiocyanate. The efficiency of the apoCIII immunoaffinity separation (percentage of ligand removed from plasma by the resin) was 99%. Subsequently, apoCIII-bound and -unbound fractions were ultracentrifuged to isolate the very low-density ( $d < 1.006$  g/mL), low-density ( $1.006 < d < 1.063$  g/mL) and high-density ( $d > 1.063$  g/mL) lipoproteins (24). Apolipoprotein B (apoB) and apoC-III were measured in whole plasma and in VLDL and LDL with and without apoC-III by enzyme-linked immunosorbent assay, and cholesterol and triglycerides were measured by enzymatic methods (Infinity Kit, Thermo Fischer Scientific, Waltham, Massachusetts).

In order 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 IDs. These controls were included and ran in every batch of real samples so that each control went through the whole process at least 8 times. The coefficients of variation were for NHS: 9% for apoB in LDL without apoC-III and 17% for apoB in LDL with apoC-III; and for HPFS: 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 excluding participants with insufficient sample or missing data for apoB in at least one of the LDL and VLDL fractions, the dataset consisted of 1476 participants (320 female and 419 male cases and 320 female and 419 male controls). The cases were identified and the controls selected in two-year cycles. Two men selected as

controls in one cycle later developed CHD and were selected in a subsequent cycle, this time as cases. For this reason the study sample contains 1476 and not 1478 individuals. For these two participants we used the same baseline measurements in the pair where they were cases and in the pair where they were controls.

Because there is only one 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 (3) was the concentration of LDL with apoC-III, as reflected by the concentration of apoB in this fraction, i.e. “apoB in LDL with apoC-III”.

### Statistical analysis

Data from the two 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, on the basis of 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 (25). We analyzed our exposures in four logistic models: model one conditioned on matching factors only, model two with additional adjustment for parental history of CHD before the age of 60 years, alcohol intake and personal history of hypertension at baseline; model three with adjustment for all the variables in model two plus body mass index and personal history of diabetes; and model four adding 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 two-tailed, and P values below 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). Participants in the highest quintile of LDL with apoC-III, as compared with participants in the lowest quintile, had a significantly increased risk of CHD (relative risk for highest versus lowest quintile 2.58, 95 percent confidence interval, 1.78 to 3.74, P for trend<0.001), conditioning on 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 percent confidence interval, 1.14 to 2.61). Adjustment for other risk factors in models 2, 3, and 4, including personal history of diabetes 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 (Supplemental Table 1 and Table 2). It was not possible to perform multivariate logistic analyses in the diabetic subgroup (n=126) due to 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 vs bottom quintile of LDL with apoC-III 2.39, 95% CI: 1.56–3.67).

### **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, the total cholesterol-to-HDL cholesterol ratio or non-HDL cholesterol, one-by-one, and 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, the total cholesterol to HDL cholesterol ratio or non-HDL cholesterol (Supplemental Figure 1).

### **No effect modification by hypertriglyceridemia**

To address the strength of the relationship between LDL with apoCIII and CHD at different TG levels, first we 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 apo-CIII in the participants who had normal or high TG, according to the standard cutpoint, 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 $\geq$ 150 mg/dL,  $p$  for interaction=0.63. Thus the finding on LDL CIII 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 2, panel A), only LDL with apoC-III was associated with CHD (relative risk 2.38, 95 percent confidence interval, 1.54 to 3.68; compared with relative risk 1.25, 95 percent confidence interval 0.76 to 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 2, panel B). The association with CHD was still significantly higher for LDL with apoC-III ( $P$  for difference in slopes=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 percent confidence interval, 1.35 to 2.99), but not cholesterol in LDL without apoC-III (relative risk for highest versus lowest quintile 1.30, 95 percent confidence interval, 0.86 to 1.95) was significantly associated with CHD (Supplemental Figure 2).

### **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 in model 3 attenuated the relative risks for both VLDL types but both were still significant (Supplemental Table 2).

### Relative size of VLDL and LDL with and without apoC-III

The mean triglyceride:apoB molar ratio, an indicator of VLDL particle size, was higher for VLDL with apoC-III than for VLDL without apoC-III (11724 vs 7988) in the study sample. The mean cholesterol:apoB molar ratio, an indicator of LDL particle size, was higher for LDL with apoC-III than for LDL without apoC-III (2934 vs 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, higher plasma triglycerides, lower HDL cholesterol levels, slightly higher body-mass indexes, and a higher prevalence of hypertension (Supplemental Table 3). Contrastingly, higher levels of LDL without apoC-III were not associated with any of these conditions, but with higher LDL cholesterol and plasma apoB.

### 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 percent confidence interval 0.87 to 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 non-users (0.85 mg/dL for users versus 0.81 mg/dL for non-users,  $p=0.53$ ). The same was observed for LDL without apoC-III (77.6 mg/dL for users versus 77.5 mg/dL for non-users,  $p=0.96$ ).

## 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, 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 sub-fractions that contains apoC-III. This result extends our previous finding in patients with pre-existing CHD and type 2 diabetes (3). 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 still 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 (26), and through induction of insulin resistance and inflammatory signaling pathways governed by nuclear factor kappa-B in endothelial cells (12,27). 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 (28). All this evidence provides biological plausibility for a direct involvement of LDL with apoC-III in atherosclerosis.

There are on average 10–20 molecules of apoC-III, but only one molecule of apoB in each LDL with apoC-III (2). 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, where 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 (13). It is conceivable that this site may only be exposed in LDL, 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, while 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 (29). 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 from the CARE trial (30): statin treatment reduced LDL with apoC-III by 29% and LDL without apoC-III by 30%. Also, all traditional lipid risk factors were associated with CHD in our study sample (Supplementary Table 4), 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 cholesterol:apoB molar ratio 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 like a proxy for small, dense LDL. ApoC-III has been reported to transfer from VLDL to HDL during freezing or storage (31). 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 degrees Celsius. The distribution of apoC-III changed only very slightly and nonsignificantly (from 14% in VLDL, 16% in LDL, 70% in HDL when analyzed fresh; to 12% in VLDL, 15% in LDL, 73% in HDL after freezing/storage/thawing), so we believe this effect is unlikely to have had an important influence in 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. Even though 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. Another relevant limitation is that we performed multiple statistical tests needed 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 of 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 (13), that may influence the effect of LDL with apoC-III on atherosclerosis. Nevertheless, when our results are put together with the evidence of reduced atherosclerosis in humans with genetically reduced apoC-III (28) and with the abundant documentation of direct proatherogenic effects of apoC-III (11–13, 27); these results encourage further research into apoC-III as a potentially causative factor in atherosclerosis and CVD, 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.

In summary, 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 (32–34), we think that our results may endorse LDL with apoC-III as a highly attractive target for the prevention or treatment of atherosclerotic diseases.

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 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 LDL that contains or does not contain apoC-III and the appearance of coronary heart disease (CHD) events in more than 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 it, 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 to 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 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.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

### Funding information

This work was supported by NIH/NHLBI grant HL070159.

#### Disclosures

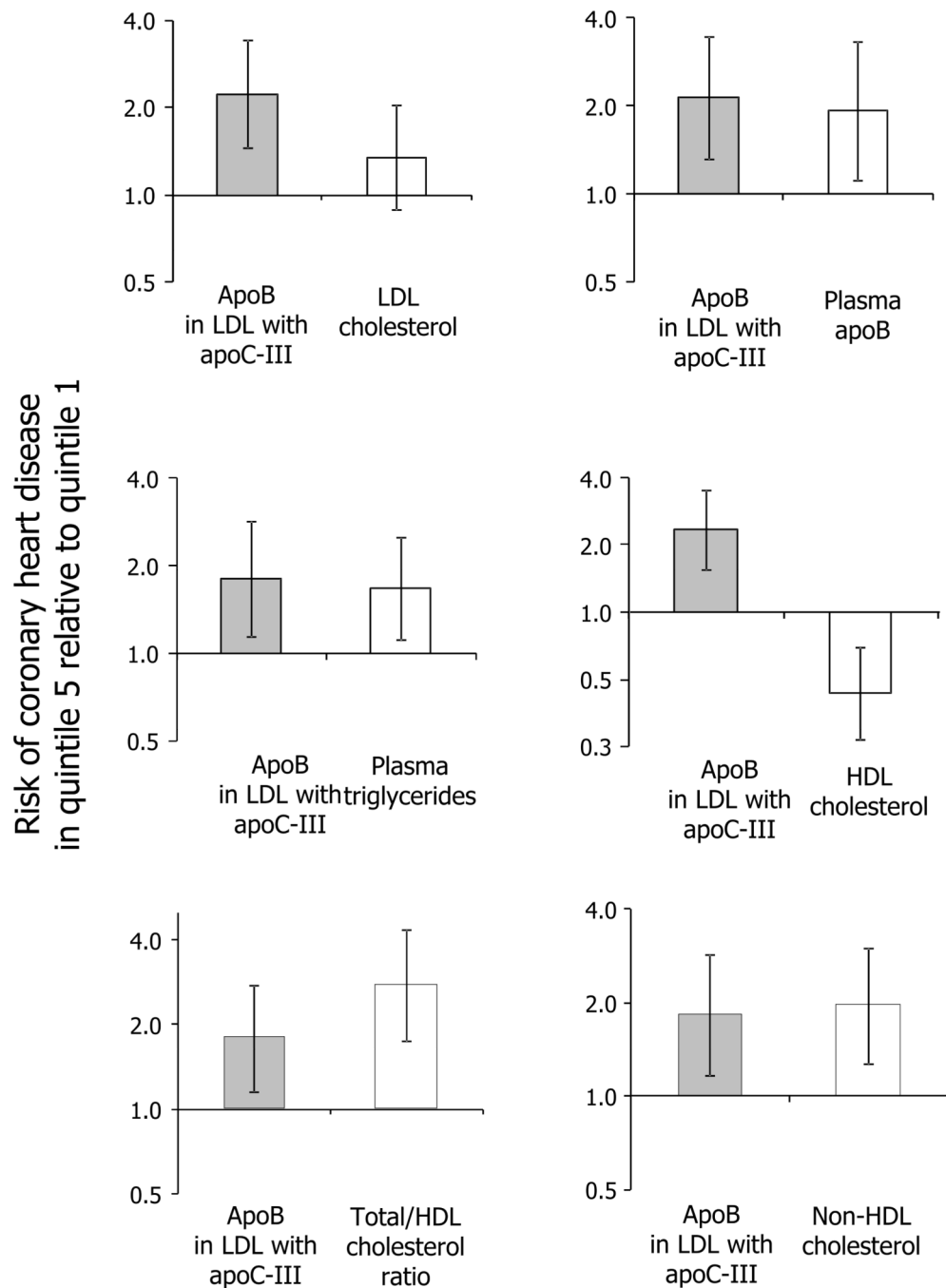
Dr. Sacks has received research funding from ISIS pharmaceuticals to investigate the effect of a biotechnology product on apoC-III levels.

## References

1. Alaupovic P. Significance of apolipoproteins for structure, function, and classification of plasma lipoproteins. *Methods Enzymol.* 1996; 263:32–60. [PubMed: 8748999]
2. Campos H, Perlov D, Khoo C, Sacks FM. Distinct patterns of lipoproteins with apoB defined by presence of apoE or apoC-III in hypercholesterolemia and hypertriglyceridemia. *J Lipid Res.* 2001; 42:1239–1249. [PubMed: 11483625]
3. Lee SJ, Campos H, Moye LA, Sacks FM. LDL containing apolipoprotein CIII is an independent risk factor for coronary events in diabetic patients. *Arterioscler Thromb Vasc Biol.* 2003; 23:853–858. [PubMed: 12637336]
4. Ooi EM, Barrett HM, Chan D, Watts GF. Apolipoprotein C-III: understanding an emerging cardiovascular risk factor. *Clin Science.* 2008; 114:611–624.
5. Zheng C, Khoo C, Ikewaki K, Sacks FM. Rapid turnover of apolipoprotein C-III-containing triglyceride-rich lipoproteins contributing to the formation of LDL subfractions. *J Lipid Res.* 2007; 48:1190–1203. [PubMed: 17314277]
6. Mendivil CO, Zheng C, Furtado J, Lel J, Sacks FM. Metabolism of Very-Low-Density lipoprotein and Low-Density Lipoprotein containing apolipoprotein C-III and not other small apolipoproteins. *Arterioscler Thromb Vasc Biol.* 2010; 30:239–245. [PubMed: 19910636]
7. Kawakami A, Yoshida M. Apolipoprotein CIII links dyslipidemia with atherosclerosis. *J Atheroscler Thromb.* 2009; 16:6–11. [PubMed: 19262004]
8. Sehayek E, Eisenberg S. Mechanisms of inhibition by apolipoprotein C of apolipoprotein E dependent cellular metabolism of human triglyceride rich lipoproteins through the low density lipoprotein receptor pathway. *J Biol Chem.* 1991; 266:18259–18267. [PubMed: 1917954]
9. Zheng C, Khoo C, Furtado J, Sacks FM. Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense low-density lipoprotein phenotype. *Circulation.* 2010; 121:1722–1734. [PubMed: 20368524]
10. Kawakami A, Aikawa M, Alcaide P, Luscinskas FW, Libby P, Sacks FM. Apolipoprotein CIII induces expression of vascular cell adhesion molecule-1 in vascular endothelial cells and increases adhesion of monocytic cells. *Circulation.* 2006; 114:681–687. [PubMed: 16894036]
11. Kawakami A, Aikawa M, Libby P, Alcaide P, Luscinskas FW, Sacks FM. Apolipoprotein CIII in apolipoprotein B lipoproteins enhances the adhesion of human monocytic cells to endothelial cells. *Circulation.* 2006; 113:691–700. [PubMed: 16461842]
12. Kawakami A, Osaka M, Tani M, Azuma H, Sacks FM, Shimokado K, Yoshida M. Apolipoprotein CIII links hyperlipidemia with vascular endothelial function. *Circulation.* 2008; 118:731–742. [PubMed: 18663085]
13. Hiukka A, Ståhlman M, Pettersson C, Levin M, Adiels M, Teneberg S, Leinonen ES, Hultén LM, Wiklund O, Oresic M, Olofsson SO, Taskinen MR, Ekroos K, Borén J. ApoCIII-Enriched LDL in type 2 diabetes displays altered lipid composition, increased susceptibility for sphingomyelinase, and increased binding to biglycan. *Diabetes.* 2009; 58:2018–2026. [PubMed: 19502413]
14. Blankenhorn DH, Alaupovic P, Wickham E, Chin HP, Azen SP. Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts. Lipid and nonlipid factors. *Circulation.* 1990; 81:470–476. [PubMed: 2404631]
15. Hodis HN, Mack WJ, Azen SP, Alaupovic P, Pogoda JM, LaBree L, Hemphill LC, Krams DM, Blankenhorn DH. Triglyceride- and cholesterol-rich lipoproteins have a differential effect on mild/moderate and severe lesion progression as assessed by quantitative coronary angiography in a controlled trial of lovastatin. *Circulation.* 1994; 90:42–49. [PubMed: 8026027]
16. Luc G, Fievet C, Arveiler D, Evans AE, Bard JM, Cambien F, Fruchart JC, Ducimetiere P. Apolipoproteins C-III and E in apoB- and non-apoB containing lipoproteins in two populations at

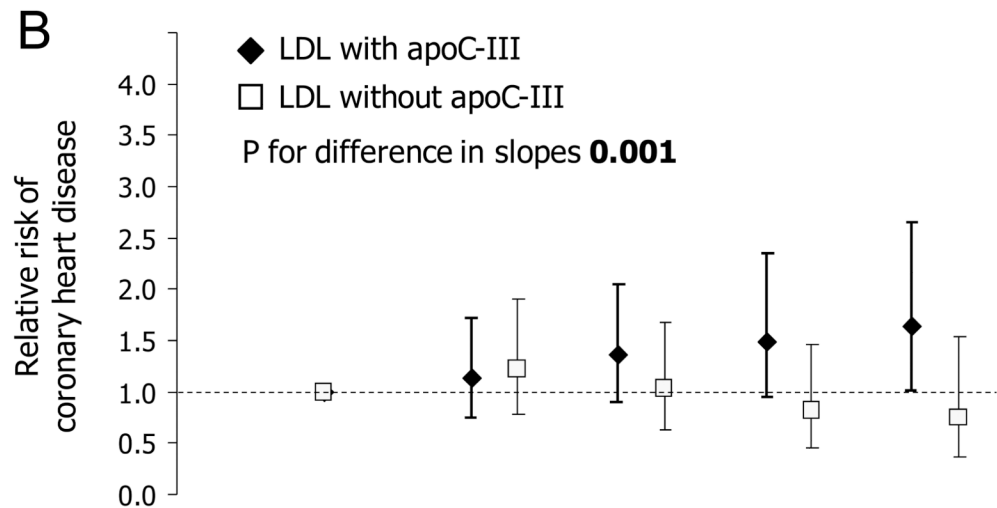
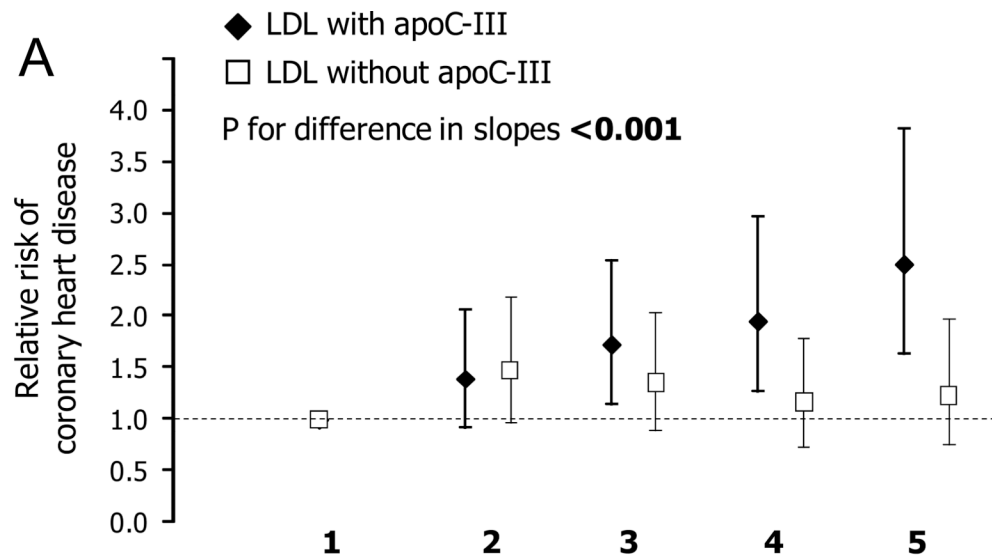
- contrasting risk for myocardial infarction: the ECTIM study. Etude Cas Temoins sur 'Infarctus du Myocarde. *J Lipid Res.* 1996; 37:508–517. [PubMed: 8728314]
17. Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, Pfeffer MA, Braunwald E. VLDL, apolipoproteins B, CIII and E and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. *Circulation.* 2000; 102:1886–1892. [PubMed: 11034934]
  18. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. *Am J Epidemiol.* 1992; 135:1114–1126. [PubMed: 1632423]
  19. Colditz GA, Manson JE, Hankinson SE. The Nurses' Health Study: 20-year contribution to the understanding of health among women. *J Womens Health.* 1997; 6:49–62. [PubMed: 9065374]
  20. Giovannucci E, Colditz G, Stampfer MJ, Rimm EB, Litin L, Sampson L, Willett WC. The assessment of alcohol consumption by a simple self-administered questionnaire. *Am J Epidemiol.* 1991; 133:810–817. [PubMed: 2021148]
  21. Chasan-Taber S, Rimm EB, Stampfer MJ, Spiegelman D, Colditz GA, Giovannucci E, Ascherio A, Willett WC. Reproducibility and validity of a self-administered physical activity questionnaire for male health professionals. *Epidemiology.* 1996; 7:81–86. [PubMed: 8664406]
  22. Prentice RL, Breslow NE. Retrospective studies and failure time models. *Biometrika.* 1978; 65:153–158.
  23. Khoo C, Judge H, Sacks FM. Effects of estrogenic oral contraceptives on the lipoprotein B particle system defined by apolipoproteins E and C-III content. *J Lipid Res.* 1999; 40:202–212. [PubMed: 9925648]
  24. Pietzsch J, Subat S, Nitzsche S, Leonhardt W, Schentke KU, Hanefeld M. Very fast ultracentrifugation of serum lipoproteins: influence on lipoprotein separation and composition. *Biochim Biophys Acta.* 1995; 1254:77–88. [PubMed: 7811751]
  25. Prentice RL, Breslow NE. Retrospective studies and failure time models. *Biometrika.* 1978; 65:153–158.
  26. Kawakami A, Osaka M, Aikawa M, Uematsu S, Akira S, Libby P, Shimokado K, Sacks FM, Yoshida M. Toll-like receptor 2 mediates apolipoprotein CIII-induced monocyte activation. *Circ Res.* 2008; 103:1402–1409. [PubMed: 18974386]
  27. Kawakami A, Aikawa M, Nitta N, Yoshida M, Libby P, Sacks FM. Apolipoprotein CIII-induced THP-1 cell adhesion to endothelial cells involves pertussis toxin-sensitive G protein- and protein kinase C alpha-mediated nuclear factor-kappaB activation. *Arterioscler Thromb Vasc Biol.* 2007; 27:219–225. [PubMed: 17038637]
  28. Pollin TI, Damcott CM, Shen H, Ott SH, Shelton J, Horenstein RB, Post W, McLenithan JC, Bielak LF, Peyser PA, Mitchell BD, Miller M, O'Connell JR, Shuldiner AR. A null mutation in human APOC3 confers a favorable plasma lipid profile and apparent cardioprotection. *Science.* 2008; 322:1702–1705. [PubMed: 19074352]
  29. Alaupovic P, Mack WJ, Knight-Gibson C, Hodis HN. The role of triglyceride-rich lipoprotein families in the progression of atherosclerotic lesions as determined by sequential coronary angiography from a controlled clinical trial. *Arterioscler Thromb Vasc Biol.* 1997; 17:715–722. [PubMed: 9108785]
  30. Lee SJ, Sacks FM. Effect of pravastatin on intermediate-density and low-density lipoproteins containing apolipoprotein CIII in patients with diabetes mellitus. *Am J Cardiol.* 2003; 92:121–124. [PubMed: 12860210]
  31. Cohn JS, Rodriguez C, Jacques H, Tremblay M, Davignon J. Storage of human plasma samples leads to alterations in the lipoprotein distribution of apoC-III and apoE. *J Lipid Res.* 2004; 45:1572–1579. [PubMed: 15145987]
  32. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation.* 1998; 97:1837–1847. [PubMed: 9603539]
  33. Assmann G, Cullen P, Schulte H. Simple scoring scheme for calculating the risk of acute coronary events based on the 10-year follow-up of the prospective cardiovascular Münster (PROCAM) study. *Circulation.* 2002; 105:310–315. [PubMed: 11804985]

34. Ridker PM, Buring JE, Rifai N, Cook NR. Development and validation of improved algorithms for the assessment of global cardiovascular risk in women: the Reynolds Risk Score. *JAMA*. 2007; 297:611–619. [PubMed: 17299196]



**Figure 1.**

Relative risk of coronary heart disease during follow up in the complete study sample, according to levels of apoB in LDL with 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 adjusted for matching factors, presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake and personal history of hypertension.



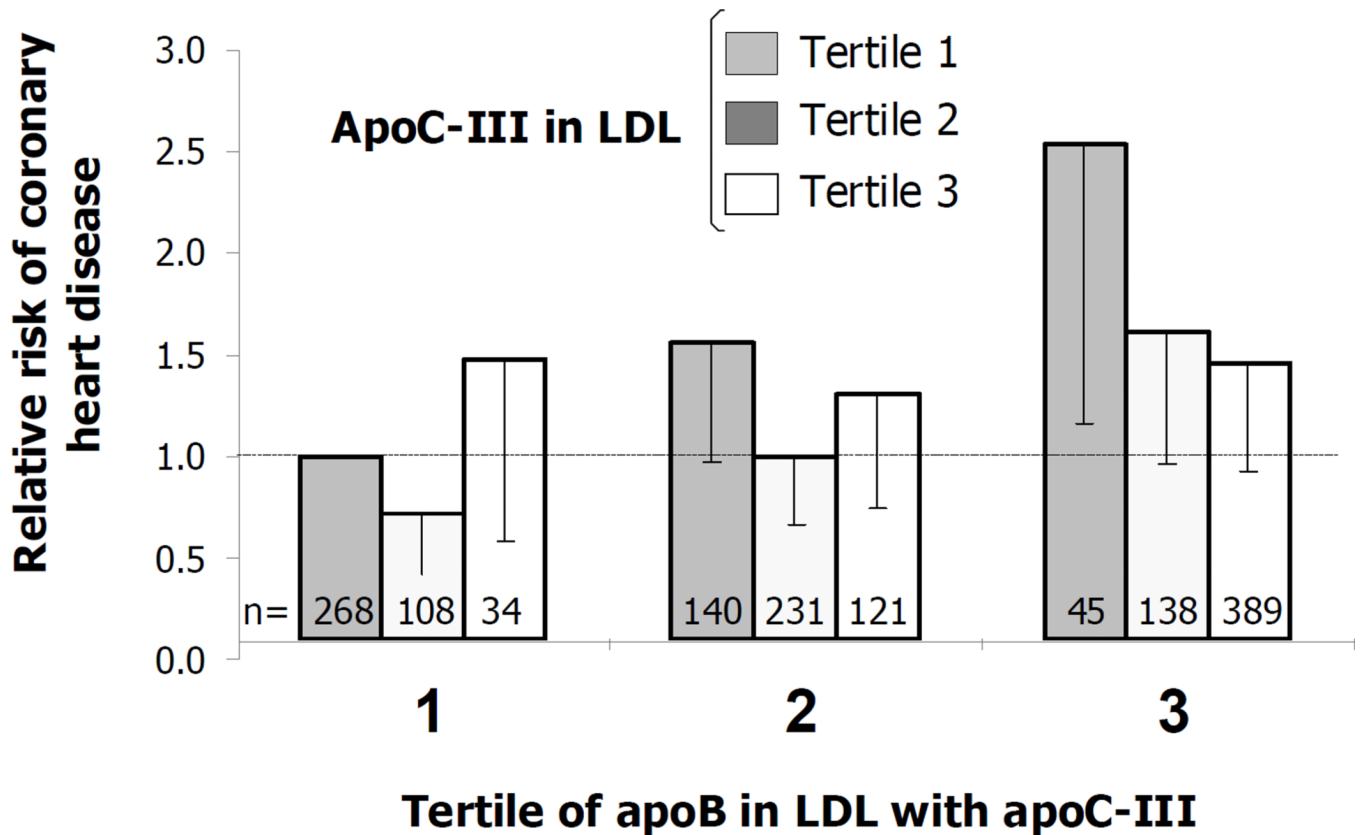
Quintile	1	2	3	4	5
<b>LDL with apoC-III n=</b>	240	270	297	318	351
<b>LDL without apoC-III n=</b>	265	295	317	288	311

**Figure 2.**

Relative risk of CHD during follow-up in the complete study sample, mutually adjusting apoB in LDL with and without apoC-III. Relative risks and 95% confidence intervals are given for each quintile compared to the lowest quintile. In panel A, the model was also adjusted for matching factors, presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake and personal history of hypertension. In panel B, the model was adjusted for all variables in panel A plus personal history of diabetes and plasma triglycerides.

Dark diamonds represent LDL with apoC-III, p for trend<0.001 in panel A, p for trend=0.07 in panel B. Light squares represent LDL without apoC-III, p for trend=0.97 in panel A, p for

trend=0.22 in panel B.  $P < 0.001$  for difference in slopes in panel A,  $p = 0.001$  for difference in slopes in panel B.



**Figure 3.**

Relative risk of coronary heart disease during follow-up, according to the tertile of apoB in LDL with apoC-III and the tertile of apoC-III in LDL at baseline. Subjects in tertile 1 of apoB in LDL with apoC-III and tertile 1 of apoC-III in LDL served as the reference group. The model was also adjusted for matching factors, presence or absence of a parental history of coronary heart disease before the age of 60 years, alcohol intake, personal history of hypertension, personal history of diabetes and plasma triglycerides.



Table 1

Baseline characteristics of the study sample.

Characteristic	Women*		Men*		P Value †
	Cases (n=320)	Controls (n=320)	Cases (n=419)	Controls (n=419)	
Age (yr)	60±6	60±6	64±8	64±8	-
Current smoker (%)	25	25	9	8	-
Body-mass index (Kg/m <sup>2</sup> )	26.8±5.6	25.4±4.2	26.1±3	25.5±3	0.017
Parental history of CHD before 60 years of age (%)	20	14	15	12	<0.001
Postmenopausal (%)	91	88	-	-	-
Postmenopausal hormone therapy among postmenopausal women (%)	33	35	-	-	-
Aspirin use ‡ (%)	26	30	40	33	<0.001
History of hypertension (%)	53	31	37	28	<0.001
History of diabetes (%)	17	6	9	3	<0.001
Alcohol consumption (g/day)					
Median	0.9	1.1	4.4	7.5	0.009
Q1-to-Q3	0-4.1	0-6.4	0-15.0	1.0-18.2	
Total cholesterol (mmol/L)	6.11±1.19	6.01±1.22	5.62±1.01	5.41±0.93	<0.001
LDL cholesterol (mmol/L)	3.81±1.04	3.65±1.01	3.47±0.85	3.26±0.83	<0.001
HDL cholesterol (mmol/L)	1.35±0.36	1.53±0.44	1.09±0.28	1.22±0.34	<0.001
Total-to-HDL cholesterol ratio	3.7±1.5	3.4±1.6	3.7±1.1	3.4±1.4	<0.001
Triglycerides (mmol/L)	1.59±1.11	1.23±0.65	1.63±0.92	1.34±0.76	<0.001
C-reactive protein (mg/liter)					
Median	0.52	0.38	0.34	0.26	0.83
Q1-to-Q3	0.20-1.45	0.14-1.02	0.13-1.07	0.08-0.87	
Total plasma apoB (g/L)	0.92±0.28	0.88±0.31	0.97±0.28	0.9±0.26	<0.001
ApoB in VLDL with apoC-III (g/L)	0.012±0.011	0.009±0.008	0.023±0.016	0.02±0.015	0.003
ApoB in VLDL without apoC-III (g/L)	0.036±0.028	0.03±0.024	0.038±0.027	0.031±0.024	<0.001
ApoB in LDL with apoC-III (g/L)	0.09±0.084	0.075±0.056	0.135±0.085	0.117±0.08	<0.001
ApoB in LDL without apoC-III (g/L)	0.78±0.24	0.77±0.28	0.77±0.23	0.73±0.22	<0.001

Characteristic	Women*			Men*		
Total plasma apoC-III (g/L)	0.18±0.11	0.16±0.1	0.30	0.17±0.09	0.16±0.09	0.16
ApoC-III in VLDL (g/L)	0.02±0.019	0.017±0.016	0.028	0.027±0.018	0.024±0.016	0.019
ApoC-III in LDL (g/L)	0.034±0.03	0.028±0.022	0.001	0.032±0.023	0.028±0.019	0.003
ApoC-III:ApoB molar ratio in VLDL	131±117	161±240	0.18	80±48	85±62	0.12
ApoC-III:ApoB molar ratio in LDL	27±20	28±23	0.35	16±7	16±7	0.35

\* Data on women are from the Nurses' Health Study, and 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. Plus-minus values are means ±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. CHD denotes CHD coronary heart disease, and MET-hr metabolic equivalent-hours. The body-mass index is the weight in kilograms divided by the square of the height in meters.

† P values for the difference between patients and controls (unadjusted) were determined by paired student's t-test for variables expressed as means ±SD, by the signed rank test for variables expressed as medians, and by the McNemar chi-square test for variables expressed as percentages.

‡ Current aspirin use was defined as every one to four days per week for women and as two or more times per week for men.

Table 2

Relative risks of coronary heart disease during follow-up in the complete study sample, according to the quintile of LDL types (as measured by the apoB concentration in each fraction) or apolipoprotein concentrations at baseline. Relative risks and 95% confidence intervals are given for each quintile compared to the lowest quintile of each apolipoprotein measurement. The group of women included 320 patients and 320 controls with fourteen years of follow-up. The group of men included 419 patients and 419 controls with ten years of follow-up. Quintiles and median values of apolipoprotein levels are based on values in controls. For each relative risk, quintile 1 served as the reference group. Matching factors were age, smoking status, and the month of blood sampling. Among women, data were also adjusted for fasting status at the time of blood sampling.

	Quintile categories					P for difference between sexes †	P for trend ‡
	1	2	3	4	5		
<b>LDL with apolipoprotein CIII</b>							
n=	240	270	297	318	351		
Model 1 *	1.00 (ref)	1.38 (0.96–1.98)	1.71 (1.2–2.44)	2.12 (1.46–3.08)	2.58 (1.78–3.74)	<0.001	0.74
Model 2	1.00 (ref)	1.32 (0.89–1.98)	1.65 (1.11–2.45)	1.99 (1.32–3.00)	2.41 (1.60–3.64)	<0.001	0.97
Model 3	1.00 (ref)	1.28 (0.86–1.91)	1.57 (1.06–2.32)	1.87 (1.24–2.81)	2.23 (1.48–3.37)	<0.001	0.72
Model 4	1.00 (ref)	1.16 (0.77–1.74)	1.35 (0.90–2.03)	1.50 (0.97–2.31)	1.64 (1.03–2.60)	<0.001	0.56
<b>LDL without apolipoprotein CIII</b>							
n=	265	295	317	288	311		
Model 1	1.00 (ref)	1.4 (0.98–2.00)	1.60 (1.11–2.32)	1.38 (0.94–2.03)	1.72 (1.14–2.61)	0.06	0.09
Model 2	1.00 (ref)	1.58 (1.05–2.38)	1.58 (1.04–2.40)	1.46 (0.95–2.25)	1.78 (1.11–2.84)	0.12	0.053
Model 3	1.00 (ref)	1.58 (1.06–2.36)	1.57 (1.04–2.38)	1.49 (0.97–2.28)	1.72 (1.08–2.75)	0.13	0.039
Model 4	1.00 (ref)	1.53 (1.02–2.31)	1.45 (0.96–2.21)	1.36 (0.88–2.10)	1.44 (0.89–2.32)	0.29	0.032
<b>Apolipoprotein C-III in LDL</b>							
n=	274	270	282	308	342		
Model 1	1.00 (ref)	1.03 (0.72–1.47)	1.16 (0.81–1.67)	1.56 (1.07–2.29)	2.10 (1.41–3.14)	<0.001	0.88
Model 2	1.00 (ref)	1.1 (0.75–1.63)	1.25 (0.84–1.84)	1.53 (1.01–2.32)	2.19 (1.42–3.38)	<0.001	0.65
Model 3	1.00 (ref)	1.03 (0.69–1.53)	1.15 (0.77–1.71)	1.45 (0.95–2.21)	1.97 (1.26–3.06)	0.002	0.50
Model 4	1.00 (ref)	0.98 (0.66–1.46)	0.96 (0.64–1.46)	1.15 (0.74–1.80)	1.33 (0.81–2.20)	0.004	0.43
<b>Total plasma apolipoprotein C-III</b>							
n=	279	296	285	286	330		
Model 1	1.00 (ref)	1.21 (0.85–1.72)	1.11 (0.78–1.58)	1.15 (0.79–1.67)	1.60 (1.09–2.37)	0.016	0.23

	Quintile categories					P for difference between sexes <sup>‡</sup>	P for trend <sup>†</sup>
	1	2	3	4	5		
Model 2	1.00 (ref)	1.17 (0.80–1.71)	1.11 (0.76–1.64)	1.12 (0.75–1.68)	1.62 (1.06–2.48)	0.36	0.023
Model 3	1.00 (ref)	1.18 (0.80–1.75)	1.12 (0.76–1.66)	1.11 (0.73–1.68)	1.48 (0.96–2.28)	0.40	0.085
Model 4	1.00 (ref)	1.01 (0.68–1.51)	0.90 (0.6–1.36)	0.79 (0.50–1.24)	0.96 (0.60–1.56)	0.18	0.45

\* Model one is conditioned only on matching factors, model 2 is 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. Model three is adjusted for all variables in model two plus body-mass index and personal history of diabetes. Model four is adjusted for all variables in model three plus plasma triglycerides.

<sup>†</sup> P values for trend are based on the median levels of apolipoproteins in quintiles of the controls.

<sup>‡</sup> Calculated as the P value for the interaction between sex and median apolipoprotein levels in quintiles of the controls.