Applipoprotein C-III and High-Density Lipoprotein Subspecies Defined by Apolipoprotein C-III in Relation to Diabetes Risk

Sarah A. Aroner*, Ming Yang, Junlong Li, Jeremy D. Furtado, Frank M. Sacks, Anne Tjønneland, Kim Overvad, Tianxi Cai, and Majken K. Jensen

* Correspondence to Dr. Sarah A. Aroner, Department of Nutrition, Harvard T. H Chan School of Public Health, 655 Huntington Avenue, Building 2, Room 302, Boston, MA 02115 (e-mail: saroner@mail.harvard.edu).

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Apolipoprotein C-III (apoC-III) is a potentially novel biomarker that may play an important role in the pathogenesis of diabetes, particularly when present on the surface of high-density lipoprotein (HDL). In a case-cohort study carried out among 434 incident diabetes cases occurring before 2007 and 3,101 noncases in the Danish Diet, Cancer, and Health Study, we examined associations of baseline (1993–1997) plasma concentrations of apoC-III and subspecies of HDL defined by the presence or absence of apoC-III with risk of diabetes using Cox regression. ApoC-III was strongly associated with risk of diabetes (for top quintile vs. bottom quintile, hazard ratio (HR) = 3.43, 95% confidence interval (CI): 1.75, 6.70; P-trend < 0.001). The cholesterol concentration of HDL without apoC-III was inversely associated with risk of diabetes (HR = 0.48, 95% CI: 0.27, 0.85; P-trend = 0.002), more so than total HDL-C (HR = 0.60, 95% CI: 0.35, 1.03; P-trend = 0.04), whereas HDL-C with apoC-III was not associated (HR = 1.05, 95% CI: 0.50, 2.21; P-trend = 0.44) (for HDL-C with apoC-III vs. HDL-C without apoC-III, P-heterogeneity = 0.002). ApoC-III itself is a strong risk marker for diabetes, and its presence on HDL may impair the antidiabetogenic properties of HDL. ApoC-III has potential to be a therapeutic target for the prevention of diabetes.

Original Contribution

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Abbreviations: apoC-III, apolipoprotein C-III; CHD, coronary heart disease; CI, confidence interval; DCH, Diet, Cancer, and Health; ELISA, enzyme-linked immunosorbent assay; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; HR, hazard ratio; TARF, Turkish Adult Risk Factor.

Despite knowledge of key lifestyle determinants of diabetes risk and the availability of a number of effective preventive agents, diabetes rates continue to soar worldwide (1). As current preventive strategies are focused primarily on the management of plasma glucose levels, further strides in the prevention of this multifactorial disease are likely to arise from strategies targeting novel pathophysiological mechanisms. Because disturbances in lipid metabolism and inflammation precede and possibly contribute to the development of diabetes (2, 3), investigation of these pathways may lead to the identification of novel biomarkers pointing towards new treatment targets. Apolipoprotein C-III (apoC-III), a small atherogenic and proinflammatory protein found on the surface of lipoproteins (very low-density lipoprotein, low-density lipoprotein, and high-density lipoprotein (HDL)), is one such factor that holds particular promise given its potentially wide-ranging effects on a number of diabetogenic mechanisms (4).

Apoc-III has become increasingly recognized for its hypothesized role in the development of cardiovascular disease through the promotion of dyslipidemia and atherogenesis (5–7). In vitro, animal, and human studies have shown that apoC-III blocks the interaction of apolipoprotein B and apolipoprotein E on chylomicrons and very low-density lipoprotein with cellular receptors (8, 9), impairs clearance of these lipoproteins from plasma (9–13), and may at high levels impair the action of lipoprotein lipase (14–16), all leading to hypertriglyceridemia. Further, genetic studies support the effects of apoC-III on triglycerides and cardiovascular disease as being
causal, since genetically elevated and reduced apoC-III levels have been associated with correspondingly higher and lower levels of triglycerides and risk of cardiovascular disease (17–22).

Emerging evidence suggests that apoC-III may also influence the risk of diabetes, not only by causing hypertriglyceridemia but by stimulating pancreatic β-cell apoptosis (23, 24), inducing insulin resistance (17, 25), and promoting inflammation (26). Several cross-sectional studies have found positive associations of circulating apoC-III levels with diabetes (27, 28) and components of the metabolic syndrome (29). In one prospective analysis conducted within the Turkish Adult Risk Factor (TARF) Study cohort, Onat et al. (30) found that concentrations of apoC-III were strongly associated with incident diabetes. However, the results were based on a limited number of cases (n = 57) in a population with a high prevalence of dyslipidemia.

Beyond having diabetogenic properties itself, apoC-III may contribute to the risk of diabetes by modulating the metabolism and function of the lipoproteins on which it resides (7, 31). In the TARF Study, Onat et al. also found that concentrations of apoC-III in the HDL fraction were particularly strongly associated with diabetes risk (30). In prior work, we sought to expand the concept of HDL speciation further by separating HDL containing apoC-III from HDL that does not have any apoC-III. We have shown that subspecies of HDL defined on the basis of apoC-III are differentially associated with risk of coronary heart disease (CHD) (32). HDL with apoC-III, although comprising only 11%–16% of HDL cholesterol (HDL-C), was found to be associated with a higher risk of CHD, while HDL without apoC-III was found to be inversely associated with CHD risk, more so than total HDL (32). We have also found associations with prevalent diabetes, as well as metabolic risk factors such as obesity and blood glucose, to differ in a similar way, showing harmful or no associations for HDL with apoC-III and beneficial associations only for HDL without apoC-III (32, 33). Therefore, we hypothesize that HDL with apoC-III may constitute a dysfunctional subspecies of HDL that is adversely, or at least not beneficially, associated with diabetes risk. To further assess the relationship of apoC-III and apoC-III-defined HDL subspecies with future risk of diabetes, we performed a prospective analysis in a large Danish cohort.

We investigated associations of total apoC-III, apoC-III in the HDL fraction, and apoC-III-based HDL subclasses with risk of diabetes in a case-cohort study (35) nested within the DCH Study. The case-cohort study was originally designed for analyses of incident nonfatal myocardial infarction and fatal CHD and included all confirmed cases of CHD diagnosed from study entry through April 2008 (n = 1,949), as well as a random subcohort of participants who were free of CHD at baseline (n = 1,750) (see Web Figure 1, available at https://academic.oup.com/aje). To examine diabetes as our endpoint in the present analysis, we excluded participants with prevalent diabetes at baseline (n = 133) and identified incident diabetes cases occurring between baseline and December 31, 2006, among the remaining members of the original case-cohort. After additional exclusion of participants with missing covariate values, the case cohort included 434 cases with incident diabetes (138 within the reference subcohort) and 3,101 noncases (total n = 3,535).

**Assessment of diabetes**

Diabetes cases were identified via the Danish National Diabetes Registry (36) on the basis of an inpatient or outpatient hospital diagnosis of diabetes, chiropody for diabetes, repeated blood glucose measurements, or the purchase of diabetes medication. While the National Diabetes Registry does not contain information on type of diabetes, we assumed that cases included in our study represented cases of type 2 diabetes, given the rare adult onset of type 1 diabetes (37). Further details on case ascertainment in the National Diabetes Registry are available in the Web Appendix.

**Biochemical measurements**

Using previously published methods (32), total plasma apoC-III was measured in 2009–2010 using an enzyme-linked immunosorbent assay (ELISA) with anti-human apoC-III antibody. Plasma triglyceride concentrations were measured using an enzymatic assay reagent (Thermo Fisher Scientific, Inc., Waltham, Massachusetts). Whole plasma was fractionated into lipoproteins containing apoC-III and lipoproteins deficient of apoC-III using immunoaffinity column chromatography with anti-human apoC-III bound to Sepharose 4B resin (Academy Biomedical Company, Inc., Houston, Texas). Thawed plasma samples were gently vortexed, and 200 mL was incubated for 16 hours with rotation at 4°C on a column bed of 2.5 mL of anti-apoC-III resin. The unbound fraction was eluted with phosphate-buffered saline, and the bound fraction was freed from the antibodies and eluted with 3 mL sodium thiocyanate. A portion of the bound and unbound fractions was further processed with dextran sulfate and magnesium chloride to precipitate lipoproteins with apolipoprotein B (i.e., low-density lipoprotein and very low-density lipoprotein), leaving only HDL in the supernatant fraction. We then measured concentrations of apoC-III in the resulting HDL fraction by ELISA using rabbit and goat anti-human apoC-III antibodies and horseradish peroxidase antibody conjugates for detection and measured HDL-C using an enzymatic assay reagent (Thermo Fisher Scientific). Liquid transfer for 96-well plate loading and ELISA dilutions were handled robotically with a Multiprobe II.

**METHODS**

**Study population and design**

The Danish Diet, Cancer, and Health (DCH) Study is an ongoing prospective study that began in 1993–1997 with the enrollment of 57,053 Danish-born residents between the ages of 50 and 65 years who were free of cancer. At baseline, participants filled out self-administered food frequency and lifestyle questionnaires, and technicians obtained anthropometric and blood pressure measurements, as well as collected nonfasting blood samples at the study clinic. Blood specimens were separated into plasma, serum, lymphocytes, and erythrocytes and frozen at −150°C within 2 hours of collection. Details on the DCH cohort have been published previously (34). The study was approved by the National Committee on Health Research Ethics and the Danish Data Protection Agency. Informed consent was obtained from all participants.
We evaluated the baseline characteristics of participants who developed diabetes during follow-up and members of the randomly selected subcohort separately. Partial Spearman correlations, adjusted for age and sex, were calculated for the correlation between apoC-III and HDL-C exposures. Inverse probability-weighted Cox proportional hazards regression was used to estimate associations between apolipoprotein variables and risk of diabetes, with weights accounting for the sampling probability of the original study design based on CHD status (38). This analysis included the additional noncases that occurred outside of the subcohort, and a resampling procedure was used to appropriately adjust the variance under a stratified case-cohort design (39). Because registry data were used, participants were only censored due to death or loss to follow-up from emigration.

Because associations using continuous (per standard deviation or log-transformed) apolipoprotein measures did not appear to be linear, results are presented for quintiles, with quintiles estimated from the subcohort. Wald tests for trend were performed with apolipoprotein variables modeled as the log-transformed median value of each quintile. While we did not observe differences in the association by sex, statistical power was limited in female-specific analyses, as only 138 of the diabetes events occurred among women. To account for potential sex differences in diabetes risk and modest variability in apolipoprotein measurements by batch, we fitted sex- and batch-stratified Cox models. Multivariable models included adjustment for the following potential confounders: age (years), smoking (never smoker, former smoker, or current smoker of <15, 15–24, or ≥25 g of tobacco per day), duration of education (short (<8 years), medium (8–10 years), or long (>10 years)), body mass index (weight (kg)/height (m²)), alcohol intake (nondrinker or drinker of <5, 5–9, 10–19, 20–39, or ≥40 g of alcohol per day), and self-reported hypertension. Because results were nearly identical in sensitivity analyses substituting waist circumference for body mass index, we included only body mass index in our final models. We did not adjust for use of lipid-lowering medication, as the prevalence of such use was less than 1% at baseline in the DCH Study. Measures of glucose and insulin were not available in the DCH Study; thus, we could not assess the potential impact of adjustment for these important intermediates.

Because concentrations of HDL-C with and without apoC-III sum to total HDL-C, models simultaneously included the 2 fractions, and likelihood ratio tests were used to assess heterogeneity of trends. To examine whether the proportion of HDL-C with apoC-III might predict diabetes risk beyond total HDL-C concentration, we tested whether the log-transformed proportion of HDL-C with apoC-III was significantly associated with diabetes in models including total log-transformed HDL-C.

While triglyceride levels are known to affect risk of diabetes (40), they are also substantially increased by apoC-III (5, 7, 41). To examine how much of the association between apoC-III and diabetes is explained by triglycerides, we additionally adjusted for log-transformed triglyceride level in a subsequent model. All analyses were performed using R, version 3.1.2 (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

The median age at baseline was 56 years for the random subcohort members and 58 years for the participants who developed diabetes during the 13 years of follow-up (the average age length of follow-up was 10 years). In comparison with the subcohort, participants who developed diabetes during follow-up were more likely to be male, to be postmenopausal, to have greater adiposity, to have less education, to currently smoke, and to have been diagnosed with hypertension or hypercholesterolemia. Future cases had lower concentrations of HDL-C and higher concentrations of total apoC-III and triglycerides (Table 1).

Correlations between lipid measures

HDL-C was strongly correlated with HDL-C without apoC-III (Spearman’s ρ = 0.94), reflecting the predominance of HDL-C without apoC-III, and apoC-III in HDL was moderately correlated with total apoC-III (ρ = 0.48) (Table 2). The correlation between the two apoC-III-based HDL-C subspecies was modest (ρ = 0.25), as were most other correlations between apoC-III and HDL-C variables.

Association of apoC-III concentrations with diabetes risk

Higher total plasma apoC-III concentrations were strongly associated with a greater risk of diabetes (Table 3). In multivariable models, participants in the top quintile of apoC-III concentrations had a nearly 3.5-fold higher risk of diabetes than those in the bottom quintile (hazard ratio (HR) = 3.43, 95% confidence interval (CI) 1.75, 6.70; P-trend < 0.001). After additional adjustment for triglycerides, the association for apoC-III remained significant but was attenuated (top quintile vs. bottom quintile: HR = 1.94, 95% CI: 1.00, 3.77; P-trend = 0.04). A nonsignificant positive association was present for apoC-III in the HDL fraction (upon depletion of apolipoprotein B from plasma) in the multivariable model (top quintile vs. bottom: HR = 1.73, 95% CI: 0.89, 3.38; P-trend = 0.10)
After adjustment for triglycerides, the hazard ratio was lowered to 1.12 (95% CI: 0.58, 2.18; \( P \)-trend = 0.69).

Associations of apoC-III-based HDL-C subspecies with diabetes risk

On average, 15% of HDL-C had any amount of apoC-III among the randomly selected subcohort members, with the proportion being slightly higher among future cases (Table 1). In multivariable models, concentrations of HDL-C with and without apoC-III were differentially associated with risk of diabetes (\( P \)-heterogeneity = 0.002). HDL-C without apoC-III was inversely associated with diabetes risk (top quintile vs. bottom: HR = 0.48, 95% CI: 0.27, 0.85; \( P \)-trend = 0.002), while HDL-C with apoC-III was unassociated with risk (top quintile vs. bottom: HR = 1.05, 95% CI: 0.50, 2.21; \( P \)-trend = 0.44). The association for HDL-C without apoC-III was stronger than that for total HDL-C (top quintile vs. bottom: HR = 0.60, 95% CI: 0.35, 1.03; \( P \)-trend = 0.04) (Figure 1 and Web Table 1).

As a sensitivity analysis, we repeated analyses of HDL subspecies restricted to the subcohort, where 138 diabetes cases occurred. Estimates in the subcohort were quite consistent with those in the full sample (top quintile vs. bottom: for HDL-C without apoC-III, HR = 0.36 (95% CI: 0.19, 0.66), and for HDL-C with apoC-III, HR = 1.16 (95% CI: 0.54, 2.51)) but were less precise due to the more limited numbers of cases in the subcohort. The proportion of HDL-C that had apoC-III was statistically significant in a multivariable model that also adjusted for total HDL-C (\( P \)-trend = 0.01). Subspecies of HDL-C according to apoC-III were no longer differentially associated with risk of diabetes after additional adjustment for triglycerides (HDL without apoC-III: HR = 0.87 (95% CI: 0.47, 1.61); HDL with apoC-III: HR = 0.80 (95% CI: 0.40, 1.60); \( P \)-heterogeneity = 0.40).

TABLE 1. Baseline Characteristics of Participants Who Developed Diabetes During Follow-up and Subcohort Members in the Danish Diet, Cancer, and Health Study, 1993–2006

<table>
<thead>
<tr>
<th>Variable</th>
<th>Diabetes Cases (n = 434)</th>
<th>Subcohort (n = 1,717)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Median (IQR)</td>
<td>% Median (IQR)</td>
</tr>
<tr>
<td>Age, years</td>
<td>58 (54–62)</td>
<td>56 (53–60)</td>
</tr>
<tr>
<td>Female sex</td>
<td>32</td>
<td>47</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>74</td>
<td>59</td>
</tr>
<tr>
<td>Currently using estrogen</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Body mass index(^a)</td>
<td>29 (26–32)</td>
<td>26 (23–28)</td>
</tr>
<tr>
<td>Waist circumference, cm</td>
<td>100 (92–107)</td>
<td>89 (80–98)</td>
</tr>
<tr>
<td>Short education (&lt;8 years)</td>
<td>44</td>
<td>33</td>
</tr>
<tr>
<td>Current smoker</td>
<td>51</td>
<td>38</td>
</tr>
<tr>
<td>Alcohol intake, g/day</td>
<td>13 (3–33)</td>
<td>14 (6–32)</td>
</tr>
<tr>
<td>Hypertension(^f)</td>
<td>41</td>
<td>18</td>
</tr>
<tr>
<td>Hypercholesterolemia(^f)</td>
<td>15</td>
<td>8</td>
</tr>
<tr>
<td>Lipid concentration, mg/dL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides</td>
<td>134 (90–195)</td>
<td>80 (56–123)</td>
</tr>
<tr>
<td>Total apoC-III</td>
<td>12.0 (8.5–16.8)</td>
<td>11.4 (7.9–15.3)</td>
</tr>
<tr>
<td>ApoC-III in HDL</td>
<td>6.0 (3.7–9.5)</td>
<td>5.6 (3.6–8.4)</td>
</tr>
<tr>
<td>HDL-C</td>
<td>45 (33–57)</td>
<td>52 (40–65)</td>
</tr>
<tr>
<td>HDL-C with apoC-III</td>
<td>7.6 (4.4–11.7)</td>
<td>7.7 (4.8–11.4)</td>
</tr>
<tr>
<td>HDL-C without apoC-III</td>
<td>35 (25–46)</td>
<td>43 (33–55)</td>
</tr>
<tr>
<td>Proportion of HDL-C with apoC-III, %</td>
<td>16.6 (11.2–25.6)</td>
<td>14.8 (10.3–20.6)</td>
</tr>
</tbody>
</table>

Abbreviations: apoC-III, apolipoprotein C-III; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol; IQR, interquartile range.

\(^a\) Participants were not asked to fast before the blood drawing.
\(^b\) Per the case-cohort study design, the 138 cases that occurred within the subcohort were included in both the case count and the subcohort count.

\(^c\) 25th–75th percentiles.

\(^d\) Among postmenopausal women.

\(^e\) Weight (kg)/height (m)\(^2\).

\(^f\) Self-reported physician’s diagnosis of hypertension or hypercholesterolemia.
The association between apoC-III and diabetes risk, HDL-C with apoC-III was not associated with incident diabetes.

Our findings contribute to growing evidence for the involvement of apoC-III in the pathophysiology of diabetes. One of the important downstream metabolic effects of apoC-III is the regulation of plasma triglyceride levels (5, 7, 8, 41–43). At physiological concentrations in humans, the primary mechanism by which apoC-III influences triglyceride levels appears to be delaying the clearance of triglyceride-rich lipoproteins (9–13), although some evidence suggests that apoC-III may also stimulate triglyceride synthesis and the formation of triglyceride-rich lipoproteins (42, 43) and at high levels impair the action of lipoprotein lipase (7, 14–16). Supporting associations of apoC-III and diabetes through hypertriglyceridemia, mice overexpressing the apolipoprotein C-III gene (APOC3) have higher plasma triglyceride levels and are predisposed to fatty liver disease and hepatic insulin resistance (25), conditions associated with the development of diabetes (44). In human studies, APOC3 gene variants have been identified that are associated with both plasma apoC-III and triglyceride levels (17–21, 45). Some (17, 18), but not all (19–21, 43) of these studies additionally found these variants to be associated with insulin resistance and nonalcoholic fatty liver disease.

Our results also support triglycerides as an important mediator of the association between apoC-III and diabetes, as we found that accounting for the concentration of triglycerides attenuated the risk of diabetes attributed to apoC-III. However, associations for total apoC-III remained statistically significant after adjustment for triglycerides, supporting evidence for additional diabetogenic functions of apoC-III beyond effects on circulating triglycerides. In vitro (46) and in vivo (23) experiments suggest that apoC-III may promote pancreatic β-cell apoptosis, leading to impaired insulin secretion. In addition, apoC-III may cause insulin resistance in the liver resulting from triglyceride

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**Table 2.** Partial Spearman Correlations Between Apolipoprotein C-III and High-Density Lipoprotein Measures in the Subcohort, Danish Diet, Cancer, and Health Study, 1993–2006

<table>
<thead>
<tr>
<th>Total ApoC-III</th>
<th>ApoC-III in HDL</th>
<th>HDL-C</th>
<th>HDL-C With ApoC-III</th>
<th>HDL-C Without ApoC-III</th>
</tr>
</thead>
<tbody>
<tr>
<td>ApoC-III in HDL</td>
<td>1.00 0.48 0.10</td>
<td>0.27 0.03^b</td>
<td>0.95</td>
<td>1.00 0.26 0.35</td>
</tr>
<tr>
<td>HDL-C</td>
<td>1.00 0.52</td>
<td>0.94</td>
<td>1.00 0.25</td>
<td></td>
</tr>
<tr>
<td>HDL-C with apoC-III</td>
<td>1.00</td>
<td>0.25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL-C without apoC-III</td>
<td>1.00</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: apoC-III, apolipoprotein C-III; HDL, high-density lipoprotein; HDL-C, high-density lipoprotein cholesterol.

^b^ Spearman correlations were adjusted for age and sex. All units were mg/dL.

^P^ = 0.23; all other ^P^ values were less than 0.001.

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**Table 3.** Risk of Diabetes According to Apolipoprotein C-III Concentrations in Whole Plasma and in the High-Density Lipoprotein Fraction, Danish Diet, Cancer, and Health Study, 1993–2006

<table>
<thead>
<tr>
<th>Quintile of apoC-III^a^</th>
<th>Median Value, mg/L</th>
<th>No. of Diabetes Cases</th>
<th>Total ApoC-III</th>
<th>Crude Model</th>
<th>Multivariable Model^e^</th>
<th>ApoC-III in HDL^b^</th>
<th>Crude Model</th>
<th>Multivariable Model^e^</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median Value, mg/L</td>
<td>HR^d^ 95% CI</td>
<td>HR^d^ 95% CI</td>
<td>Median Value, mg/L</td>
<td>HR^d^ 95% CI</td>
<td>HR^d^ 95% CI</td>
</tr>
<tr>
<td>1</td>
<td>5.2</td>
<td>73</td>
<td>1.00 Referent</td>
<td>1.00 Referent</td>
<td>2.3 87 1.00 Referent 1.00 Referent</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8.6</td>
<td>85</td>
<td>1.27 0.69,2.34</td>
<td>1.20 0.66,2.20 4.0 84 1.16 0.67,1.99 0.94 0.53,1.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>11.4</td>
<td>74</td>
<td>2.05 1.19,3.52</td>
<td>1.99 1.13,3.50 5.7 79 1.23 0.70,2.14 1.14 0.65,2.00</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14.5</td>
<td>89</td>
<td>2.24 1.23,3.98</td>
<td>1.80 0.99,3.26 7.8 72 1.21 0.65,2.26 1.10 0.59,2.04</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>5</td>
<td>19.6</td>
<td>113</td>
<td>3.50 1.83,6.68</td>
<td>3.43 1.75,6.70 12.0 112 2.30 1.19,4.45 1.73 0.89,3.38</td>
<td></td>
<td></td>
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</tbody>
</table>

P-trend^g^ < 0.001 < 0.001 0.03 0.10

Abbreviations: apoC-III, apolipoprotein C-III; CI, confidence interval; HDL, high-density lipoprotein; HR, hazard ratio.

^a^ Quintiles were based on the distribution in the random subcohort.

^b^ Analyses of apoC-III concentration in the HDL fraction also adjusted for total HDL cholesterol.

^c^ The multivariable model adjusted for age, smoking, education, body mass index, alcohol intake, and hypertension.

^d^ HRs were obtained from Cox proportional hazards regression models stratified by sex and batch.

^e^ P-trend was calculated using the median values of the log-transformed apoC-III variables.
Onat et al. observed between apoC-III in HDL and diabetes risk in the TARF Study led them to speculate that apoC-III may have rendered HDL “dysfunctional” (30). Given our findings, it would be of interest to explore whether elevated levels of HDL with apoC-III may have contributed to the lack of association for total HDL in the dyslipidemic TARF population.

The divergent associations that we observed for HDL-C with and without apoC-III in the DCH Study support our hypothesis that apoC-III defines distinct subspecies of HDL with respect to risk of diabetes. Because apolipoproteins mediate downstream interactions of HDL with receptors and enzymes involved in cardiometabolic disease processes, defining lipoprotein subspecies by the presence or absence of apolipoproteins might provide additional insight into specific lipoprotein functions beyond the traditional classification by lipid content and size (31). We previously demonstrated that while HDL without apoC-III displays a strong inverse relationship with risk of CHD, HDL with apoC-III is associated with a slightly elevated risk (32). Since we are (to our knowledge) the first to assess these HDL subspecies in relation to future risk of diabetes, our findings require confirmation in additional prospective studies.

Our study had several strengths. It was the second prospective study to investigate apoC-III in relation to diabetes risk and included over 7 times as many cases as the prior investigation (30). In addition, our assessment of apoC-III-based HDL subspecies is novel and expands upon the prior study, which measured apoC-III in HDL. Our results suggest that it is the presence of any apoC-III on HDL rather than the concentration of apoC-III in HDL that is important for future risk of diabetes and CHD (32). Limitations of our study include the predominantly Caucasian population, the measurement of lipids at a single point in time, and insufficient numbers of participants to evaluate possible differences by sex.

While our measurement of lipids in the nonfasting state might be viewed as an additional drawback, since nonfasting lipids are more complicated to measure and are subject to more variability than fasting lipids (54), nonfasting lipid levels may be more clinically relevant. Because the majority of people’s time is spent in a postprandial state, nonfasting measurements are more reflective of usual lipid levels (54). Furthermore, nonfasting lipoprotein and apolipoprotein levels have been shown to be as predictive of incident cardiovascular disease as fasting levels (55, 56), and nonfasting levels may also provide a better indicator of dyslipidemia in the insulin-resistant state (57, 58). Thus, although both fasting and nonfasting measures are acceptable, the use of nonfasting samples may provide another explanation for the differences between our results and those obtained from fasting samples in the TARF population.

Additional study will also be needed to understand whether apoC-III is causally related to diabetes. Mechanisms through which apoC-III might be involved in insulin resistance are complex and potentially bidirectional, since apoC-III may promote the development of insulin resistance (18, 25, 59) and, in turn, insulin resistance may enhance production of apoC-III (60). The influence of apoC-III on dyslipidemia and inflammation particularly warrants further study, as these are increasingly becoming recognized as novel pathways in the pathophysiology of diabetes (61, 62).
The elevation in apoC-III levels many years before the clinical onset of diabetes has important implications for the prevention of diabetes, particularly given the potential involvement of apoC-III in mechanisms distinct from glucose regulation. Current diagnostic tests for diabetes rely almost exclusively on indicators of glucose metabolism, which are effective at early detection among persons with underlying metabolic abnormalities but may not perform as well in populations without metabolic risk factors (63). Thus, apoC-III may be a marker for risk of diabetes in a true primordial prevention setting. Further novel treatments that inhibit the action of apoC-III might expand the current repertoire of antidiabetic agents, which primarily target glucose metabolism. Fibrates, currently used for the treatment of dyslipidemia (64), and antisense oligonucleotide inhibitors for apoC-III, now in late-stage clinical trials (5), are 2 such promising options that reduce apoC-III gene expression (64).

In conclusion, concentrations of total apoC-III were associated with a higher risk of diabetes in this study, and the presence of apoC-III on HDL appeared to define a dysfunctional HDL subspecies not protective against diabetes. Our results suggest that apoC-III offers promise as a novel diabetogenic factor and potential target for the development of new diabetes treatment options.

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Author affiliations: Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts (Sarah A. Aroner, Jeremy D. Furtado, Frank M. Sacks, Majken K. Jensen); Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, Massachusetts (Ming Yang, Tianxi Cai); Analysis Group, Inc., Boston, Massachusetts (Junlong Li); Channing Division of Network Medicine, Department of Medicine, Brigham and Women’s Hospital, Harvard Medical School, Boston, Massachusetts (Frank M. Sacks, Majken K. Jensen); Danish Cancer Society Research Center, Copenhagen, Denmark (Anne Tjønneland); Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark (Kim Overvad); and Section for Epidemiology, Department of Public Health, Aarhus University, Aarhus, Denmark (Kim Overvad).

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