Title

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Serum apolipoproteins and apolipoprotein-defined lipoprotein subclasses:

a hypothesis-generating prospective study of cardiovascular events in type 1 diabetes

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ABSTRACT:

Apolipoproteins APOB, APOC3, and APOE, and apolipoprotein-defined lipoprotein subclasses (ADLS; based on qualitative apolipoprotein complement) have been associated with dyslipidemia and cardiovascular disease (CVD). Our main objective was to define associations of serum apolipoproteins and ADLS with 'any CVD' and 'major atherosclerotic cardiovascular events' (MACE) in a prospective study of T1D. Serum apolipoproteins and ADLS (14 biomarkers in total) were measured in sera (obtained 1997-2000) from a subset (n=465) of the Epidemiology of Diabetes Interventions and Complications (EDIC) cohort. Prospective associations of 'any CVD' (myocardial infarction, stroke, confirmed angina, silent MI, revascularization, or congestive heart failure) and MACE (fatal or nonfatal myocardial infarction or stroke), over 5942 and 6180 patient-years follow-up respectively, were investigated using Cox proportional hazards models, unadjusted and adjusted for risk factors. During 15 years follow-up, 50 'any CVD' and 24 MACE events occurred. Nominally significant positive univariate associations with 'any CVD' were APOB, APOC3 and its sub-fractions [heparin precipitate (HP), heparin soluble (HS)], and ADLS-defined Lp-B. In adjusted analyses, APOC3-HS remained nominally significant. Nominally significant positive univariate associations with MACE were APOC3 and sub-fractions, and Lp-B:C; those with total APOC3 and APOC3-HS persisted in adjusted analyses. However, these associations did not reach significance after adjustment for multiple testing. There were no significant associations of APOA1, APOA2, APOE, or other ADLS with either 'any CVD' or MACE. These hypothesis-generating data suggest that total serum APOC3 and APOC3 in HDL are potentially important predictive biomarkers for 'any CVD' and MACE in T1D adults.

Key words: apolipoprotein-determined lipoprotein subclasses, APOC3, major adverse cardiac event, type 1 diabetes

INTRODUCTION

The incidence and prevalence of type 1 diabetes (T1D) is increasing globally, and despite modern management of associated risk factors, it remains associated with greatly increased morbidity and mortality from cardiovascular disease (CVD) (1-3). Serum lipids and apolipoprotein concentrations are important biomarkers of CVD, and several studies have identified the clinical utility of serum apolipoprotein levels and measurements of apolipoprotein-defined lipoprotein subclasses (ADLS) in predicting vascular complications of Type 2 diabetes (4-8), complementing known associations of CVD with conventional lipids; however, such data are limited in the T1D population.

In studies of apolipoprotein concentrations in plasma in non-diabetic cohorts, APOB has been positively associated with CVD events (9, 10). Similar associations have been observed with APOC3, which is involved in the transport and catabolism of triacylglycerols (11). The distribution of APOC3 between APOB- and nonAPOB-containing particles can be elucidated by heparin precipitation. Thus, APOC3 in APOB-containing lipoproteins can be precipitated using heparin (APOC3-HP), while that in APOA1-containing lipoproteins remains in the supernatant, i.e. is 'heparin-soluble' (APOC3-HS). A higher APOC3-HS: APOC3-HP ratio ('APOC3 ratio') is considered a useful index of peripheral catabolism of triacylglycerol (TG)-rich lipoproteins (6). Serum APOE is less clearly associated with atherogenic risk (12): it enhances uptake of triglyceride-rich particles by remnant receptors primarily in the liver (13). APOB-containing lipoproteins include LDL, IDL and VLDL, while APOA1-containing lipoproteins are predominantly associated with HDL.

Apolipoprotein-defined lipoprotein subclass (ADLS) analysis relates particle function and metabolism: it defines particles according to their qualitative apolipoprotein complement, and the subclass names reflect the apolipoproteins present on the particle (14). ADLS thus provides information about the distribution of apolipoproteins among particle classes, in contrast to 'simple' quantification of

apolipoproteins in whole plasma which lacks this information. Based on the ADLS nomenclature, lipoproteins can be categorized into two 'families': the APOA1 family overlaps with HDL, includes two subclasses (Lp-A1, Lp-A1:A2), and usually has anti-atherogenic potential; the APOB family includes VLDL, IDL, and LDL, includes five subclasses (Lp-B, Lp-B:E, Lp-B:C, Lp-B:C:E and Lp-A2:B:C:D:E), and generally has pro-atherogenic effects (15). The Monitored Atherosclerosis Regression Study (MARS) and the Cholesterol Lowering Atherosclerosis Study (CLAS) demonstrated that high levels of Lp-B and Lp-B:C particles predict coronary events even when adjusted for conventional lipid profiles (16, 17). Prospective data on the associations of serum apolipoproteins and ADLS and with CVD events in T1D are lacking and are the subject of our current analysis.

In 1993, the Diabetes Control and Complications Trial (DCCT, 1983-1993) demonstrated that intensive diabetes management for an average of 6.5 years, with an emphasis on glycemic control, dramatically reduced the onset and progression of the microvascular complications of diabetes (18). Few CVD events had occurred by the time of DCCT close-out in 1993 because the study cohort was still young (34 ± 7 years, mean ± SD) and with relatively short duration of T1D (12±4 years, mean ± SD) (19). Furthermore, risk of early events was reduced by the original DCCT entry criteria: these mandated an absence of CVD events or CVD risk factors such as hypertension and hyperlipidemia. In the continuing follow-up phase of DCCT, the Epidemiology of Diabetes Interventions and Complications (EDIC) study, implemented in 1994, HbA1c levels converged and became almost identical between the former DCCT randomization groups, but despite this, over ensuing years carotid intima media thickness (IMT) progressed more slowly in the former intensive vs 'conventional' management group, and there were fewer clinical CVD events (20, 21), a phenomenon dubbed 'metabolic memory' (19). We previously reported cross-sectional and some longitudinal associations between novel lipoprotein-related risk factors and carotid intima-media thickness (IMT) in the DCCT/EDIC cohort (5, 22, 23), but the longitudinal associations of these detailed metrics with macrovascular disease events in T1D have not been explored. Now with 27-

years follow-up from DCCT baseline, we report longitudinal associations of serum ADLS and individual

apolipoproteins with 'any CVD' and Major Adverse Cardiac Events (MACE) in this T1D cohort.

METHODS

The DCCT/EDIC study cohort and related methods have been previously described (18). Briefly, starting in 1983, the DCCT was a randomized study designed to compare the rates of microvascular complications between participants assigned to receive intensive therapy (n=711) aimed at lowering glycemic values to near the non-diabetic range, and participants assigned to conventional therapy (n=730) aimed at maintaining clinical well-being with no specific glucose targets. At baseline, the DCCT study cohort consisted of a primary prevention cohort (1-5 years of diabetes duration, no retinopathy based on fundus photography and albumin excretion rate (AER) less than 40 mg/24 h) and a secondary intervention cohort (1-15 years of diabetes duration, minimal to moderate non-proliferative retinopathy and AER < 200 mg/24 h). In 1993, at the end of DCCT, all participants were instructed in intensive therapy and referred back to their healthcare providers. Then 97% of the surviving DCCT cohort enrolled in the EDIC study, the DCCT follow-up observational study, and 94% of the surviving DCCT participants were still actively participating after an additional 20 years follow-up.

In 1996, a collaboration between the Medical University of South Carolina (MUSC) and DCCT/EDIC was initiated to identify vascular risk factors. Twenty-five of 28 DCCT/EDIC clinical centers participated, and in 1997-2000 (EDIC Years 4-6), serum samples were shipped overnight on dry ice to MUSC; on arrival, aliquots were promptly prepared and stored at -70°C until analysis. The study, which meets Declaration of Helsinki guidelines, was approved by the Institutional Review Boards at MUSC and all participating DCCT/EDIC centers. Each participant gave written informed consent. Of the 1,441 DCCT participants, 968 agreed to participate in the MUSC study, but cost and resource considerations precluded determination of ADLS and apolipoprotein concentrations in all of these participants. The present study therefore utilized a previously-described subset (n=465) (24-26). Briefly, all those with abnormal albuminuria (albumin excretion rate (AER) >40 mg/24 hours), increased ETDRS retinopathy score (53/<53

or higher), or elevated carotid atherosclerosis (≥25% stenosis at a carotid lesion) were included (i.e., all available cases meeting one or more of these definitions were sampled), together with a larger group of subjects free of all of these complications. The three disease categories (albuminuria, retinopathy, carotid stenosis ≥25%) were combined and reweighted to reflect the demographic and vascular disease status of the entire EDIC cohort at EDIC Year 6 (see Statistical Analysis, below).

CVD Risk Factors. DCCT/EDIC study visits included a detailed medical history including demographic and behavioral risk factors, medical outcomes, and a physical examination which included measurements of height, weight, sitting blood pressure, and pulse rate. Blood samples were collected at each visit and assayed centrally at the DCCT/EDIC Central Biochemistry Laboratory (University of Minnesota) for HbA1c, using high-performance ion-exchange liquid chromatography. Fasting lipids (triglycerides, LDL and HDL cholesterol) were measured annually during DCCT and in alternate years during EDIC and were evaluated centrally (27). Total cholesterol, triglyceride, and HDL-C levels were determined using previously reported enzymatic methods (23). LDL-C was estimated according to the Friedewald equation (27).

Variables were fixed (e.g. sex) or time-dependent (e.g. HbA1c), the latter captured either as the current (most recent) value, or as the updated mean from baseline. The updated mean is the weighted average of prior values using weights proportional to the time interval between the measurements.

Apolipoproteins and Apolipoprotein-Defined Lipoprotein Subclasses Measurements

We evaluated a subset (n=465) of the DCCT/EDIC cohort using fasting samples collected at EDIC Years 4-6 (1997-2000), with participants selected based on retinopathy, nephropathy and carotid stenosis status. For all measures, sampling weights were calculated based on the relative distribution of retinopathy, nephropathy and carotid stenosis in this sub-cohort and in the full EDIC cohort, and were further adjusted for sex and original DCCT cohort (primary prevention vs. secondary intervention).

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Apolipoproteins were quantified by electro-immunoassays for APOA1, APOA2, APOB, APOC3, and APOE (14). APOC3 was measured in whole serum before and after heparin precipitation. The precipitation step enables determination of APOC3 bound to APOA1-containing lipoproteins (heparin-soluble, APOC3-HS) and APOC3 bound to APOB-containing lipoproteins (heparin precipitate, APOC3-HP) (28). We also report APOC3-HS/HP or "APOC3 ratio" (APOC3-R) as a useful index of catabolism of TG-rich lipoproteins (29).

For determination of APOB-containing ADLS, 100µL of whole plasma was mixed with buffer solution and then sequentially treated with polyclonal antisera to APOA2, followed by antisera to APOE, and finally with antisera to APOC3, with overnight incubations at each step followed by centrifugation to separate the precipitates and supernatants (30). Determination of APOB in the first precipitate and all supernatants enabled calculation of Lp-B, Lp-B:C, [Lp-B:E + Lp-B:C:E] and Lp-A2:B:C:D:E subclasses, each expressed according to its APOB content (30). Lp-A1 and Lp-A1:A2 were measured by differential turbidimetry and defined according to APOA1 content (31). ADLS assays were conducted in the Lipid and Lipoprotein Laboratory at the Oklahoma Medical Research Foundation using previously described procedures (30, 32).

Cardiovascular outcomes

CVD events were ascertained based on medical history, electrocardiogram and available medical records, and were adjudicated by a committee masked to DCCT treatment group and HbA1c levels. The primary CVD outcome ("any CVD") was defined as the time to the first occurrence of CVD death, non-fatal myocardial infarction, non-fatal stroke, subclinical myocardial infarction on ECG, angina confirmed by ischemic changes with exercise tolerance testing or by clinically significant obstruction on coronary angiography, revascularization (with angioplasty or coronary artery bypass) or congestive heart failure (paroxysmal nocturnal dyspnea, orthopnea or marked limitation of physical activity caused by heart

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disease) (33-36). The secondary CVD outcome, major atherosclerotic cardiovascular events (MACE) included only the time to fatal or nonfatal myocardial infarction or stroke, whichever occurred first. Participants free of a CVD event were administratively censored as of 12/31/2013 (the date of the last CVD data lock). Participants with 'any CVD' events (n=11) and MACE events (n=6) prior to the study sample collection time-point were excluded from these analyses, while all subsequent incident CVD events until censoring were included in the analyses.

Statistical analysis

Summary statistics (counts and percentages for binary variables, and medians and quartiles for continuous variables) of the baseline (i.e., EDIC Year 4-6) characteristics were used to describe the participants and to assess whether, after adjustment for the sampling weights, the 465 participants with available apolipoprotein and ADLS measurements included in these analyses were representative of the entire DCCT/EDIC cohort. All statistical analyses employed the sampling weights computed based on retinopathy, nephropathy and carotid stenosis status, further adjusted for sex and the original DCCT cohort.

Summary statistics were also used to describe the lipoprotein measures both overall and separately by the initial DCCT treatment group, while the Wilcoxon test allowed for the sampling weights as employed to test for differences between the two DCCT treatment groups. Kaplan-Meier survival curves were used to describe the time-to-event outcomes (i.e. any CVD and MACE). Cox proportional hazards models (separately for any CVD and MACE) were employed to assess the association between each lipoprotein measure and the risk of CVD. The power to detect associations in time-to-event analyses is dictated by the number of events. Given the relatively small number of incident any CVD events among the 465 participants in our study, and informed by our previous work on risk factors for CVD in the DCCT/EDIC cohort (35), a set of pre-specified models was considered: Model 1 (unadjusted); Model 2 (age and mean

HbA1c); Model 3 (age, mean HbA1c and log triglycerides); Model 4 (age, mean HbA1c and LDL); and Model 5 (age, mean HbA1c, log triglycerides, systolic BP and pulse rate). HbA1c, LDL, pulse rate and systolic blood pressure (SBP) were defined using the updated mean values, with weights proportional to the time between visits. For triglycerides, we used the most recent value, as this has previously been shown to be most strongly associated with CVD events (35), and data were log-transformed. Given the lower number of MACE events, only models 1 to 4 were considered for the MACE outcome. All analyses were performed using R, and p-values ≤0.05 were considered nominally significant. Given the exploratory nature of our analyses, the reported p-values were computed without adjustment for multiple testing.

RESULTS

Table 1 presents the characteristics of the 465 participants at EDIC Years 4-6 (1997-2000), with values presented after adjustment for sampling weights, and compares these to the characteristics of the full DCCT/EDIC cohort at the same time-point. For the 465 participants, sampling weights were computed based on retinopathy, nephropathy, and carotid stenosis status, and further adjusted for sex and original DCCT cohort (primary or secondary prevention). After weight adjustment, 47% of the participants were from the original DCCT intensive treatment group, 50% were from the original primary prevention cohort, 53% were males, and 17% were current smokers, and the study sub-set was representative of the full cohort. Among the 465 participants in this study, by the end of 2013, there were 50 incident 'any CVD' events over a total follow-up of 5,942 patient-years (rate of 8.4 events (any) per 1,000 individuals at risk for one year), and 24 MACE events over a total follow-up of 6,180 patient-years (rate of 3.9 events per 1,000 individuals at risk for one year). Figure 1 shows the survival probability curves for the time to 'any CVD' event and time to MACE in our cohort.

Summaries of the apolipoprotein and ADLS biomarkers stratified by initial DCCT randomization groups (intensive vs. conventional) are presented in Table 2. Only Lp-A1 was borderline nominally

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different, being higher in the former conventional vs. intensive group (p=0.049), and APOA1 also tended to be higher in the former conventional vs. intensive group (p=0.06).

Table 3 shows associations of fourteen apolipoprotein and ADLS biomarkers with the risk of 'any CVD' in T1D. In Model 1 (unadjusted), higher levels of APOB (P=0.004), APOC3 total (P<0.0001), APOC3-HP (P=0.001), APOC3-HS (P<0.0001) and Lp-B (P=0.001) were nominally associated with higher risk of 'any CVD'. After adjustment for age and mean HbA1c (Model 2), all these variables remained nominally associated with 'any CVD', except APOC3-HP which revealed a positive trend (p=0.07). When further adjusted for triglyceride levels (Model 3), only APOC3-HS remained nominally associated with 'any CVD' (P=0.019), and when adjusted for LDL-cholesterol (Model 4), both APOC3 total and APOC3-HS emerged nominally significant (P=0.022 and P=0.001, respectively). In the final adjusted model for age, mean HbA1c, mean SBP, mean pulse and current (log) triglycerides (Model 5), only APOC3-HS was nominally associated with the risk of 'any CVD' (P=0.012).

The results were somewhat similar for the association of apolipoprotein and ADLS biomarkers with MACE, as shown in Table 4. In Model 1, higher levels of APOC3 total (P=0.001), APOC3-HP (P=0.007), APOC3-HS (P=0.004) and Lp-B:C (P=0.046) were nominally associated with MACE. When adjusted for age and mean HbA1c (Model 2), APOC3 total and APOC3-HS remained nominally significant (P=0.013 and P=0.026, respectively) and Lp-B:C showed a positive trend (p=0.06). When further adjusted for serum triglycerides (Model 3), none of the biomarkers was nominally significant, and when adjusted for LDL-cholesterol (Model 4), both APOC3 total and APOC3-HS emerged as nominally significant (P=0.026 and P=0.027, respectively).

Additional analyses (not shown) addressed the association between the biomarkers and the risk of CVD after further adjustment for the initial DCCT treatment group, AER, duration of diabetes, hypertension, smoking status and use of statins, all assessed at EDIC Year 6. Due to the small number of

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events, these potential risk factors were included separately as additional covariates in the models described in Table 3, Model 5 for 'any CVD event' and in Table 4, Model 4 for MACE. The results were little different from those above.

DISCUSSION

Overall, our analyses revealed that APOC3 and its subfractions showed the most consistent and nominally significant prospective associations with risks of 'any CVD' event and MACE in models adjusted for traditional cardiovascular risks and conventional lipids. Among the other apolipoproteins measured in our cohort, APOB showed a positive association with 'any CVD' event, but not with MACE, after adjustment for age and mean HbA1c. Among the ADLS biomarkers, only Lp-B showed a positive association with 'any CVD event', but none was associated with MACE after adjustment for age and mean HbA1c. The findings add new data on the role of APOC3 in predicting CVD risks in individuals with T1D.

Apolipoprotein C3 (APOC3) is synthesized in the liver. It is found on the surface of VLDL, LDL and HDL particles, and has been independently associated with hypertriglyceridemia and cardiovascular events. Various mechanisms for APOC3 atherogenicity have been proposed: it leads to decreased clearance of APOB from the circulation (37), formation of small dense LDL (38), and stimulation of hepatic formation of triglyceride-rich VLDL (39). In a meta-analysis of eleven studies that included 2,832 cases with cardiovascular events, each 5mg/dL increase in total APOC3 was associated with a 33% increase in risk of cardiovascular events (40). In our study, we observed risk to increase by approximately 10% for every mg/dL increase in total APOC3 for 'any CVD' event and for MACE. In a recent report of 4,659 participants with CVD risk factors in the Multi-Ethnic Study of Atherosclerosis (MESA), HDL particles containing APOC3 were positively associated with coronary artery calcification in women (41). Our findings are consistent with these previous observations, as we observed nominally significant

positive associations of the HDL-containing sub fraction of APOC3 (APOC3- HS) with 'any CVD' event and with MACE: this persisted in our most rigorously-adjusted model which adjusted for systolic blood pressure, and pulse, both pre-specified variables in our analytic plan. Recently, again in DCCT/EDIC, we reported prospective associations of APOC3-HP with carotid IMT in men (17); similar positive associations of APOC3 with carotid intima-media thickness (IMT) have been reported in a healthy population (42), and with coronary heart disease in high-risk adults, including those with type 2 diabetes (43).

We did not observe any association of APOC3 ratio with CVD events or MACE, possibly due the absence of associations of 'APOC3 in LDL and VLDL' (i.e. APOC3-HP) and APOB with cardiovascular outcomes in adjusted models. Our findings in this study add to the existing literature, and suggest that APOC3, as well as APOC3 content of HDL, may represent a therapeutic target in people with Type 1 diabetes.

Among the other apolipoproteins measured, we observed a nominally significant positive association of APOB with 'any CVD' event, but not with MACE in models adjusted for age and HbA1c. We previously found a positive association of APOB with IMT in DCCT/EDIC (17), and APOB has been associated with risk for stroke and increased IMT in other populations with and without diabetes (44, 45). No significant associations of HDL-containing APOA1, A2, or APOE with either 'any CVD' or MACE were found in our cohort; this may be explained by the overall low prevalence of dyslipidemia at baseline and the low number of events in the follow-up period.

Among the ADLS biomarkers, Lp-B was nominally associated with 'any CVD' (Models 1 & 2), and Lp-B:C was positively associated with MACE (Model 1, p=0.046; Model 2, p=0.06). APOB-containing ADLS subclasses, especially Lp-B and Lp-B:C have shown positive correlations with risk for coronary artery disease in subjects with hypercholesterolemia (46), as well as with macrovascular disease

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in subjects with type 2 diabetes (47). Elevated Lp-B:C was shown to be highly correlated with the progression of atherosclerosis, reflected by coronary artery calcium (CAC) scores, in patients with rheumatoid arthritis (48). We previously showed that Lp-B was prospectively associated with increased IMT in men in DCCT/EDIC, but no previous studies have reported associations with CVD events and MACE in a T1D population. In our study, in models adjusted for blood pressure and/or conventional lipids, ADLS were not significantly associated with future CVD, perhaps because of strong collinearity among atherogenic lipids that contribute to CVD.

The strengths of our study include the detailed clinical characterization and long-term follow-up of DCCT/EDIC participants, the rigorous design of the parent study, the detailed measures of apolipoproteins and ADLS, and the definition and rigorous validation of CVD and MACE end-points. Blood and urine samples were collected and maintained under stringent conditions since collection, and ADLS and apolipoprotein assays were conducted in a single laboratory with robust quality control.

Study limitations include the necessity, due to assay cost, of including only a subset of the EDIC cohort, and the fact that the DCCT/EDIC study comprises predominantly Caucasian (North American) participants and thus has limited 'generalizability' to other populations. ADLS assays require large investments of time, labor, and funds, and standardization across laboratories is challenging: therefore, ADLS assays are currently applicable only for research, and not for clinical application. The power to detect associations between risk factors and the subsequent risk of outcomes in Cox proportional hazards (PH) models is a function of the effect size (i.e., hazard ratio) and the number of observed events. The relatively small number of CVD events in our study (50 'any CVD' events and 24 MACE events) may have limited our power to detect associations between the biomarkers and the risk of CVD. Adjustment for confounding factors in the prospective analyses may have been imperfect. No adjustment for multiple testing was performed, and therefore the results should be interpreted with caution: applying the

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Holm correction for fourteen tests would require that the smallest p-value be ≤ 0.05/14= 0.0035 to reach significance, with larger cutoff values for the other p-values. For example, APOB, APOC3 total, APOC3-HP and APOC3-HS and Lp-B would remain significant after adjustment for the 14 tests (i.e., biomarkers) in the unadjusted models for any CVD.

In conclusion, we provide new, biologically-plausible evidence of prospective associations of serum 'total APOC3' and APOC3 in HDL (APOC3- HS) with cardiovascular events in people with Type 1 diabetes. These associations provide information beyond that yielded by conventional lipid/lipoprotein measures, and may help to elucidate new biomarkers, pathogenic mechanisms and therapeutic targets for the prevention of cardiovascular events in type 1 diabetes.

Competing interests

The authors declare no competing interests.

Authors' contributions

AB, IB, AJJ, JAS. and YZ analyzed data, wrote, reviewed and edited the manuscript. RLK, MF L-V,

WTG, MJB, and PA reviewed and edited the manuscript. TJL wrote, reviewed and edited the manuscript.

TJL is the guarantor of this work. All authors read and approved the final manuscript.

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References:

1.   Laing, S. P., A. J. Swerdlow, S. D. Slater, A. C. Burden, A. Morris, N. R. Waugh, W. Gatling, P. J. Bingley, and C. C. Patterson. 2003. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. *Diabetologia* **46**: 760-765.

2.   Soedamah-Muthu, S. S., J. H. Fuller, H. E. Mulnier, V. S. Raleigh, R. A. Lawrenson, and H. M. Colhoun. 2006. High risk of cardiovascular disease in patients with type 1 diabetes in the U.K.: a cohort study using the general practice research database. *Diabetes Care* **29**: 798-804.

3.   Pease, A., A. Earnest, S. Ranasinha, N. Nanayakkara, D. Liew, N. Wischer, S. Andrikopoulos, and S. Zoungas. 2018. Burden of cardiovascular risk factors and disease among patients with type 1 diabetes: results of the Australian National Diabetes Audit (ANDA). *Cardiovasc. Diabetol.* **17**: 77.

4.   Alaupovic, P., J. M. Bard, M. Tavella, and D. Shafer. 1992. Identification of apoB-containing lipoprotein families in NIDDM. *Diabetes* **41 Suppl 2**: 18-25.

5.   Basu, A., A. J. Jenkins, J. A. Stoner, Y. Zhang, R. L. Klein, M. F. Lopes-Virella, W. T. Garvey, D. S. Schade, J. Wood, P. Alaupovic, and T. J. Lyons. 2018. Apolipoprotein-defined lipoprotein subclasses, serum apolipoproteins, and carotid intima-media thickness in T1D. *J. Lipid Res.* **59**: 872-883.

6.   Blackett, P. R., K. S. Blevins, E. Quintana, M. Stoddart, W. Wang, P. Alaupovic, and E. T. Lee. 2005. ApoC-III bound to apoB-containing lipoproteins increase with insulin resistance in Cherokee Indian youth. *Metabolism* **54**: 180-187.

7.   Sasongko, M. B., T. Y. Wong, T. T. Nguyen, R. Kawasaki, A. J. Jenkins, J. Shaw, C. Robinson, and J. J. Wang. 2012. Serum apolipoproteins are associated with systemic and retinal microvascular function in people with diabetes. *Diabetes* **61**: 1785-1792.

8.   Ley, S. H., S. B. Harris, P. W. Connelly, M. Mamakeesick, J. Gittelsohn, T. M. Wolever, R. A. Hegele, B. Zinman, and A. J. Hanley. 2010. Association of apolipoprotein B with incident type 2 diabetes in an aboriginal Canadian population. *Clin. Chem.* **56**: 666-670.

9.   Kastelein, J. J., W. A. van der Steeg, I. Holme, M. Gaffney, N. B. Cater, P. Barter, P. Deedwania, A. G. Olsson, S. M. Boekholdt, D. A. Demicco, M. Szarek, J. C. LaRosa, T. R. Pedersen, and S. M. Grundy. 2008. Lipids, apolipoproteins, and their ratios in relation to cardiovascular events with statin treatment. *Circulation* **117**: 3002-3009.

10.   Ballantyne, C. M., J. S. Raichlen, and V. A. Cain. 2008. Statin therapy alters the relationship between apolipoprotein B and low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol targets in high-risk patients: the MERCURY II (Measuring Effective Reductions in Cholesterol Using Rosuvastatin) trial. *J. Am. Coll. Cardiol.* **52**: 626-632.

11.   Ooi, E. M., P. H. Barrett, D. C. Chan, and G. F. Watts. 2008. Apolipoprotein C-III: understanding an emerging cardiovascular risk factor. *Clin Sci (Lond)* **114**: 611-624.

12.   Sofat, R., J. A. Cooper, M. Kumari, J. P. Casas, J. P. Mitchell, J. Acharya, S. Thom, A. D. Hughes, S. E. Humphries, and A. D. Hingorani. 2016. Circulating Apolipoprotein E Concentration and Cardiovascular Disease Risk: Meta-analysis of Results from Three Studies. *PLoS Med.* **13**: e1002146.

13.   Kowal, R. C., J. Herz, K. H. Weisgraber, R. W. Mahley, M. S. Brown, and J. L. Goldstein. 1990. Opposing effects of apolipoproteins E and C on lipoprotein binding to low density lipoprotein receptor-related protein. *J. Biol. Chem.* **265**: 10771-10779.

14.   Alaupovic, P. 2003. The concept of apolipoprotein-defined lipoprotein families and its clinical significance. *Curr. Atheroscler. Rep.* **5**: 459-467.

15.   Alaupovic, P. 1996. Significance of apolipoproteins for structure, function, and classification of plasma lipoproteins. *Methods Enzymol.* **263**: 32-60.

16.   Blankenhorn, D. H., P. Alaupovic, E. Wickham, H. P. Chin, and S. P. Azen. 1990. Prediction of angiographic change in native human coronary arteries and aortocoronary bypass grafts. Lipid and nonlipid factors. *Circulation* **81**: 470-476.

17.   Alaupovic, P., H. N. Hodis, C. Knight-Gibson, W. J. Mack, L. LaBree, L. Cashin-Hemphill, C. N. Corder, D. M. Kramsch, and D. H. Blankenhorn. 1994. Effects of lovastatin on ApoA- and ApoB-containing lipoproteins. Families in a subpopulation of patients participating in the Monitored Atherosclerosis Regression Study (MARS). *Arterioscler. Thromb.* **14**: 1906-1913.

18.   Nathan, D. M., S. Genuth, J. Lachin, P. Cleary, O. Crofford, M. Davis, L. Rand, and C. Siebert. 1993. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N. Engl. J. Med.* **329**: 977-986.

19.   Lachin, J. M., N. H. White, D. P. Hainsworth, W. Sun, P. A. Cleary, and D. M. Nathan. 2015. Effect of intensive diabetes therapy on the progression of diabetic retinopathy in patients with type 1 diabetes: 18 years of follow-up in the DCCT/EDIC. *Diabetes* **64**: 631-642.

20.   Nathan, D. M., J. Lachin, P. Cleary, T. Orchard, D. J. Brillon, J. Y. Backlund, D. H. O'Leary, and S. Genuth. 2003. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N. Engl. J. Med.* **348**: 2294-2303.

21.   Polak, J. F., J. Y. Backlund, P. A. Cleary, A. P. Harrington, D. H. O'Leary, J. M. Lachin, and D. M. Nathan. 2011. Progression of carotid artery intima-media thickness during 12 years in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study. *Diabetes* **60**: 607-613.

22.   Basu, A., A. J. Jenkins, Y. Zhang, J. A. Stoner, R. L. Klein, M. F. Lopes-Virella, W. T. Garvey, and T. J. Lyons. 2016. Nuclear magnetic resonance-determined lipoprotein subclasses and carotid intima-media thickness in type 1 diabetes. *Atherosclerosis* **244**: 93-100.

23.   Lyons, T. J., A. J. Jenkins, D. Zheng, R. L. Klein, J. D. Otvos, Y. Yu, D. T. Lackland, D. McGee, M. B. McHenry, M. Lopes-Virella, and W. T. Garvey. 2006. Nuclear magnetic resonance-determined lipoprotein subclass profile in the DCCT/EDIC cohort: associations with carotid intima-media thickness. *Diabetic Med.* **23**: 955-966.

24.   Lopes-Virella, M. F., N. L. Baker, K. J. Hunt, T. J. Lyons, A. J. Jenkins, and G. Virella. 2012. High concentrations of AGE-LDL and oxidized LDL in circulating immune complexes are associated with progression of retinopathy in type 1 diabetes. *Diabetes Care* **35**: 1333-1340.

25.   Hunt, K. J., N. Baker, P. Cleary, J. Y. Backlund, T. Lyons, A. Jenkins, G. Virella, and M. F. Lopes-Virella. 2013. Oxidized LDL and AGE-LDL in circulating immune complexes strongly predict progression of carotid artery IMT in type 1 diabetes. *Atherosclerosis* **231**: 315-322.

26.   Lopes-Virella, M. F., N. L. Baker, K. J. Hunt, J. Lachin, D. Nathan, and G. Virella. 2011. Oxidized LDL immune complexes and coronary artery calcification in type 1 diabetes. *Atherosclerosis* **214**: 462-467.

27.   The DCCT Research Group. 1992. Lipid and lipoprotein levels in patients with IDDM diabetes control and complication. Trial experience. *Diabetes Care* **15**: 886-894.

28.   Burstein, M., H. R. Scholnick, and R. Morfin. 1970. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. *J. Lipid Res.* **11**: 583-595.

29.   Alaupovic, P. 1981. David Rubinstein Memorial Lecture: the biochemical and clinical significance of the interrelationship between very low density and high density lipoproteins. *Can. J. Biochem.* **59**: 565-579.

30.   Hilpert, K. F., S. G. West, P. M. Kris-Etherton, K. D. Hecker, N. M. Simpson, and P. Alaupovic. 2007. Postprandial effect of n-3 polyunsaturated fatty acids on apolipoprotein B-containing lipoproteins and vascular reactivity in type 2 diabetes. *Am. J. Clin. Nutr.* **85**: 369-376.

31.   Marz, W., M. Trommlitz, and W. Gross. 1988. Differential turbidimetric assay for subpopulations of lipoproteins containing apolipoprotein A-I. *J. Clin.Chem. Clin. Biochem.* **26**: 573-578.

32.   Attman, P. O., P. Alaupovic, and A. Gustafson. 1987. Serum apolipoprotein profile of patients with chronic renal failure. *Kid. Internat.* **32**: 368-375.

33.   Lachin, J. M., T. J. Orchard, and D. M. Nathan. 2014. Update on cardiovascular outcomes at 30 years of the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. *Diabetes Care* **37**: 39-43.

34.   DCCT/EDIC Research Group. 2016. Intensive Diabetes Treatment and Cardiovascular Outcomes in Type 1 Diabetes: The DCCT/EDIC Study 30-Year Follow-up. *Diabetes Care* **39**: 686-693.

35.   DCCT/EDIC Research Group. 2016. Risk Factors for Cardiovascular Disease in Type 1 Diabetes. *Diabetes* **65**: 1370-1379.

36.   Bebu, I., B. H. Braffett, R. Pop-Busui, T. J. Orchard, D. M. Nathan, and J. M. Lachin. 2017. The relationship of blood glucose with cardiovascular disease is mediated over time by traditional risk factors in type 1 diabetes: the DCCT/EDIC study. *Diabetologia* **60**: 2084-2091.

37.   Sehayek, E., and S. Eisenberg. 1991. 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.* **266**: 18259-18267.

38.   Zheng, C., C. Khoo, J. Furtado, and F. M. Sacks. 2010. Apolipoprotein C-III and the metabolic basis for hypertriglyceridemia and the dense low-density lipoprotein phenotype. *Circulation* **121**: 1722-1734.

39.   Sundaram, M., S. Zhong, M. Bou Khalil, P. H. Links, Y. Zhao, J. Iqbal, M. M. Hussain, R. J. Parks, Y. Wang, and Z. Yao. 2010. Expression of apolipoprotein C-III in McA-RH7777 cells enhances VLDL assembly and secretion under lipid-rich conditions. *J. Lipid Res* **51**: 150-161.

40.   Wyler von Ballmoos, M. C., B. Haring, and F. M. Sacks. 2015. The risk of cardiovascular events with increased apolipoprotein CIII: A systematic review and meta-analysis. *J. Clin. Lipidol.* **9**: 498-510.

41.   Aroner, S. A., M. Koch, K. J. Mukamal, J. D. Furtado, J. H. Stein, M. C. Tattersall, R. L. McClelland, and M. K. Jensen. 2018. High-Density Lipoprotein Subspecies Defined by Apolipoprotein C-III and Subclinical Atherosclerosis Measures: MESA (The Multi-Ethnic Study of Atherosclerosis). *J. Am. Heart Assoc.* doi: 10.1161/JAHA.117.00782442.

42.   Yamamoto, R., F. M. Sacks, F. B. Hu, B. Rosner, J. D. Furtado, S. A. Aroner, E. Ferrannini, S. Baldi, M. Kozakova, B. Balkau, A. Natali, and M. K. Jensen. 2018. High density lipoprotein with apolipoprotein C-III is associated with carotid intima-media thickness among generally healthy individuals. *Atherosclerosis* **269**: 92-99.

43.   Jensen, M. K., S. A. Aroner, K. J. Mukamal, J. D. Furtado, W. S. Post, M. Y. Tsai, A. Tjonneland, J. F. Polak, E. B. Rimm, K. Overvad, R. L. McClelland, and F. M. Sacks. 2018. High-Density Lipoprotein Subspecies Defined by Presence of Apolipoprotein C-III and Incident Coronary Heart Disease in Four Cohorts. *Circulation* **137**: 1364-1373.

44.   Wallenfeldt, K., L. Bokemark, J. Wikstrand, J. Hulthe, and B. Fagerberg. 2004. Apolipoprotein B/apolipoprotein A-I in relation to the metabolic syndrome and change in carotid artery intima-media thickness during 3 years in middle-aged men. *Stroke* **35**: 2248-2252.

45.   Huang, F., Z. Yang, B. Xu, Y. Bi, M. Xu, Y. Xu, J. Lu, Y. Liu, M. Dai, W. Zhou, W. Wang, and Y. Chen. 2013. Both serum apolipoprotein B and the apolipoprotein B/apolipoprotein A-I ratio are associated with carotid intima-media thickness. *PloS One* **8**: e54628.

46.   Alaupovic, P., W. J. Mack, C. Knight-Gibson, and H. N. Hodis. 1997. 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.* **17**: 715-722.

47.   Gervaise, N., M. A. Garrigue, G. Lasfargues, and P. Lecomte. 2000. Triglycerides, apo C3 and Lp B:C3 and cardiovascular risk in type II diabetes. *Diabetologia* **43**: 703-708.

48.   Knowlton, N., J. A. Wages, M. B. Centola, J. Giles, J. Bathon, C. Quiroga, and P. Alaupovic. 2012. Apolipoprotein B-containing lipoprotein subclasses as risk factors for cardiovascular disease in patients with rheumatoid arthritis. *Arthritis Care Res (Hoboken)* **64**: 993-1000.

Table 1. Participant characteristics of the subgroup full cohort (EDIC 1997-2000)

Variables	Study subgroup (n=465) Weighted[#]	Entire EDIC cohort (n=1389)[*]
Group, intensive % (n)	10 (47)	3.6 (50)
Cohort, primary % (n)	11 (50)	3.6 (50)
Sex, men % (n)	11 (53)	3.8 (53)
Smoking % (n)	3.6 (17)	1.2 (17)
Age (years)	40.8 (34.5, 46.4)	40.5 (35.2, 45.5)
BMI (kg/m^2)	26.3 (23.9, 28.8)	26.4 (24.0, 29.1)
Mean HbA1c (%)	8.2 (7.4, 9.3)	8.0 (7.3, 8.8)
Mean Total Cholesterol (mg/dL)	185 (165, 202)	183 (164, 203)
Mean HDL (mg/dL)	50 (44, 59)	51 (45, 60)
Mean LDL (mg/dL)	115 (98, 133)	113 (97, 131)
Triglycerides (mg/dL)	74 (53, 104)	72 (53, 106)
Mean SBP (mm/Hg)	117 (112, 123)	116 (111, 122)
Mean DBP (mm/Hg)	75 (72, 78)	74 (71, 78)
Mean Pulse Rate (bpm)	74 (70, 78)	74 (69, 78)
Any CVD[¥] % (n)	11 (50)	12 (174)
MACE[¥] % (n)	5 (24)	6 (83)

MACE: major atherosclerotic cardiovascular events
Percentages (n) for binary variables. Median (1st, 3rd quartiles) for continuous variables
[*]Numbers vary depending on availability.
[#]Weighted to account for complications, sex, and the DCCT cohort (primary prevention vs. secondary intervention)
[¥]Incident events (events after EDIC Year 6)

Table 2. Study subset: serum levels of serum apolipoproteins and ADLS, EDIC Year 4-7, stratified by initial DCCT randomization group

Biomarker	Overall (n=465)	Intensive Group (n=219)	Conventional Group (n=246)	p
APOB (mg/dL)	72.5 (62.4, 84.8)	73.3 (64.4, 84.4)	71.8 (61.4, 85.3)	0.53
APOA1 (mg/dL)	146.1 (126.8, 163.6)	141.3 (121.9, 163.7)	149.9 (133.9, 162.9)	0.06
APOA2 (mg/dL)	35.1 (27.0, 43.2)	33.2 (25.5, 42.7)	36.5 (28.4, 43.9)	0.13
APOE (mg/dL)	4.3 (3.6, 4.8)	4.2 (3.5, 4.8)	4.4 (3.7, 4.9)	0.55
APOC3 total (mg/dL)	9.3 (7.6, 11.1)	9.3 (7.6, 11.1)	9.3 (7.7, 11.2)	0.79
APOC3-HP (mg/dL)	2.3 (1.8, 3.1)	2.4 (1.8, 3.1)	2.3 (1.8, 3.1)	0.87
APOC3-HS (mg/dL)	6.8 (5.6, 8.1)	6.8 (5.6, 8.1)	6.8 (5.7, 8.1)	0.57
APOC3 Ratio	2.8 (2.2, 3.5)	2.8 (2.1, 3.4)	2.9 (2.3, 3.5)	0.25
Lp-B (mg APOB/dL)	35.6 (29.2, 41.4)	36.2 (30.0, 42.6)	34.8 (28.7, 41.0)	0.31
Lp-B:C (mg APOB/dL)	11.6 (9.0, 14.4)	11.9 (9.2, 14)	11.1 (8.9, 14.6)	0.40
Lp-A2:B:C:D:E (mg APOB/dL)	12.9 (10.3, 15.6)	12.3 (10.2, 15.6)	13.0 (10.3, 15.6)	0.92
Lp-B:E + Lp-B:C:E (mg APOB/dL)	11.8 (8.9, 15.2)	11.7 (8.9, 15.1)	11.9 (8.9, 15.2)	0.95
Lp-A1 (mg APOA1/dL)	39.9 (34.8, 44.4)	38.9 (33.4, 43.7)	40.8 (36.0, 44.8)	**0.05**
Lp-A1:A2 (mg APOA1/dL)	107.4 (92.4, 120.4)	103.5 (87.5, 120.4)	109.6 (96.5, 120.8)	0.09

[*]Using the weights accounting for complications, sex and the DCCT cohort (primary prevention vs. secondary intervention)
Data presented as: Median (1st, 3rd quartiles) for each variable

Table 3. Cox models: hazard ratios (95% confidence intervals): associations of apolipoproteins and ADLS with risk of 'any CVD'

Variable	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]		Model 5[e]	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
APOB (mg/dL)	1.02 (1.01, 1.03)	**0.004**	1.02 (1.00, 1.04)	**0.027**	1.01 (0.99, 1.03)	0.30	1.01 (0.98, 1.04)	0.25	1.01 (0.98, 1.03)	0.39
APOA1 (mg/dL)	1.01 (0.99, 1.02)	0.19	1.00 (0.98, 1.01)	0.92	1.00 (0.98, 1.02)	0.77	1.00 (0.98, 1.02)	0.86	1.00 (0.98, 1.02)	0.79
APOA2 (mg/dL)	1.02 (0.99, 1.04)	0.15	1.01 (0.98, 1.03)	0.44	1.01 (0.98, 1.04)	0.37	1.01 (0.98, 1.04)	0.36	1.01 (0.98, 1.04)	0.28
APOE (mg/dL)	1.13 (0.87, 1.46)	0.35	0.99 (0.73, 1.35)	0.96	0.92 (0.67, 1.24)	0.57	1.01 (0.72, 1.43)	0.94	0.88 (0.64, 1.23)	0.47
APOC3 total (mg/dL)	1.15 (1.08, 1.22)	**<0.0001**	1.10 (1.03, 1.18)	**0.003**	1.07 (0.98, 1.18)	0.12	1.10 (1.01, 1.19)	**0.022**	1.07 (0.98, 1.17)	0.11
APOC3-HP (mg/dL)	1.18 (1.07, 1.32)	**0.001**	1.11 (0.98, 1.23)	0.07	1.00 (0.87, 1.15)	0.97	1.08 (0.95, 1.21)	0.21	0.99 (0.86, 1.15)	0.97
APOC3-HS (mg/dL)	1.28 (1.14, 1.45)	**<0.0001**	1.24 (1.11, 1.39)	**0.0002**	1.19 (1.03, 1.37)	**0.019**	1.26 (1.11, 1.45)	**0.001**	1.18 (1.04, 1.35)	**0.012**
APOC3 Ratio	0.73 (0.48, 1.12)	0.15	0.76 (0.51, 1.14)	0.18	0.98 (0.57, 1.69)	0.94	0.83 (0.55, 1.26)	0.38	1.05 (0.62, 1.77)	0.84
Lp-B (mg APOB/dL)	1.04 (1.01, 1.06)	**0.001**	1.03 (1.01, 1.06)	**0.014**	1.02 (0.98, 1.05)	0.22	1.02 (0.98, 1.06)	0.16	1.02 (0.98, 1.05)	0.32
Lp-B:C (mg APOB/dL)	1.06 (0.99, 1.13)	0.08	1.05 (0.97, 1.14)	0.18	1.02 (0.94, 1.09)	0.64	1.02 (0.92, 1.14)	0.64	1.02 (0.94, 1.11)	0.67
Lp-A2:B:C:D:E (mg APOB/dL)	1.06 (0.99, 1.14)	0.12	1.05 (0.97, 1.14)	0.22	1.03 (0.94, 1.11)	0.51	1.04 (0.95, 1.13)	0.39	1.03 (0.95, 1.11)	0.44
Lp-B:E + Lp-B:C:E (mg APOB/dL)	1.03 (0.97, 1.08)	0.26	1.00 (0.95, 1.05)	0.91	0.99 (0.93, 1.05)	0.74	0.98 (0.92, 1.05)	0.57	0.98 (0.92, 1.05)	0.69
Lp-A1 (mg APOA1/dL)	1.01 (0.97, 1.05)	0.64	0.98 (0.94, 1.02)	0.35	0.98 (0.94, 1.02)	0.42	0.98 (0.94, 1.03)	0.46	0.98 (0.94, 1.02)	0.38
Lp-A1:A2 (mg APOA1/dL)	1.01 (0.99, 1.03)	0.12	1.00 (0.98, 1.02)	0.64	1.00 (0.98, 1.02)	0.50	1.00 (0.98, 1.02)	0.61	1.00 (0.98, 1.03)	0.49

P values in bold if nominally significant (<0.05)
ADLS: apolipoprotein-defined lipoprotein subclasses
[a]Model 1: unadjusted
[b]Model 2: adjusted for age and mean HbA1c
[c]Model 3: adjusted for age, mean HbA1c and triglycerides (log transformed)
[d]Model 4: adjusted for age, mean HbA1c and LDL-cholesterol
[e]Model 5: adjusted for age, mean HbA1c, mean systolic blood pressure, mean pulse rate and current triglycerides (log transformed)

Table 4. Cox models for the associations of serum apolipoprotein and ADLS biomarkers and the risk of MACE

Variable	Model 1[a]		Model 2[b]		Model 3[c]		Model 4[d]	
	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P	HR (95% CI)	P
APOB (mg/dL)	1.01 (0.99, 1.03)	0.12	1.01 (0.99, 1.04)	0.27	1.00 (0.98, 1.03)	0.80	1.01 (0.97, 1.05)	0.45
APOA1 (mg/dL)	1.00 (0.99, 1.02)	0.30	0.99 (0.98, 1.01)	0.41	0.99 (0.98, 1.01)	0.55	0.99 (0.98, 1.00)	0.37
APOA2 (mg/dL)	1.00 (0.97, 1.04)	0.66	0.98 (0.95, 1.02)	0.44	0.98 (0.91, 1.07)	0.48	0.98 (0.95, 1.02)	0.44
APOE (mg/dL)	1.20 (0.85, 1.69)	0.28	1.07 (0.64, 1.80)	0.78	0.99 (0.61, 1.62)	0.98	1.09 (0.63, 1.88)	0.75
APOC3 total (mg/dL)	1.15 (1.05, 1.25)	**0.001**	1.10 (1.02, 1.19)	**0.013**	1.07 (0.95, 1.19)	0.22	1.11 (1.01, 1.22)	**0.026**
APOC3-HP (mg/dL)	1.16 (1.04, 1.29)	**0.007**	1.08 (0.96, 1.22)	0.17	0.93 (0.77, 1.13)	0.50	1.07 (0.94, 1.22)	0.26
APOC3-HS (mg/dL)	1.28 (1.08, 1.53)	**0.004**	1.19 (1.02, 1.39)	**0.026**	1.13 (0.93, 1.36)	0.19	1.22 (1.02, 1.45)	**0.027**
APOC3 Ratio	0.82 (0.46, 1.43)	0.48	0.81 (0.47, 1.37)	0.43	1.05 (0.51, 2.15)	0.90	0.83 (0.49, 1.39)	0.48
Lp-B (mg APOB/dL)	1.02 (0.99, 1.05)	0.11	1.02 (0.98, 1.06)	0.30	1.00 (0.96, 1.03)	0.94	1.01 (0.97, 1.06)	0.51
Lp-B:C (mg APOB/dL)	1.08 (1.00, 1.17)	**0.046**	1.09 (0.99, 1.20)	0.06	1.05 (0.97, 1.14)	0.21	1.09 (0.96, 1.25)	0.16
Lp-A2:B:C:D:E (mg APOB/dL)	1.02 (0.92, 1.13)	0.72	1.01 (0.89, 1.15)	0.81	0.98 (0.87, 1.12)	0.83	1.00 (0.88, 1.15)	0.92
Lp-B:E + Lp-B:C:E (mg APOB/dL)	1.02 (0.95, 1.09)	0.46	0.99 (0.92, 1.07)	0.91	0.98 (0.91, 1.07)	0.74	0.98 (0.89, 1.07)	0.69
Lp-A1 (mg APOA1/dL)	1.01 (0.96, 1.07)	0.58	0.97 (0.92, 1.03)	0.35	0.97 (0.92, 1.03)	0.42	0.97 (0.92, 1.03)	0.37
Lp-A1:A2 (mg APOA1/dL)	1.01 (0.99, 1.03)	0.15	0.99 (0.98, 1.01)	0.66	0.99 (0.98, 1.02)	0.81	0.99 (0.97, 1.01)	0.57

P values in bold if nominally significant (<0.05)

ADLS: apolipoprotein-defined lipoprotein subclasses; MACE: major atherosclerotic cardiovascular events

[a]Model 1: unadjusted

[b]Model 2: adjusted for age and mean HbA1c

[c]Model 3: adjusted for age, mean HbA1c and triglycerides (log transformed)

[d]Model 4: adjusted for age, mean HbA1c and LDL-cholesterol

Figure 1. Kaplan-Meier survival curves for the time to any CVD and time to MACE.