Published in final edited form as:
J Diabetes Complications. 2013 ; 27(5): 447–453. doi:10.1016/j.jdiacomp.2013.06.002.

Apolipoprotein-defined Lipoproteins and Apolipoproteins: Associations with Abnormal Albuminuria in Type 1Diabetes in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications Cohort

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Duality of Interest: There are no relevant conflicts of interest.

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Abstract

Aims—Dyslipoproteinemia has been associated with nephropathy in diabetes, with stronger correlations in men than in women. We aimed to characterize and compare plasma lipoprotein profiles associated with normal and increased albuminuria in men and women using apolipoprotein-defined lipoprotein subclasses and simple apolipoprotein measures.

Methods—This is a cross-sectional study in a subset (154 women and 282 men) of the Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) cohort, using samples obtained in 1997-9. Immunochemical methods were used to quantify plasma apolipoprotein-based lipoprotein subclasses and individual apolipoprotein levels.

Results—In adjusted analyses, elevated Lipoprotein-B (Lp-B) was significantly associated with macroalbuminuria in men [odds ratios (OR) and 95% confidence interval (CI): 2.13 (1.15-3.97)] and women [3.01 (1.11-8.12)], while association with Lp-B:C was observed only in men [1.84 (1.19-2.86)]. For individual apolipoproteins the following significant associations with macroalbuminuria were observed in men only: Apolipoprotein B (ApoB) [1.97 (1.20-3.25)], Apo-AII [0.52 (0.29-0.93)], ApoC-III [1.95 (1.16-3.30)], “ApoC-III in VLDL” (heparin-manganese precipitate) [1.88 (1.16-3.04)], and “ApoCIII in HDL” (heparin-manganese supernatant) [2.03 (1.27-3.26)], all $P<0.05$).

Conclusions—Atherogenic apolipoprotein-based profiles are associated with nephropathy in Type 1 diabetic men and to a lesser extent in women. The difference could result from the greater prevalence and severity of dyslipoproteinemia, and from the greater prevalence of renal dysfunction, in men vs women.

Keywords

Type 1 diabetes; nephropathy; albuminuria; lipoproteins; apolipoproteins

Introduction

In diabetes, albuminuria is associated with progression of nephropathy, retinopathy, and cardiovascular disease (CVD).[1, 2] Control of hyperglycaemia, smoking, hypertension, and dyslipoproteinaemia can prevent, retard, and even reverse renal damage.[1-4] While links between renal disease and dyslipoproteinaemia are recognized[1, 2, 4], the associations between renal dysfunction and specific lipoprotein subclasses are not fully elucidated. The present study seeks new detail concerning associations between nephropathy and dyslipoproteinaemia in Type 1 diabetes mellitus (T1DM).

Non-obese people with T1DM with “near normal” glycemia and normal renal function tend to exhibit normal “conventional” lipid profiles.[2] With renal damage, total and LDL-Cholesterol (-C), intermediate density lipoproteins (IDL-C), triglycerides, and ApoB levels rise, and HDL-C and ApoA-I levels fall, resulting in a profile that more closely resembles that found in people with Type 2 diabetes (T2DM).[2] Lipoprotein changes with renal damage have been demonstrated in the Diabetes Control and Complications Trial/Epidemiology of

Diabetes Interventions and Complications (DCCT/EDIC) cohort.[5, 6] In these studies, pro-atherogenic lipoprotein associations with nephropathy were stronger in men than women. Methods included (density-based) ultracentrifugation[5] and nuclear magnetic resonance (NMR) spectroscopy, the latter reflecting particle number and size.[6]

Apolipoproteins are major constituents of lipoprotein particles, with structural, enzymatic, and receptor-binding roles.[2, 7] The present study employs a relatively rarely-used method for lipoprotein sub-classing. Intact lipoproteins can be categorized by their apolipoprotein complement, a critical determinant of lipoprotein function and metabolism, rather than by their size, density, or charge. It is important to distinguish this characterization of intact particles from "raw" apolipoprotein concentrations (also used), which provide different information, since the same apolipoprotein may be found in several lipoprotein subclasses.

Lipoproteins can be divided into two major classes, one containing ApoB (density 0.92-1.063 g/mL) and the other, ApoA-I (1.063-1.21 g/mL).[7] Lipoproteins may therefore be classified and named according to their qualitative apolipoprotein content, recognizing that there is never more than one ApoB per particle. There are five ApoB-containing subclasses, of which two are cholesterol-rich: Lipoprotein B (Lp-B) and Lipoprotein B:E (Lp-B:E), and three are triglyceride-rich: Lipoprotein B:C (Lp-B:C), Lipoprotein B:C:E (Lp-B:C:E) and Lipoprotein A-II:B:C:D:E (Lp-A-II:B:C:D:E). These groupings of cholesterol- and triglyceride-rich ApoB-containing particles approximate density-defined LDL and VLDL respectively. Levels of each are expressed in terms of ApoB (mg/dL). There are two ApoA-I-containing subclasses that are in essence, HDL subclasses: Lipoprotein A-I (Lp-A-I) and Lipoprotein AI:A-II (Lp-A-I:A-II). Levels are expressed in terms of ApoA-I (mg/dL).[7]

Of the "raw" apolipoproteins, attention has focused on ApoCIII owing to its atherogenicity.[8, 9] Triglyceride-rich particles (mainly VLDL) with ApoCIII are designated as "ApoC-III-HP" [precipitated by heparin-manganese (Mn)]. HDL particles with ApoCIII that remain in the supernatant after heparin-Mn precipitation are designated as "ApoC-III-HS". The ApoC-III-HS to ApoC-III-HP ratio is called the "ApoC-III ratio" (ApoC-IIIR), and a lower value reflects slower catabolism of triglyceride-rich lipoproteins.[7]

Apolipoprotein-defined subclasses differ in pathogenicity. The Cholesterol Lowering Atherosclerosis Study[10] and Monitored Atherosclerosis Regression Study [11] showed that increased triglyceride-rich lipoproteins (Lp-B:C and Lp-AII:B:C:D:E) or, in general, the amount of ApoC-III bound to ApoB-containing lipoproteins, was associated with progression of atheroma. Particles containing both ApoCIII and ApoB have also been associated with insulin resistance[12], T2DM[13], and renal failure.[14] Levels of these particles, as well as ApoB-only containing lipoproteins (Lp-B), have been associated with the progression of coronary artery[11] and renal disease.[15]

Thus, evidence links dyslipoproteinemia, particularly ApoB- and ApoCIII-containing lipoproteins, with renal damage and atherosclerosis. Since these associations have not been studied in T1DM, we assessed relationships between nephropathy and apolipoprotein-defined lipoprotein subclasses and individual apolipoprotein levels in a cross-sectional sub-study of the DCCT/EDIC cohort.

Methods

Subjects

The DCCT involved 1,441 patients with T1DM and tested whether intensive therapy of T1DM aimed at achieving near normal glycemic levels would ameliorate complications compared with conventional therapy. After a mean of 6.5 years, the DCCT was stopped because of major benefit of intensive vs. conventional therapy on microvascular damage.[3] All subjects were invited to join EDIC[16], an ongoing observational study. Each EDIC subject has an annual clinical and biochemical assessment with determination of fasting lipid profiles in alternate years. Also, as described previously, AER and calculated creatinine clearance are determined using 4hr urine collections obtained the same day as the blood draw.[6, 16] Retinopathy is assessed using photography at the visit alternate from that at which fasting blood is taken.[17]

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 centers participated, and in 1997-9, samples were sent to MUSC. The study, which meets Declaration of Helsinki guidelines, was approved by the Institutional Review Boards at MUSC and all participating DCCT/EDIC centers. Each subject gave written informed consent. Of the 1,441 DCCT participants, 1063 agreed to participate in the MUSC program. Within the group of participants, those with albumin excretion rate (AER) above 40 mg/24 hr at the previous visit were selected as cases, and 3 to 4 participants without abnormal albuminuria at the previous visit were selected as controls. Thus, in the selection of these 436 subjects, those with abnormal albuminuria (AER > 40 mg/24 hrs) at the previous visit were oversampled; resulting in 95 of the 436 patients having increased AER and 341 of the patients having normal AER values.

Sample collection

Venous blood, collected after an overnight fast prior to insulin, was processed as previously reported.[16] Samples were stored (-70°C) until analysis. The DCCT/EDIC Central Laboratory performed the assays for conventional lipids, HbA_{1c} and serum creatinine.[18]

Estimated glucose disposal rate (eGDR)

Estimated glucose disposal rate (eGDR), an estimate of insulin sensitivity was calculated: $24.31 - 12.22(WHR) – 3.29(HT) – 0.57(HbA_{1c})$ $mg.kg^{-1}.min^{-1}$, where HT is hypertension (no=0, yes=1).[19]

Conventional lipids

Total and HDL-cholesterol, and triglyceride, were determined by autoanalyzer, and LDL-C was estimated by Friedewald's formula if triglycerides<400mg/dL. If triglycerides were >400mg/dL, LDL-C was determined after VLDL removal.[20]

Apolipoproteins and apolipoprotein-defined lipoprotein subclasses

Apolipoproteins were quantified by electroimmunoassays for ApoA-I, ApoA-II, ApoB, ApoC-III, and ApoE. [21] Quantification of ApoC-III bound to ApoA- and to ApoB-

containing lipoproteins was performed on heparin-Mn supernatants (ApoC-III-HS) and precipitates (ApoC-III-HP), respectively.[21] Quantification of ApoB-containing subclasses was by immunoprecipitation of plasma with polyclonal antisera to ApoA-II, ApoE and ApoC-III.[22] Lp-B, Lp-B:C, Lp-B:E + Lp-B:C:E and Lp-A-II:B:C:D:E particle levels were expressed according to ApoB content. For ApoA1-containing lipoproteins, Lp-A-I and Lp-A-I:A-II were measured by differential turbidimetry.[23]

Statistics

Concentrations of lipoproteins were measured at samples taken from 1997-1999 and examined in relation to the odds of concurrent increased levels of albumninuria. Biomarkers were standardized such that a one unit change corresponded to a standard deviation for each biomarker. It is also well known that gender has an impact on lipoprotein levels; thus, in addition to analysis of the entire cohort, analyses were also stratified by gender.[6, 17, 18, 24]

Baseline covariates for the current analyses were obtained from the concurrent physical examination and laboratory data (fasting lipids and renal function). Outcomes of interest were defined as normal AER (all AER < 40 mg/24 hrs); microalbuminuria (40 mg/24 hrs ≤ AER < 300 mg/24 hrs); and macroalbuminuria (AER ≥ 300 mg/24 hrs). Standard descriptive statistics were used to summarize the general demographic and clinical data. A linear trend test was used to evaluate continuous demographic and clinical measures across albuminuria outcomes; the Cochran-Armitage trend test was used to assess the relationship for categorical variables. Similarly, raw differences in the measured lipoprotein levels across albuminuria outcomes were examined using an analysis of variance framework.

To account for the uneven sampling (oversampling of those with increased AER) inverse probability weighted generalized logistic regression models (with 95% CI's) were used to quantify the association of lipoprotein levels and of clinical and demographic characteristics on the presence of micro and macroalbuminuria. The primary parameter of interest in the logistic regression models is the change in the log-odds (with 95% Wald CI) for the presence of micro or macroalbuminuria as compared to those with normal albuminuria levels. Models are adjusted for design variables and known risk factors of abnormal albunimuria; adjusted models contain DCCT randomized treatment, baseline retinopathy cohort, gender, smoking status, diabetes duration, eGDR, the use of any ACE/ARB drugs, the use of lipid lowering drugs, and the DCCT baseline levels of AER.

All statistical analyses were performed using SAS System version 9.3 (SAS Institute, Cary, NC, USA). A type I error rate was controlled for significance at 0.05 for all analysis.

Results

Baseline Characteristics

Table 1 shows demographic and clinical characteristics by albuminuria status. Those with elevated AER (micro and macroalbuminuria) were less likely to have been in the experimental treatment group, had higher systolic and diastolic blood pressures, HbA1c, triglycerides, total and LDL cholesterol (macroalbuminuria only), Early Treatment Diabetic Retinopathy Study (ETDRS) scores and percentage of smokers, lower eGDR and standard

creatinine clearance rate (macroalbuminuria only), and were more likely to be taking ACE inhibitors or lipid lowering drugs in comparison to those with normal AER status (P≤0.01). Due to over-sampling (inclusion of all available samples from study subjects with micro/macro-albuminuria), our sub-set differed from the remainder of the DCCT/EDIC cohort. At the time of sampling, they had significantly higher HbA1c (8.3 vs. 8.1%; p=0.002) and longer duration of T1DM (19.0 vs. 17.6 y; p<0.001), but similar age, lipid levels, systolic and diastolic blood pressure, body mass index, and proportion of smokers. They were less likely to have been complication-free at baseline (37.6 vs. 55.9%; p<0.001), more likely to be male (64.7 vs. 47.7%; p<0.001), but equally distributed between former DCCT treatment randomization groups (data not shown).

Apolipoprotein-Defined Lipoprotein Subclasses (Unadjusted Analyses)

In a univariate analysis, mean Lp-B levels were significantly higher in both men and women with macroalbuminuria when compared to microalbuminuria and normal urinary albumin groups (p<0.05, Table 2). Lp-B:C was significantly higher, while Lp-A-I:A-II was significantly lower, only in men with macroalbuminuria and microalbuminuria, respectively, versus normal AER group ((p<0.05, Table 2). No other differences were noted in the remaining apolipoprotein-defined lipoprotein subclasses.

Individual Apolipoproteins (Unadjusted Analyses)

For the individual apolipoproteins, levels of ApoB, ApoC-III, ApoC-IIIHP, and ApoC-IIIHS were significantly elevated in men with macroalbuminuria when compared to microalbuminuria and normal urinary albumin groups (P<0.001; Table 2). In contrast, ApoC-III Ratio (HS/HP) was significantly lower in men with elevated versus normal AER levels. However, no significant differences in any of these individual apolipoproteins were noted in women. In case of Apo-AI, levels were significantly lower only in men with microalbuminuria, while Apo-AII was lower in men with elevated AER and in women with macroalbuminuria versus normal AER group (p<0.01, Table 2). Finally, ApoE was lower in both men and women with macroalbuminuria versus normal AER subjects (Table 2).

Apolipoprotein-Defined Lipoprotein Subclasses (Adjusted Analyses)

In adjusted model, elevated Lp-B was significantly associated with higher odds of macroalbuminuria both men and women (p=0.017 and p=0.03, respectively, Table 3), while the association with Lp-B:C was evident only in males (p=0.007). No significant relationships were seen for other subclasses with either micro or macroalbuminuria (Table 3).

Individual Apolipoproteins (Adjusted Analyses)

In adjusted model, significant associations of individual apolipoproteins with abnormal albuminuria were observed only in men but none in women. Elevated ApoB and measures of the triglyceride rich ApoC-III were significantly associated with macroalbuminuria (ApoB: p=0.008; ApoC-III: p=0.012; ApoC-III HP: p=0.010; ApoC-IIIHS: p=0.003, Table 3). Although the direction of the HS to HP ratio of ApoC-III shows evidence of protection when the levels of HS are greater than HP, their associations with abnormal albuminuria

were not statistically significant in either men or women. Reduced Apo-AII concentrations were significantly associated with the presence of both micro- and macroalbuminuria in men ($p<0.001$ and $p=0.026$, respectively), thus suggesting a protective effect. No such associations were noted in cases of Apo-AI or ApoE in either gender (Table 3).

Secondary Covariates

Additional risk factors associated with the presence of macroalbuminuria included intensive vs. conventional DCCT treatment group (0.18 (0.07-0.45), $p<0.001$), increased levels of AER at DCCT baseline (1.03 (1.01-1.05), $p=0.002$) and increased concurrent eGDR was associated with decreased odds of macroalbuminuria (0.67 (0.56-0.82), $p<0.001$). Additionally, there was an increased odds of both micro- and macroalbuminuria associated with increased mean DCCT HbA_{1C} (1.56 (1.20-2.01), $p<0.001$ and 2.52 (1.82-3.48), $p<0.001$, respectively).

Discussion

Detailed apolipoprotein-defined lipoprotein subclass and individual apolipoprotein levels were analyzed in a subset of the DCCT/EDIC cohort selected for the presence or absence of abnormal albuminuria (micro- and macroalbuminuria) reflected by AER. In our study, we noted significant gender-related differences in profiles of apolipoprotein-defined lipoprotein subclasses and individual apolipoproteins associated with the presence of nephropathy. For men, ApoB levels and two ApoB-containing subclasses, Lp-B and Lp-B:C, were higher in the presence of nephropathy, while LpAI:AII was lower among those with microalbuminuria only. Among ‘raw’ apolipoprotein measures, multiple associations with nephropathy were observed in men, but fewer in women. The lower ApoC-III Ratio in nephropathic males may suggest impaired lipolysis of triglyceride-rich lipoproteins. Interestingly, all three measures of ApoC-III were significantly elevated in men with nephropathy when compared to those with micro- and normal albumin levels, while no such differences were observed in women. In women, significantly higher Lp-B, but lower ApoA-II and ApoE were associated with nephropathy in comparison to normal AER group; but only Lp-B remained significant in adjusted analyses. The gender differences might be explained by the smaller numbers and smaller differences between those with and without the complication in women versus men.

The stronger association of dyslipoproteinemia with AER in men than in women in the DCCT/EDIC, as revealed in the present study, and in the previously reported NMR-based findings by our group[6], may partly explain the higher susceptibility of men to nephropathy. Previous T1DM studies, in DCCT/EDIC and in other cohorts, also confirm unfavorable albuminuria outcomes in men compared with women.[25-28] These differences might stem from sex differences in “nephrotoxic” or “nephroprotective” lipoprotein effects, perhaps mediated by hormonal effects on lipoprotein receptors or enzymes, or other factors such as blood pressure or smoking. The associations of the chemical and metabolic characteristics of lipoproteins with nephropathy, and the effects of gender, are addressed by our present study. Importantly, apolipoprotein-defined lipoprotein subclass analysis has the potential to

provide novel information beyond that obtained with conventional lipid profiles, as well as clues to mechanisms of renal damage, and the identification of therapeutic targets.

Our new findings involve particle characteristics of all three major 'conventional' lipoprotein classes (VLDL, LDL, HDL) that cannot be discerned by conventional lipid analyses, measurement of individual apolipoproteins, or NMR analysis.[6] These various methods to describe plasma lipoproteins are thus complementary to one another. In the present study, we show that, in men and women, cholesterol-rich particles that contain only ApoB (i.e. Lp-B, which may represent small dense LDL) are strongly associated with nephropathy. This is consistent with our NMR finding of a significant positive association of the smallest LDL subclass with nephropathy.[6] Concerning 'raw' ApoB levels, in our earlier DCCT/EDIC-based cross-sectional study, ApoB levels (by nephelometry) were positively related to renal damage in men and in the total cohort (borderline in women).[6] Elevated ApoB confers increased coronary artery disease risk, and has been associated with accelerated decline in glomerular filtration rate in nephropathic T1DM subjects.[29] In the present study, elevated ApoB levels were associated with macroalbuminuria in men only. Several other reports from the DCCT/EDIC cohort have also identified significant associations between conventional lipid profiles and/or ApoB and diabetic renal disease.[25, 30, 31] Ongoing DCCT/EDIC cohort follow-up will further identify which standard lipid and apolipoprotein measures, NMR, and apolipoprotein-defined lipoprotein subclasses are most predictive of progression or regression of nephropathy in T1DM.

In spite of absence of HDL-C differences according to renal status in the current study, or clear-cut differences by NMR-based analyses in our earlier work[6], apolipoprotein-defined subclass analysis in the present study revealed lower LpAI:AII particle levels in men with micro-, but not macroalbuminuria versus normal AER group. In contrast, LpA-I particles did not differ according to AER. "Raw" apolipoprotein data revealed significantly lower ApoA-II levels in both men and women in the presence of nephropathy versus normal urinary albumin levels. These differences across HDL-related subclasses and apolipoproteins may be the result of the small sample size of participants with nephropathy in our study. In cross-sectional studies, plasma Apo-AI and Apo-A-II were shown to be significantly lower in type 1 diabetic patients with nephropathy versus those without nephropathy,[32] and in (non-diabetic) patients with chronic renal failure versus non-diabetic and type 2 diabetic controls.[21] In a 10-year prospective type 1 diabetes study, low HDL-C predicted renal disease[33], perhaps attributable to the vaso-protective, anti-oxidant, anti-inflammatory, anti-thrombotic and reverse cholesterol transport functions of HDL.[34] However, none of these previous studies involved detailed lipoprotein analyses, such as NMR-derived measures or measurements of detailed apolipoproteins. Thus, our current findings of inverse associations between Apo-A-II levels and nephropathy status, and our previously reported data on modest differences in levels of NMR-derived HDL subclasses in association with nephropathy,[6] warrant further investigation in longitudinal studies of larger sample size and detailed HDL-related subclass analyses in T1DM.

ApoE, which is synthesized in many tissues, including kidney, liver, and adrenal glands, is known to associate with VLDL, IDL, and HDL, and modulates their affinity with 'remnant' (ApoE-) and LDL (ApoB-) receptors.[35] There are three common ApoE isoforms (2, 3, and

4) with frequencies of 0.08, 0.78, and 0.14, respectively. ApoE genotype has been associated with combined hyperlipidemia (ApoE2), atherosclerosis (ApoE4), and in some but not all studies with diabetic nephropathy (increased ApoE2, decreased ApoE4).[36-38] In the Pittsburgh Epidemiology of Diabetes Complications study, the presence of ApoE2 or ApoE4 allele was significantly associated with overt nephropathy in T1DM. [39] In our study, levels of ApoE-containing lipoproteins (LpA-II:B:C:D:E and Lp-B:E + Lp-B:C:E) did not differ significantly by AER, but plasma ApoE levels (which include ApoE from several lipoprotein subclasses combined) were significantly lower in men and women with macroalbuminuria vs. normal AER. In a small cross-sectional study using proteomic and western blot analyses, serum ApoE was lower in subjects with vs. without renal damage in type 2 diabetes.[40] Thus, the associations of ApoE (protection vs. susceptibility) with nephropathy in T1DM, revealed by ApoE levels, or ApoE polymorphisms and their interaction with other risk factors of macroalbuminuria remain to be clarified.

We found increased ApoC-III-containing particles in diabetic men, but not in women with nephropathy. This is consistent with our earlier report in DCCT/EDIC subjects in which ‘raw’ serum ApoC-III levels (by ELISA) were related to AER in adjusted analyses including gender.[41] ApoB-containing lipoproteins enriched in ApoC-III inhibit lipoprotein lipase (LPL), retarding triglyceride-rich lipoprotein catabolism. These particles also interfere with LDL receptor binding, potentially increasing lipoprotein circulation time and (pathogenic) glycation.[42] ApoC-III can also induce (pro-inflammatory) cell adhesion molecules (CAMs)[9], which play a critical role in the process of atherogenesis. Both circulating and renal CAM levels are increased in diabetic nephropathy.[43, 44] Thus, the atherogenic and nephrotoxic effects of ApoB-containing lipoproteins that also contain ApoC-III may be enhanced in T1DM.

Our cross-sectional study cannot address the extent to which dyslipoproteinemia is a cause or effect of increased AER in diabetes. Our study limitations also include a small sample size, especially in the micro- and macroalbuminuria groups, when compared to those with normal AER, among males and females. However, this could be explained by the fact that our sub-set was selected from the larger DCCT/EDIC prospective cohort that recruited young type 1 diabetic patients with absence of dyslipidemia and renal diseases at baseline.[3] Previous cross-sectional and longitudinal studies in the DCCT/EDIC have shown that total cholesterol, triglyceride, and ApoB levels[6, 25, 30, 31], and low HDL-C[31], predict nephropathy; our cross-sectional study provides evidence on the lipoprotein subclasses that may be involved. Also, our observed associations between elevated cholesterol-rich- (Lp-B and ApoB) and triglyceride-rich (Lp-B:C and ApoC-III) subclasses and individual apolipoproteins, and presence of nephropathy, remained significant following adjustments of several factors known to influence lipid metabolism. In general, the lipoprotein characteristics associated with nephropathy in the present study are associated with increased CVD risk, obesity, and insulin resistance, which in turn are emerging as risk factors for nephropathy in T1DM. [26, 45]

Renal disease is ameliorated by lipid-lowering drugs[2], including in patients with T1DM.[46] In the Fenofibrate and Event Lowering in Diabetes (FIELD) study of T2DM, fenofibrate, which decreases triglyceride levels and increases HDL-C and LDL size, ameliorated

diabetic nephropathy.[47] Of note, LDL-C levels of our study subjects at sample acquisition were above the current recommended LDL-C target (100mg/dl)[48], hence more subjects today may be treated with 'statins', which may protect against micro- and macrovascular complications.[1, 2]

In conclusion, in men with T1DM, nephropathy, as assessed by AER, is associated with multiple features of "atherogenic dyslipoproteinemia" (elevated Lp-B, Lp-B:C, ApoB, ApoC-III measures and lower ApoC-III Ratio and ApoE). Fewer associations were observed in women. Protective associations of ApoA-containing subclasses with nephropathy were also observed in men. Future clinical and basic studies are needed to clarify the role of lipoproteins in renal disease, to improve understanding of disease mechanisms, and to develop new treatments. Relevant genotypes and lipoprotein-associated enzyme activities should also be evaluated. Apolipoprotein-based subclass analysis is currently costly and laborious for clinical application, but in the research context it can facilitate understanding of mechanisms, not only of diabetic nephropathy but also of other vascular complications of diabetes.

Acknowledgments

A complete list of participants in the DCCT/EDIC Research Group can be found in New England Journal of Medicine, 2011;365:2366-2376.

The authors thank DCCT/EDIC patients, study nurses and co-ordinators. Technical assistance of Karina Moller, Kevin Joyce, Yanis Bellil, Lyle Walton, Leslie Potter, Andrea Semler, Jenny Smith, Leslie Nichols, Hillarie Stecker and Azar Dashti is acknowledged.

Grants were provided by: the American Diabetes Association, the National Institutes of Health NIH (PO1 HL55782), Juvenile Diabetes Research Foundation (# 4-1998-272, 996001, and 197028), the Diabetes Research and Wellness Foundation, Inc. (Fairfax, Virginia), and the Presbyterian Health Foundation of Oklahoma City. The DCCT/EDIC is sponsored through research contracts from the Division of Diabetes, Endocrinology and Metabolic Diseases of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). Additional support was provided by the NIH (NCRR) Grants M01-RR-1070 and M01 RR-14467 to the General Clinical Research Centers at MUSC and OUHSC, and by Genentech, Inc. through a Cooperative Research and Development Agreement with the NIDDK.

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Abbreviations

Apo	Apolipoprotein
CVD	Cardiovascular Disease
-C	-Cholesterol
DCCT	Diabetes Control and Complications Trial
EDIC	Epidemiology of Diabetes Intervention and Complications
FIELD	Fenofibrate Intervention and Event Lowering in Diabetes
Lp	Lipoprotein
T1DM	Type 1 diabetes mellitus

Table 1

Demographic and clinical characteristics of analysis sample by concurrent albuminuria status.

Variable	All Subjects (n=436)	Albuminuria Group			P Value
		Normal (n=341)	Micro (n=50)	Macro (n=45)	
Age (yrs)	40.2 ± 7.1	40.4 ± 6.9	39.6 ± 7.7	39.5 ± 8.2	0.764
Male % (n)	64.7 (282)	62.8 (214)	66.0 (33)	77.8 (35)	0.137
Intensive Treatment % (n)	47.9 (209)	54.0 (184)	30.0 (15)	22.2 (10)	***<0.001***
Primary Prevention Cohort % (n)	37.6 (164)	39.9 (136)	30.0 (15)	28.9 (13)	0.179
Duration of T1DM (yrs)	19.0 ± 4.4	19.0 ± 4.5	19.1 ± 4.5	19.5 ± 4.1	0.591
BMI (kg/m^2)	26.6 ± 3.8	26.5 ± 3.7	26.9 ± 4.0	26.4 ± 4.1	0.609
Waist to Hip Ratio	0.86 ± 0.09	0.85 ± 0.09	0.86 ± 0.10	0.88 ± 0.06	0.080
Systolic BP (mm Hg)	75.3 ± 9.5	118.2 ± 12.2	124.3 ± 14.2	137.0 ± 21.7	***<0.001***
Diastolic BP (mm Hg)	120.9 ± 14.9	73.9 ± 8.7	79.6 ± 10.2	81.2 ± 10.3	***<0.001***
Hypertension % (n)	28.0 (253)	66.0 (225)	44.0 (22)	13.3 (6)	***<0.001***
Smoker % (n)	19.3 (84)	16.1 (55)	30.0 (15)	31.1 (14)	***0.007***
Total Cholesterol (mg/dl)	191.3 ± 36.3	188.6 ± 35.8	188.1 ± 30.3	212.8 ± 39.5	***<0.001***
HDL Cholesterol (mg/dl)	54.6 ± 14.5	55.0 ± 14.3	52.5 ± 15.3	54.2 ± 15.2	0.352
LDL Cholesterol (mg/dl)	117.2 ± 30.3	115.4 ± 30.1	115.7 ± 29.2	130.6 ± 30.9	***0.004***
Triglycerides (mg/dl)*	72 (53-114)	66.0 (50-100)	89.0 (58-130)	122 (81-165)	***<0.001***
AER (mg/day) (DCCT Baseline)*	12.2 (7.2-21.6)	11.5 (7.2-18.7)	16.6 (8.6-28.8)	17.3 (8.6-27.4)	***0.002***
AER (mg/day) (Current)*	11.5 (7.2-30.2)	8.7 (5.8-15.8)	127 (68-203)	720 (449-1935)	***<0.001***
HbA1c % (Current)	8.31 ± 1.37	8.12 ± 1.30	9.03 ± 1.45	8.88 ±1.44	***<0.001***
HbA1C % (Study Mean)	8.29 ±1.50	7.96 ±1.35	9.09 ±1.48	9.86 ±1.35	***<0.001***
Gluc. Disp. Rate ($mg.kg^{-1}.min^{-1}$)	7.72 ± 2.40	8.15 ± 2.31	6.77 ± 2.38	5.67 ±1.54	***<0.001***
ETDRS Score (DCCT Baseline)*	2.0 (1.0-3.0)	2.0 (1.0-3.0)	3.0 (1.0-4.0)	3.0 (1.0-4.0)	***0.008***
ETDRS Score (Concurrent)*	4.0 (3.0-7.0)	3.0 (3.0-5.0)	8.0 (4.0-13.0)	7.5 (5.0-14.0)	***<0.001***
Standard Creatinine clearance (mL/sec)	115.7 ± 24.9	117.1 ± 21.3	120.9 ± 27.0	99.9 ± 39.2	***0.004***
Use of ACE/ARB drugs % (n)	17.7 (77)	10.3 (35)	30.0 (15)	60.0 (27)	***<0.001***
Use of lipid-lowering drugs % (n)	6.0 (26)	4.4 (15)	8.0 (4)	15.6 (7)	***0.011***

*Means ± SD or Median (IQR).

P < 0.05 shown in boldface.

Table 2

Apolipoprotein levels by concurrent albuminuria status. Data are shown as mean ± standard deviation for the entire cohort as well as stratified by males and females.

Lipoprotein	All Subjects			Male Subjects			Female Subjects		
	Normal (n=341)	Micro (n=50)	Macro (n=45)	Normal (n=214)	Micro (n=33)	Macro (n=35)	Normal (n=127)	Micro (n=17)	Macro (n=10)
Apolipoprotein-defined lipoprotein subclasses									
Lp-B mg ApoB/dl	36.0 ± 9.6	36.6 ±11.8	**43.6 ± 12.3**†	36.6 ± 10.0	37.8 ± 12.3	**44.2 ± 12.2**†	34.8 ± 8.9	34.1 ± 10.7	**41.6 ± 13.2**†
Lp-B:C mg ApoB/dl	11.8 ± 3.9	11.7 ± 3.6	**13.4 ± 3.6**†	12.1 ± 3.9	12.0 ± 3.6	**13.8 ± 3.7**	11.3 ± 3.9	10.9 ± 3.5	11.8 ± 2.4
Lp-A-I mg A-I/dl	39.0 ± 8.0	38.4 ± 8.6	40.8 ± 6.7	38.7 ± 7.6	36.9 ± 7.9	40.4 ± 6.9	39.6 ± 8.5	41.1 ± 9.4	42.2 ± 5.9
Lp-A-I:A-II mg ApoA-I/dl	104 ± 23	99 ± 25	102 ± 29	102 ± 23	**92.2 ± 20.1**	98.4 ± 29.3	108 ± 23	113 ± 28	112 ± 27
Lp-A-II:B:C:D:E mg ApoB/dl	13.1 ± 4.6	13.9 ± 4.7	14.0 ± 4.4	12.9 ± 4.5	14.4 ± 4.6	14.3 ± 4.6	13.4 ± 4.7	13.3 ± 4.9	13.3 ± 3.7
Lp-B:E + Lp-B:C:E mg ApoB/dl)	12.5 ± 5.3	11.4 ± 4.3	11.5 ± 3.3	12.3 ± 5.8	11.2 ± 4.2	11.3 ± 3.3	12.7 ± 4.3	11.9 ± 3.3	11.9 ± 3.3
Individual Apolipoproteins									
ApoB mg/dl	73.1 ± 16.9	73.4 ±18.3	**82.4 ± 16.7**†	73.6 ± 17.2	75.1 ± 18.8	**83.5 ± 17.0**†	72.3 ± 16.3	70.0 ± 17.3	78.6 ± 15.8
ApoA-I mg/dl	143 ± 30	138 ± 32	142 ± 33	140 ± 29	**129 ± 26**	138 ± 33	148 ± 30	154 ± 36	154 ± 30.8
ApoA-II mg/dl	36.3 ± 11.7	**32.3 ± 9.1**	**29.4 ± 9.5**	36.2 ± 11.4	**29.6 ± 6.4**	**29.6 ± 8.9**	36.5 ± 12.2	37.5 ± 11.3	**28.9 ± 12.1**
ApoE mg/dl	4.4 ± 1.0	4.2 ± 0.8	**4.0 ± 0.8**	4.4 ± 1.0	4.1 ± 0.7	**4.1 ± 0.7**	4.3 ± 0.9	4.4 ± 1.1	**3.6 ± 0.8**†
ApoC-III mg/dl	9.5 ± 3.5	9.7 ± 2.8	**12.8 ± 4.5**†	9.3 ± 3.5	9.4 ± 2.7	**13.3 ± 4.8**†	9.9 ± 3.4	10.4 ± 3.0	11.0 ± 2.5
ApoC-IIIHP mg/dl	2.6 ± 1.5	2.8 ± 1.0	**3.7 ± 1.5**†	2.6 ± 1.4	2.9 ± 1.1	**3.9 ± 1.5**†	2.5 ± 1.7	2.7 ± 0.9	2.8 ± 0.8
ApoC-IIIHS mg/dl	6.9 ± 2.2	6.8 ± 2.2	**8.9 ± 3.0**†	6.6 ± 2.3	6.4 ± 1.9	**9.2 ± 3.3**†	7.3 ± 2.2	7.6 ± 2.6	8.0 ± 1.7
ApoC-III Ratio (HS/HP)	2.9 ± 0.9	**2.6 ± 0.9**	**2.5 ± 0.6**	2.8 ± 0.9	**2.4 ± 0.7**	**2.4 ± 0.5**	3.2 ± 0.9	3.0 ± 1.1	2.9 ± 0.7

Boldface: P<0.05 vs. Normal;

† P<0.05 vs. Microalbuminuria

J Diabetes Complications. Author manuscript; available in PMC 2014 September 01.

Table 3

Odds of the presence of micoalbuminuria (micro) or macroalbuminuria (macro) as compared to normal AER for a 1 standard deviation increase in the measured apolipoprotein. Data are presented as the log odds ratio and 95% confidence interval for the whole cohort as well as stratified by males and females. P < 0.05 denoted in boldface. Odds ratios presented are adjusted for DCCT treatment assignment, baseline retinopathy cohort, gender (in analysis of all subjects), duration of diabetes, estimated glucose disposal rate, smoking status, ACE/ARB drug usage, use of lipid lowering drugs, and DCCT baseline levels of albumin excretion rate.

Lipoprotein	All Subjects (n=436)		Male Subjects (n=282)		Female Subjects (n=154)	
	Micro (n=50)	Macro (n=45)	Micro (n=33)	Macro (n=35)	Micro (n=17)	Macro (n=10)
Apolipoprotein-defined lipoprotein subclasses						
Lp-B mg ApoB/dl	1.02 (0.66-1.59)	***2.17 (1.30-3.61)***	1.06 (0.60-1.88)	***2.13 (1.15-3.97)***	0.90 (0.41-1.97)	***3.01 (1.11-8.12)***
Lp-B:C mg ApoB/dl	0.95 (0.67-1.34)	***1.68 (1.16-2.43)***	1.01 (0.64-1.58)	***1.84 (1.19-2.86)***	0.90 (0.48-1.67)	1.17 (0.55-2.50)
Lp-A-I mg A-I/dl	0.94 (0.65-1.34)	***1.59 (1.07-2.36)***	0.71 (0.42-1.20)	1.52 (0.92-2.52)	1.44 (0.85-2.43)	1.76 (0.74-4.17)
Lp-A-I:A-II mg ApoA-I/dl	0.86 (0.63-1.16)	1.13 (0.78-1.66)	0.71 (0.48-1.04)	1.09 (0.69-1.73)	1.31 (0.73-2.35)	1.31 (0.47-3.67)
Lp-A-II:B:C:D:E mg ApoB/dl	1.09 (0.79-1.51)	1.03 (0.69-1.52)	1.16 (0.76-1.76)	1.08 (0.69-1.69)	0.90 (0.50-1.62)	0.84 (0.29-2.41)
Lp-B:E + Lp-B:C:E mg ApoB/dl	0.78 (0.49-1.22)	0.92 (0.58-1.48)	0.87 (0.50-1.49)	1.06 (0.62-1.80)	0.83 (0.33-2.07)	0.55 (0.21-1.41)
Individual Apolipoproteins						
ApoB mg/dl	0.95 (0.62-1.45)	***1.93 (1.25-2.98)***	1.04 (0.60-1.79)	***1.97 (1.20-3.25)***	0.83 (0.39-1.75)	1.79 (0.70-4.59)
ApoA-I mg/dl	0.87 (0.64-1.20)	1.24 (0.84-1.83)	0.70 (0.56-1.06)	1.19 (0.74-1.91)	1.36 (0.76-2.41)	1.43 (0.55-3.68)
ApoA-II mg/dl	***0.69 (0.51-0.94)***	***0.51 (0.32-0.81)***	***0.45 (0.29-0.71)***	***0.52 (0.29-0.93)***	1.20 (0.77-1.87)	0.34 (0.09-1.22)
ApoE mg/dl	0.83 (0.57-1.21)	***0.65 (0.43-0.97)***	0.62 (0.36-1.09)	0.69 (0.41-1.18)	1.18 (0.62-2.24)	0.47 (0.21-1.08)
ApoC-III mg/dl	0.90 (0.64-1.27)	***1.52 (1.02-2.26)***	0.79 (0.51-1.23)	***1.95 (1.16-3.30)***	1.17 (0.68-2.02)	0.74 (0.46-1.19)
ApoC-IIIHP mg/dl	0.98 (0.70-1.37)	1.25 (0.74-2.11)	0.95 (0.62-1.45)	***1.88 (1.16-3.04)***	1.02 (0.75-1.38)	0.68 (0.36-1.28)
ApoC-IIIHS mg/dl	0.86 (0.58-1.26)	***1.70 (1.18-2.45)***	0.72 (0.45-1.15)	***2.03 (1.27-3.26)***	1.25 (0.64-2.44)	0.75 (0.37-1.54)
ApoC-III Ratio (HS/HP)	0.72 (0.48-1.09)	0.77 (0.51-1.17)	0.58 (0.33-1.02)	0.65 (0.37-1.14)	0.90 (0.47-1.73)	1.11 (0.53-2.31)