Serum Lipoproteins in the Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications Cohort

Associations with gender and glycemia

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OBJECTIVE — To relate the nuclear magnetic resonance (NMR)-determined lipoprotein profile, conventional lipid and apolipoprotein measures, and in vitro oxidizibility of LDL with gender and glycemia in type 1 diabetes.

RESEARCH DESIGN AND METHODS — In the 1997–1999 Diabetes Control and Complications Trial/Epidemiology of Diabetes Intervention and Complications (DCCT/EDIC) cohort, serum from 428 women and 540 men were characterized by conventional lipids, NMR, apolipoprotein levels, and LDL susceptibility to in vitro oxidation. Simple and partial correlation coefficients were calculated for each lipoprotein-related parameter versus gender, with and without covariates (age, diabetes duration, concurrent HbA_{1c}, DCCT randomization, hypertension, BMI, waist-to-hip ratio, and albuminuria). For concurrent HbA_{1c}, data were analyzed as above, exchanging gender for HbA_{1c}. Associations were significant if $P < 0.05$.

RESULTS — Although men and women had similar total and LDL cholesterol and triglycerides, men exhibited the following significant percent differences in NMR profiles versus women: small VLDL 41; IDL −30; medium LDL 39; small LDL 21; large HDL −32; small HDL 35; LDL particle concentration 4; VLDL and HDL diameters −8 and −4, respectively. Small VLDL, small HDL, medium LDL (women only), small LDL (men only), and LDL particle concentration were positively correlated, and HDL size was inversely correlated, with concurrent HbA_{1c}. NMR profile was unrelated to prior DCCT randomization. Susceptibility of LDL to oxidation was unrelated to gender and glycemia.

CONCLUSIONS — Male gender and poor glycemia are associated with a potentially more atherogenic NMR lipoprotein profile. Neither gender nor glycemia influence LDL oxidation in vitro.

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Abbreviations: AER, albumin excretion rate; Apo, apolipoprotein; CBL, Central Biochemistry Laboratory; Lp, lipoprotein; MUSC, Medical University of South Carolina; NMR-LSP, NMR lipoprotein subclass profile; NMR, nuclear magnetic resonance.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Factors implicated in the development of the vascular complications of diabetes include long diabetes duration, poor glycemic control, smoking, hypertension, and dyslipidemia (1,2). While people with type 2 diabetes have a characteristic lipid profile (increased triglycerides, normal or high levels of LDL, reduced HDL) (3), those with type 1 diabetes usually exhibit a normal conventional lipid profile, yet atherosclerosis is still accelerated (2–4). Qualitative abnormalities of lipoproteins, such as glycation and oxidation, may be important and are not detected by conventional measures (5,6). Also, previously unrecognized quantitative factors such as alterations in lipoprotein subclass distribution may be significant contributors (7–9). Adverse effects of lipoproteins may be mediated through effects on coagulation, fibrinolysis, vascular tone, insulin resistance, or susceptibility of lipoproteins to oxidation or matrix binding.

Each major lipoprotein class (VLDL, LDL, HDL) is heterogeneous in size and density, i.e., consists of subclasses (7–10). The best characterized subclass-associated risk factor for vascular disease is small dense LDL, which is usually associated with higher triglycerides and lower HDL cholesterol, and is prevalent in the metabolic syndrome (Syndrome X) and type 2 diabetes (7). LDL cholesterol levels do not predict LDL size, and therefore at any given level of LDL, one subject may have predominantly large LDL while another may have a preponderance of potentially damaging small dense LDL. Drugs that lower LDL may differ in their ability to affect LDL size and density. HDL also can be subdivided into at least two major subclasses thought to differ in antiatherogenic effects (10). Apparently favorable HDL levels may disguise high levels of noncardioprotective small HDL. Again, no information concerning HDL

subclass distribution is gained from conventional lipoprotein profiles.

Techniques to assess LDL and HDL lipoprotein subclasses include gradient gel electrophoresis, density gradient ultracentrifugation, and laser diffraction (11–13). These techniques are time-, labor-, and cost-intensive, and with exceptions (14), are only used in small-scale research studies. A nuclear magnetic resonance (NMR) technique to measure lipoprotein subclasses in serum or plasma is available for clinical and research use (15). The detail provided by NMR analysis may reveal otherwise hidden and potentially adverse or favorable changes in lipoprotein subclass distributions and may improve the predictive power of the lipid profile.

The Diabetes Control and Complications Trial (DCCT) and the Epidemiology of Diabetes Intervention and Complications (EDIC) studies are described elsewhere (16–18). In 1993, after a mean follow-up of 6.5 years, the DCCT was stopped because of a powerful favorable effect of intensive versus conventional diabetes therapy on microvascular complications (17). DCCT subjects were invited to join the EDIC cohort (18), an observational study with the primary goal to delineate the development of macrovascular disease in type 1 diabetes. Glycemic control of EDIC subjects from the two original DCCT randomization groups is now similar at ~8%. This is 1% higher than that of the intensively treated group during the DCCT and 1% lower than the conventionally treated group. Despite this, the benefit of intensive treatment during the DCCT in reducing the incidence and progression of retinopathy and nephropathy has been maintained for at least 4 years after completion of the DCCT protocol (19).

In 1996, a collaborative project between investigators at the Medical University of South Carolina (MUSC) and the DCCT/EDIC Research Group was implemented. Its primary goal is to identify risk factors and mechanisms for vascular disease in type 1 diabetes. One of our objectives is to examine associations and the predictive power of detailed lipoprotein characterization with risk factors for, and the presence of, chronic complications of type 1 diabetes.

We now report conventional lipids, the NMR-determined lipoprotein profile, apolipoprotein and Lp(a) measures, and response to LDL oxidation (in vitro) in relation to gender and glycemia in the DCCT/EDIC cohort.

RESEARCH DESIGN AND METHODS

Subjects and sample collection

The study was approved by the Institutional Review Boards of the MUSC and the 25 participating DCCT/EDIC centers. Written informed consent was obtained from all participating subjects. Clinical data were obtained according to the EDIC protocol (18,19). To obtain serum, blood was collected into a Falcon tube (Norcross, Fisher, GA) containing polypropylene beads (Sarstedt, Newton, NC) by venepuncture after an overnight fast and before insulin administration. To obtain plasma for LDL preparation, 55 ml of blood were drawn into Falcon tubes containing a lipoprotein preservative solution comprised of 2.8 mmol/l EDTA, 62 μmol/l chloramphenicol, 50 μg/ml gentamicin sulfate, and 10 mmol/l ε-aminocaproic-acid (final concentrations). Serum and plasma were prepared by centrifugation (3,000 rpm, 20 min) and shipped overnight fresh on ice to MUSC, where LDL was isolated from plasma (as described below), beginning on day of receipt, and serum (for analysis by NMR, and for assay of apolipoproteins A-I and B and Lp(a) levels) was stored at −70°C for subsequent analyses. Plasma and serum collected on Fridays were stored at 4°C until shipment to MUSC the following Monday.

Blood samples taken at the same venepuncture were sent to the DCCT/EDIC Central Biochemistry Laboratory (CBL, located at Fairview University Medical Center, University of Minnesota) for determination of the conventional lipid profile, HbA_{1c}, and serum creatinine.

NMR analysis of lipoproteins

Lipoprotein subclass levels, LDL particle concentration, and average VLDL, LDL, and HDL particle diameters were measured on freshly thawed serum specimens (0.25 ml) using a 400 mol/l Hz proton NMR analyzer at LipoScience, Inc. (Raleigh, NC), as previously described (15). We previously demonstrated lack of adverse effects of glucose and nonenzymatic glycation on the NMR profile (20). NMR convention is that larger subclass descriptor numbers denote larger particles. This resulted in the following nine lipoprotein subclasses: large VLDL (60–200 nm), medium VLDL (35–60 nm), small VLDL (27–35 nm), IDL (23–27 nm), large LDL (21.3–23 nm), medium LDL (19.8–21.2 nm), small LDL (18.3–19.7 nm), large HDL (8.2–13 nm), and small HDL (7.3–8.2 nm). To simplify data analysis, VLDL subclasses were regrouped as large (V6, V5), medium (V4, V3), and small (V2, V1) subclasses, and HDL subclasses as large (cardioprotective [H5, H4, H3]) and small (noncardioprotective [H2, H1]) subclasses.

Measurement of standard lipid profiles, apolipoproteins, and HbA_{1c}.

At the CBL, total cholesterol was determined by a cholesterol oxidase method, and triglyceride by an enzymatic method utilizing an automated glycerol blank (Boehringer Mannheim Diagnostics) on a Roche COBAS Fara analyzer. HDL cholesterol was quantified with the above cholesterol method after precipitation of non-HDL cholesterol with magnesium/dextran (16). For samples with triglycerides <400 mg/dl, LDL cholesterol was estimated by Friedewald's formula. For samples with triglycerides >400 mg/dl, LDL cholesterol was determined after removal of VLDL by ultracentrifugation.

Also at CBL, HbA_{1c} was measured by high-performance ion exchange liquid chromatography as previously described (16).

Serum ApoA-I, ApoB, and Lp(a) were measured at MUSC by nephelometry (Beckman, Brea, CA) according to the manufacturer's instructions.

LDL oxidizibility

LDL (d = 1.019–1.063 g/ml) was isolated from plasma by sequential ultracentrifugation. Oxidizibility of the lipid component of LDL was determined by modification of the method of Esterbauer, as described (21). Briefly, EDTA was removed from LDL by size exclusion chromatography on Sephadex G-25 columns; the EDTA-free LDL sample was diluted to 100 μg/ml cholesterol and exposed to Cu^{2+} ions (5 μmol/l final concentration) at 30°C. Results are expressed as the change in absorbance (Δ absorbance) at 234 nm, measured at 1-min intervals, from baseline to peak absorbance (reflecting conjugated diene formation). Oxidizibility of the protein component was determined from the development of flu-

Table 1—*Demographics of the DCCT/EDIC cohort in 1997–1999 divided by prior DCCT treatment group and by gender*

	Intensive (n = 500)			Conventional (n = 468)		
	Women	Men	*P*	Women	Men	*P*
n	225	275		203	265	
Age (years)	39.9 ± 7.3	39.9 ± 6.7	NS	38.4 ± 6.8	40.2 ± 6.6	<0.01
Duration of type 1 diabetes (years)	17.6 ± 4.6	17.5 ± 4.8	NS	17.8 ± 5.2	16.8 ± 4.6	<0.05
BMI (kg/m^2)	26.8 ± 4.7	27.3 ± 4.2	NS	25.9 ± 3.9	27.0 ± 3.6	<0.005
Waist-to-hip ratio	0.8 ± 0.1	0.9 ± 0.1	<0.0001	0.8 ± 0.1	0.9 ± 0.1	<0.0001
HbA_{1c} (% hemoglobin)	8.2 ± 1.4	8.1 ± 1.3	NS	8.2 ± 1.4	8.3 ± 1.3	NS
Mean HbA_{1c} during DCCT (% hemoglobin)	7.3 ± 0.9	7.2 ± 0.9	NS	9.1 ± 1.4	9.0 ± 1.1	NS
Systolic blood pressure (mmHg)	117 ± 14	121 ± 12	<0.0005	115 ± 13	124 ± 14	<0.0001
Diastolic blood pressure (mmHg)	73 ± 9	76 ± 9	<0.0005	72 ± 9	77 ± 10	<0.0001
Total cholesterol (conventional profile; mg/dl)	189 ± 33	190 ± 37	NS	187 ± 33	189 ± 36	NS
HDL cholesterol (conventional profile; mg/dl)	63 ± 16	51 ± 14	<0.0001	63 ± 14	52 ± 12	<0.0001
LDL cholesterol (conventional profile; mg/dl)	111 ± 29	119 ± 31	<0.01	109 ± 29	118 ± 31	<0.005
Triglycerides (conventional profile; mg/dl)	76 ± 37	98 ± 67	<0.0001	78 ± 57	96 ± 73	<0.01
AER (mg/24 h)	49 ± 500	93 ± 696	NS	74 ± 288	160 ± 742	0.09
Serum creatinine (mg/dl)	0.8 ± 0.1	1.0 ± 0.2	<0.0001	0.8 ± 0.2	1.0 ± 0.2	<0.0001
Blood urea nitrogen (mg/dl)	13.0 ± 3.6	15.1 ± 4.9	<0.0001	13.2 ± 4.4	15.5 ± 4.8	<0.0001
Standard creatinine clearance (ml/min)	111 ± 25	121 ± 23	<0.0001	110 ± 24	119 ± 25	<0.0005
ETDRS Score	3.3 ± 2.4	3.6 ± 2.5	0.10	5.3 ± 3.7	5.7 ± 3.6	NS
Hard exudate score	2.3 ± 7.1	3.6 ± 9.5	0.09	6.2 ± 11.6	9.1 ± 13.5	<0.05
% Current smoker	20.9 (6.0)	20.3 (5.4)	NS	17.3 (6.4)	18.3 (5.6)	NS
% BMI >27.3 in women, >27.8 in men (kg/m^2)	39.3 (5.3)	38.5 (4.8)	NS	28.2 (6.0)	36.3 (4.9)	0.1
% WHR >0.85 in women, >0.90 in men	14.6 (6.2)	42.2 (4.6)	<0.0001	9.0 (6.7)	41.8 (4.7)	<0.0001
% Hypertension	26.1 (5.8)	42.1 (4.6)	<0.0005	26.2 (6.0)	47.3 (4.5)	<0.0001
% Taking any hypertensive medications	9.1 (6.4)	13.2 (5.6)	NS	17.8 (6.4)	21.4 (5.5)	NS
% AER >40 mg/24 h	6.8 (6.5)	11.5 (5.7)	0.1	16.8 (6.4)	20.5 (5.5)	NS
% Taking ACE inhibitor	6.7 (6.4)	12.4 (5.6)	<0.05	13.8 (6.5)	17.7 (5.6)	NS
% Taking lipid-lowering medications	4.0 (6.5)	7.3 (5.8)	NS	3.9 (6.9)	6.0 (6.0)	NS

Data are mean ± SD or % (SD).

orescence (Ex360 nm/Em430 nm) under the same conditions, expressed as the ratio of fluorescence at 24 h exposure to Cu^{2+} to baseline fluorescence.

Statistics. The following measurements were concurrent with NMR measurement: age, duration of diabetes, HbA_{1c}, BMI, WHR, and albumin excretion rate (AER). AER was categorized into normal albuminuria (<40 mg/24 h) and micro-/macroalbuminuria (≥40 mg/24 h). Determination of hypertension was based on previously documented hypertension, concurrent systolic/diastolic blood pressure ≥140/90 mmHg, or the patient taking antihypertensives.

NMR lipoprotein subclass profile (NMR-LSP) and clinical data provided by the Data Coordinating Center (George Washington University, Washington, DC) for the DCCT/EDIC Research Group were managed and analyzed using SAS (Version 8; SAS Institute, Cary, NC). A *t* test and χ^2 test were performed for continuous variables and categorical variables between women and men in the two original DCCT randomization groups. ANCOVA was used to determine gender difference while adjusting for covariates of age, duration of diabetes, HbA_{1c}, BMI, WHR, AER, hypertension, and DCCT randomization group.

To determine the relationship between lipids, NMR-determined parameters, apolipoproteins, LDL oxidizibility, and HbA_{1c}, Pearson simple and partial correlation coefficients were computed, while partitioned by the covariates above, less HbA_{1c}. Statistical significance was determined using a two-sided test at a significance level of $P < 0.05$.

RESULTS — The subset of subjects in whom detailed lipoprotein analyses was performed represents 69% of the original DCCT cohort and 73% of the original EDIC cohort. Relative to nonparticipants, the group studied has a higher proportion of men (56 vs. 47%, $P = 0.001$) and was slightly older (28 vs. 27 years, $P = 0.003$) at study entry. More of our study subjects were from the DCCT intensive management group (52 vs. 45%) and had a lower mean HbA_{1c} (8.1 vs. 8.4%, $P = 0.001$) during the DCCT. Retinal and renal complication status did not differ. Table 1 describes the clinical characteristics of the evaluated 3–5 year EDIC cohort categorized by gender and the former DCCT randomization group. As shown in Table 1, in the former DCCT conventional treatment group, women had a significantly lower age ($P < 0.01$), longer diabetes duration ($P < 0.05$), and lower BMI ($P < 0.005$) than men. In both prior DCCT treatment groups, women had significantly lower blood pressure ($P < 0.001$) and WHR ($P < 0.0001$). The number of subjects treated for hypertension or with lipid-lowering drugs did not differ between genders or according to former DCCT treatment group. As recently described for the entire cohort (19), compared with subjects from the former intensive treatment group, the former

Table 2—*Percentiles of the standard lipoprotein lipid profile, NMR-determined lipoprotein profile, apolipoprotein levels in sera, and response to in vitro oxidation of LDL from women and men in the DCCT/EDIC cohort in 1997–1999*

		5–95th percentiles				
		5	25	50	75	95
Women	Reference					
NMR Large VLDL subclass (TG, mg/dl)	15.7 (28.7)	0.0	0.3	2.0	5.3	30.0
NMR Medium VLDL subclass (TG, mg/dl)	34.3 (26.7)	0.0	2.5	8.3	19.4	57.2
NMR Small VLDL subclass (TG, mg/dl)	19.2 (11.3)	0.0	4.1	12.2	24.3	43.1
NMR IDL subclass (cholesterol, mg/dl)	4.7 (5.6)	0.0	0.0	0.0	2.8	12.2
NMR L3 subclass (cholesterol, mg/dl)	76.6 (33.7)	10.6	44.0	67.0	90.8	130.5
NMR L2 subclass (cholesterol, mg/dl)	33.4 (25.7)	0.0	0.0	22.2	47.2	96.8
NMR L1 subclass (cholesterol, mg/dl)	18.5 (18.9)	0.0	0.0	18.4	42.9	78.8
NMR Large HDL subclass (cholesterol, mg/dl)	37.4 (13.8)	19.3	32.5	42.1	52.5	67.9
NMR Small HDL subclass (cholesterol, mg/dl)	15.6 (4.4)	2.7	10.3	15.0	19.1	25.5
NMR VLDL particle size (nm)	44.2 (8.2)	33.2	39.6	45.8	56.2	97.6
NMR LDL particle size (nm)	21.1 (0.5)	20.2	20.7	21.1	21.4	21.8
NMR HDL particle size (nm)	9.4 (0.4)	8.6	8.9	9.2	9.6	10.0
NMR LDL particle concentration (nmol/l)	1,408 (437)	863	1,146	1,393	1,627	2,114
Men						
NMR Large VLDL subclass (TG, mg/dl)	29.2 (41.0)	0.0	0.7	3.2	9.6	59.4
NMR Medium VLDL subclass (TG, mg/dl)	46.2 (31.2)	0.0	5.7	17.0	42.3	99.1
NMR Small VLDL subclass (TG, mg/dl)	20.4 (10.8)	0.7	11.5	20.6	30.9	50.1
NMR IDL subclass (cholesterol, mg/dl)	5.0 (4.7)	0.0	0.0	0.0	0.4	8.6
NMR L3 subclass (cholesterol, mg/dl)	60.9 (34.9)	0.0	22.9	51.6	81.8	125.6
NMR L2 subclass (cholesterol, mg/dl)	40.7 (26.7)	0.0	7.6	39.8	64.8	105.4
NMR L1 subclass (cholesterol, mg/dl)	31.9 (25.9)	0.0	0.0	20.1	47.8	106.8
NMR Large HDL subclass (cholesterol, mg/dl)	23.8 (11.5)	8.4	18.8	27.2	36.8	56.3
NMR Small HDL subclass (cholesterol, mg/dl)	17.4 (4.8)	9.0	15.9	20.1	24.5	28.9
NMR VLDL particle size (nm)	48.1 (9.3)	35.2	40.0	44.6	51.2	73.1
NMR LDL particle size (nm)	20.7 (0.6)	19.5	20.5	20.9	21.3	21.7
NMR HDL particle size (nm)	8.9 (0.4)	8.3	8.6	8.8	9.1	9.6
NMR LDL particle concentration (nmol/l)	1,561 (411)	872	1,193	1,437	1,706	2,296

References for NMR data are mean (SD) for nondiabetic subjects in the Framingham Offspring Study.

conventional treatment group had a significantly higher proportion with increased urinary AER (>40 mg/day, $P < 0.005$) and more severe retinopathy ($P < 0.0001$), as assessed by the Early Treatment Diabetic Retinopathy Study grading system in the combined genders. In the standard lipid profile, total cholesterol did not differ between genders, but men had higher triglyceride, higher calculated LDL cholesterol, and lower HDL cholesterol (all $P < 0.01$). The standard lipid profile did not differ significantly according to prior DCCT treatment group for either gender.

NMR, lipid, and apolipoprotein profile in type 1 diabetes. Percentiles for each NMR-LSP parameter in women and men are shown in Table 2. Optimal LDL particle concentration is <1,100 nmol/l, and levels above 1,400 nmol/l are considered high risk for macrovascular disease. These LDL particle concentrations are chosen from NMR data relating to the Framingham Offspring Study and correspond to LDL cholesterol levels of 100 and 130 mg/dl, respectively (22). A total of 80% of men and 83% of women in this type 1 diabetic cohort have suboptimal (>1,100 nmol/l) LDL particle concentrations, while 54% of men and 49% of women have high-risk levels over 1,400 nmol/l. Recommended levels for LDL cholesterol are <100 mg/dl in diabetic patients (23). The proportion of the 3–5 year EDIC cohort with calculated conventional lipid profile LDL cholesterol levels of >100 mg/dl is 63% for women and 73% for men, while 23% of women and 31% of men have levels of >130 mg/dl, with the gender difference being significant, $P < 0.001$ at both LDL cholesterol levels. Small (≤20.5 nm) dense LDL is associated with increased vascular disease risk in the general population (7). In our diabetic cohort by NMR sizing, 16% of women and 30% of men have small LDL, again with a significant gender difference, $P < 0.0001$.

Suboptimal apoA-I (≤110 mg/dl) and ApoB (≥110 mg/dl) levels (representing respectively about the lower and upper 10% of the University of Utah Reference Laboratory reference range) occur in 7 and 12% of women and in 15 and 23% of men. Sex differences for both apolipoproteins are significant, $P < 0.0001$. Elevated (≥25 mg/dl) Lp(a) levels occur in 33% of women and 28% of men, $P =$ NS.

Comparing data from the two former DCCT randomization groups, there was no difference between them in relation to any NMR lipoprotein parameter, regardless of whether the data were controlled for age, gender, diabetes duration, BMI, WHR, AER, and hypertension (all $P > 0.05$, data not shown). Mean and SD of the conventional lipid profile, NMR-LSP,

Table 3—*Standard lipid profile, NMR-determined lipoprotein profile, apolipoprotein levels in sera, and response to in vitro oxidation of LDL from women and men of the DCCT/EDIC cohort in 1997–1999*

	Intensive (n = 500)				Conventional (n = 468)			
	Women	Men	*P*	*P1*	Women	Men	*P*	*P1*
n	225	275			203	265		
Conventional total cholesterol (mg/dl)	189.0 ± 32.9	190.0 ± 37.0	NS	NS	187.1 ± 33.4	188.7 ± 35.6	NS	NS
Conventional total triglyceride (mg/dl)	75.7 ± 36.9	98.2 ± 67.5	<0.0001	NS	78.0 ± 57.1	96.2 ± 72.7	<0.005	NS
Conventional LDL cholesterol (mg/dl)	110.9 ± 29.3	118.8 ± 31.3	<0.01	<0.05	108.8 ± 29.4	118.1 ± 31.2	<0.005	NS
Conventional HDL cholesterol (mg/dl)	63.0 ± 15.6	51.2 ± 13.8	<0.0001	<0.0001	62.7 ± 13.6	51.7 ± 11.7	<0.0001	<0.005
NMR Large VLDL subclass (TG, mg/dl)	5.8 ± 12.1	12.9 ± 27.7	<0.001	NS	6.4 ± 14.7	12.4 ± 31.6	<0.01	NS
NMR Medium VLDL subclass (TG, mg/dl)	14.9 ± 17.9	32.1 ± 39.2	<0.0001	<0.05	16.5 ± 24.9	28.9 ± 35.7	<0.0001	NS
NMR Small VLDL subclass (TG, mg/dl)	16.1 ± 14.6	22.4 ± 15.2	<0.0001	<0.01	15.5 ± 15.6	22.1 ± 15.8	<0.0001	0.08
NMR IDL subclass (cholesterol, mg/dl)	2.0 ± 4.4	1.3 ± 2.9	<0.05	NS	2.6 ± 4.8	1.5 ± 4.1	<0.01	<0.005
NMR L3 subclass (cholesterol, mg/dl)	68.4 ± 35.4	54.8 ± 42.3	0.0001	NS	69.4 ± 34.8	55.3 ± 37.6	<0.0001	NS
NMR L2 subclass (cholesterol, mg/dl)	32.0 ± 33.8	43.7 ± 37.4	<0.001	<0.05	28.6 ± 34.2	40.8 ± 33.8	0.0001	NS
NMR L1 subclass (cholesterol, mg/dl)	26.1 ± 27.1	31.6 ± 37.3	0.06	NS	26.3 ± 30.2	31.7 ± 37.0	0.08	0.08
NMR Large HDL subclass (cholesterol, mg/dl)	42.3 ± 15.4	28.7 ± 14.8	<0.0001	<0.0001	42.8 ± 14.1	29.1 ± 13.7	<0.0001	<0.001
NMR Small HDL subclass (cholesterol, mg/dl)	14.8 ± 6.6	20.0 ± 6.3	<0.0001	<0.0001	14.6 ± 6.8	19.8 ± 6.1	<0.0001	<0.001
NMR VLDL particle size (nm)	51.8 ± 20.1	47.4 ± 12.8	<0.005	<0.05	52.4 ± 22.0	48.4 ± 14.6	<0.05	<0.005
NMR LDL particle size (nm)	21.0 ± 0.5	20.8 ± 0.7	<0.0001	NS	21.1 ± 0.6	20.8 ± 0.6	<0.0001	NS
NMR HDL particle size (nm)	9.2 ± 0.5	8.8 ± 0.4	<0.0001	<0.0001	9.3 ± 0.4	8.9 ± 0.4	<0.0001	<0.05
NMR LDL particle concentration (nmol/l)	1426 ± 368	1490 ± 439	<0.05	NS	1408 ± 394	1467 ± 425	NS	<0.05
ApoB (mg/dl)	84.9 ± 24.8	93.2 ± 27.2	<0.001	NS	82.3 ± 23.2	90.2 ± 25.6	<0.001	NS
ApoA-I (mg/dl)	148.6 ± 33.5	137.5 ± 29.7	0.0001	<0.01	147.8 ± 30.1	138.0 ± 26.9	<0.001	NS

Data are mean ± SD. Subjects are divided according to gender. The first set of four columns relates to the previously intensively managed cohort, and the second set relates to the group previously receiving conventional diabetes management. *P* = univariate analysis. *P1* = ANCOVA: dependent variable is specified lipid, lipoprotein or apolipoprotein, independent parameter is gender, adjusted for age, duration of diabetes hypertension, HbA_{1c}, BMI, WHR, and AER in each DCCT randomization group.

and apolipoprotein levels are shown in Table 3, organized by prior DCCT treatment group and gender. On univariate analysis, all NMR-determined parameters and apoA-I and B levels differed between genders. In the conventional lipid profile, men had higher triglyceride and LDL cholesterol levels and lower HDL cholesterol levels, but total cholesterol levels did not differ. However, in the multivariate analyses for the total cohort (not shown), relative to women, men had significantly lower HDL cholesterol levels but similar total and LDL cholesterol and triglyceride levels. In men, NMR reveals higher levels of medium and small VLDL, smaller VLDL particles, lower IDL (VLDL remnant) levels, lower levels of cardioprotective large HDL, higher levels of noncardioprotective small HDL, and smaller HDL particles. Also, in spite of having similar (conventional profile) total and LDL cholesterol levels, men with type 1 diabetes have more medium and small LDL (L2 and L1), a higher LDL particle concentration, and (on univariate analyses) smaller LDL particles.

In the multivariate analyses, ApoA-I levels were significantly lower in men, but ApoB, Lp(a), and susceptibility of LDL to in vitro oxidation did not differ between genders. When subdivided according to prior DCCT treatment group (Table 3) the majority of these observations persists.

Lipid parameters and concurrent glycemia. Glycemia, assessed by concurrent HbA_{1c}, was associated with many NMR-determined parameters, as shown in Table 4. Emphasis will be placed on the more rigorous multivariate analyses, denoted by *P1*. Of the conventional lipids, only total triglyceride was significantly related to HbA_{1c} in women. In the NMR profile of women, HbA_{1c} was also positively correlated with levels of small VLDL, medium LDL (L2), small (noncardioprotective) HDL, and LDL particle concentration ($P < 0.05$, all). Also, HDL particle size was inversely related to HbA_{1c} ($P < 0.001$). ApoB was significantly related to HbA_{1c}, but ApoA-I and Lp(a) were not.

In men, HbA_{1c} was positively correlated with conventional measures of total and LDL cholesterol and total triglyceride. In the NMR profile, HbA_{1c} was positively associated with small VLDL, small LDL (L1), and LDL particle concentration (all $P < 0.05$) and was negatively associated with HDL particle size. In men, ApoB was significantly related to HbA_{1c}, but ApoA-I and Lp(a) levels were not. In both genders, the HbA_{1c} value at which the LDL particle concentration exceeds 1,400 nmol/l is ~8%. For men with HbA_{1c} <8% vs. HbA_{1c} ≥8%, LDL particle concentration (adjusted for age, diabetes duration, hypertension, BMI, WHR, AER, and prior DCCT treatment group) was mean (SD) 1,391 (371) vs. 1,561 (470) nmol/l, $P < 0.005$. Similarly, for women, LDL particle concentrations were 1,347 (354) vs. 1,488 (392) nmol/l, $P < 0.001$. In neither gender did LDL (lipid or protein) susceptibility to in vitro oxidation correlate with HbA_{1c}.

CONCLUSIONS — Compared with other measures of serum lipoproteins, NMR analysis provides details that are otherwise hidden, and may improve predictive power of the lipid profile for vascular damage and assessment of response

Table 4—*Correlations between concurrent HbA_{1c} and the standard lipid profile, NMR-determined lipoprotein profile, apolipoprotein levels in sera, and response to in vitro oxidation of LDL from women and men in the 1997–1999 DCCT/EDIC cohort*

	Women (n = 428)				Men (n = 540)			
	r	*P*	*r**	*P1*	*r*	*P*	*r**	*P1*
Conventional total cholesterol (mg/dl)	0.19	<0.001	0.10	0.09	0.17	<0.0001	0.18	<0.001
Conventional total triglyceride (mg/dl)	0.21	<0.0001	0.16	<0.05	0.14	<0.005	0.18	<0.001
Conventional LDL cholesterol (mg/dl)	0.18	<0.001	0.11	0.08	0.15	<0.005	0.17	<0.005
Conventional HDL cholesterol (mg/dl)	−0.08	0.10	−0.06	NS	−0.05	NS	−0.09	0.09
NMR Large VLDL subclass (TG, mg/dl)	0.14	<0.005	0.11	0.07	0.08	0.06	0.09	NS
NMR Medium VLDL subclass (TG, mg/dl)	0.12	<0.05	0.06	NS	0.10	<0.05	0.10	0.06
NMR Small VLDL subclass (TG, mg/dl)	0.22	<0.0001	0.21	<0.001	0.14	<0.005	0.13	<0.05
NMR IDL subclass (cholesterol, mg/dl)	0.03	NS	0.01	NS	0.00	NS	−0.04	NS
NMR L3 subclass (cholesterol, mg/dl)	0.05	NS	−0.02	NS	−0.03	NS	−0.01	NS
NMR L2 subclass (cholesterol, mg/dl)	0.12	<0.05	0.12	0.05	0.06	NS	0.08	NS
NMR L1 subclass (cholesterol, mg/dl)	0.04	NS	0.06	NS	0.16	<0.001	0.13	<0.05
NMR Large HDL subclass (cholesterol, mg/dl)	−0.09	0.08	−0.09	NS	−0.08	NS	−0.09	NS
NMR Small HDL subclass (cholesterol, mg/dl)	0.15	<0.005	0.15	<0.05	0.09	<0.05	0.05	NS
NMR VLDL particle size (nm)	−0.04	NS	0.00	NS	0.05	NS	0.06	NS
NMR LDL particle size (nm)	−0.03	NS	−0.07	NS	−0.12	<0.005	−0.09	0.10
NMR HDL particle size (nm)	−0.22	<0.0001	−0.23	<0.001	−0.16	<0.001	−0.14	<0.05
NMR LDL particle concentration (nmol/l)	0.20	<0.0001	0.17	<0.01	0.24	<0.0001	0.23	<0.0001
ApoB (mg/dl)	0.26	<0.0001	0.22	<0.001	0.19	<0.0001	0.22	<0.0001

*Partial correlation coefficient. *P* univariate analysis. *P1* adjusted for age, duration of diabetes, hypertension, BMI, WHR, AER, and DCCT randomization group.

to therapy. After demonstrating that there is no interference from high glucose levels and nonenzymatic glycation of lipoproteins, characteristic features of diabetes (24), and good correlation with more traditional lipid and apolipoprotein measures in diabetic subjects (20), we utilized NMR to determine the lipoprotein profile in serum from the majority of subjects in the DCCT/EDIC cohort. NMR reveals a dyslipoproteinemia in a high proportion (≥80%) of this cohort. There were potentially adverse changes in the NMR lipoprotein profile in association with male gender and concurrent HbA_{1c}, but not with prior DCCT randomization status. In general, the observed adverse changes included an increase in VLDL subclass levels, smaller VLDL, a shift toward smaller LDL, increased LDL particle concentration, a decrease in cardioprotective large HDL, an increase in noncardioprotective small HDL, and decreased HDL size.

While the standard lipid profile is relatively normal in the DCCT/EDIC cohort, a high frequency of dyslipoproteinemia is revealed by NMR. In less well-motivated or less well-controlled type 1 diabetic populations, the prevalence of dyslipoproteinemia may be even greater. LDL is generally considered the major atherogenic lipoprotein. Potentially adverse findings in the DCCT/EDIC cohort are relatively high LDL particle concentrations, despite the normal mean calculated LDL cholesterol levels (Table 1). In our study, more men than women had small (≤20.5 nm) LDL particles, and men had higher levels of medium and small LDL particles and a lower ratio of large to small LDL particles. LDL particle number and LDL particle size have been strongly associated with increased coronary artery disease and cerebrovascular disease in both cross-sectional and longitudinal studies in the general population (25). Small dense LDL has also been associated with diabetic nephropathy (11,26). With the future development of clinically evident macrovascular disease and progression of diabetic nephropathy in the DCCT/EDIC cohort, we will be able to define prospectively whether LDL particle size, concentration, or subclass distribution is associated with macrovascular disease and with the rate of progression of renal damage.

Before the development of the NMR technique, IDL levels were difficult to measure. IDL levels are increased in diabetes (3) and are particularly high in subjects with diabetic nephropathy (11,26). In the NMR analysis of sera from the general population of the Framingham Offspring Study, IDL levels were higher in men than in women (22). In contrast, in our diabetes-related study, IDL levels were higher in women. IDL levels were not correlated with HbA_{1c}. While IDL levels are low compared with LDL levels in our cohort, they may also contribute to diabetic vascular damage given their marked atherogenicity as reflected by avid foam cell formation (27) and by stimulation of atherogenic gene expression in cultured vascular endothelial cells (28). In the general population, higher IDL levels have been associated with angiographic severity of coronary artery disease (29) and are prospectively predictive of carotid intimal media thickening (30). Long-term follow up of this cohort will ascertain if IDL is predictive of vascular damage in people with type 1 diabetes.

HDL is regarded as a cardioprotective lipoprotein. A favorable HDL profile protects against the subsequent development of type 2 diabetes (31), and in subjects with type 1 diabetes, it protects against renal dysfunction over a 10-year time frame (32). Beneficial effects may relate to HDL functions, including reverse cholesterol transport, antioxidant effects, antithrombotic, and anti-inflammatory effects (33,34). There are two major subclasses of HDL—HDL_2 (large) and HDL_3 (small) as defined by electrophoretic or ultracentrifugation criteria (33,34)—and

five subclasses as discerned by NMR. In general, larger HDL is thought to be cardioprotective, while smaller HDL is less cardioprotective and may even promote atherosclerosis; however, this is controversial and may be related to methodological differences. In nondiabetic subjects, assessed by NMR, men have lower large HDL cholesterol levels than women (35), and in children and adolescents, HDL particle size is smaller in males (36). Total HDL cholesterol levels tend to decrease with poor glycemic control and renal impairment (3,4) but are otherwise normal or even elevated in type 1 diabetes (1,3). In our study, standard HDL cholesterol levels were lower in type 1 diabetic men than women as expected (Table 3). The more detailed NMR analysis revealed significant opposing differences in levels of large and small HDL subclasses between genders and a significant reduction in HDL size in men. By NMR, levels of large HDL particles were lower in men than in women and levels of small (less cardioprotective) were higher. Elevation of the less protective (or even harmful) small HDL fraction may result in a misleadingly favorable interpretation of the standard lipid profile. Changes in HDL subclasses, composition, or function may contribute to the accelerated vascular damage seen in diabetes. Few studies have examined the significance and predictive value of HDL subfractions in relation to vascular damage in diabetes, and the issue is being evaluated prospectively in this cohort.

In diabetes there is loss of the female cardioprotection seen in the nondiabetic population (37). As in other NMR studies in nondiabetic groups (22), the NMR profile was potentially more atherogenic in our male (diabetic) subjects, except that women had higher IDL levels. Relative to women, the men in our cohort also had a generally unfavorable risk factor profile with higher blood pressure, more central adiposity, and greater urinary albumin excretion (Table 1). However, the adverse lipid profile persisted even when controlling for these covariates, in keeping with an inherent dyslipidemia associated with male gender in type 1 diabetes. In our study, susceptibility of isolated LDL to oxidation by copper did not differ between genders. Estrogen has an antioxidant effect in this type of assay (38). The aqueous phase, but not the lipophilic estrogens present in vivo, would have been removed during LDL preparation (38). However, estrogen-induced rises in plasma triglyceride may produce small LDL particles that are more susceptible to oxidation (39). Thus, differences in the NMR-determined lipid profile and susceptibility of LDL to oxidation cannot account for the relative loss of female cardioprotection in diabetes. However, it remains to be seen if this clinical finding is confirmed in this cohort—new interventions may alter risks. At this stage few macrovascular events have occurred in the DCCT/EDIC cohort. Lack of female cardioprotection in diabetes as seen in earlier studies may relate to an overriding effect of the diabetic milieu. Gender differences in vascular disease risk factors, including other aspects of dyslipidemia (not assessed by NMR), hemostatic and fibrinolytic parameters, cytokines, and inflammation may contribute.

At the conclusion of the DCCT in 1993 (when differences in HbA_{1c} and AER between the randomly assigned treatment groups were evident), Purnell et al. (14) used density gradient ultracentrifugation and enzymatic cholesterol and triglyceride assays to analyze the lipid (not lipoprotein) profile. Relative to the intensive treatment group, the conventional treatment group had more cholesterol in the denser LDL fractions and less in the buoyant LDL fraction (14). Using NMR, standard lipid profile, and apolipoprotein measures 4–6 years later, we found no persisting differences between the former DCCT treatment groups. This may relate to the loss of the DCCT period difference in glycemia (measured by HbA_{1c}) (Table 1).

As expected, in both men and women in our study, glycemia, as assessed by concurrent HbA_{1c}, was associated with adverse changes in conventional measures of total and LDL cholesterol, triglyceride, and ApoB, but not with HDL cholesterol or ApoA-1 levels (Table 4). Further detail was provided by NMR analysis. On multivariate analysis, HbA_{1c} was positively associated with small VLDL and with potentially pro-atherogenic medium and small LDL, higher LDL particle concentration, and smaller HDL size. The association with large HDL was inverse but did not reach statistical significance. Glycemia affects HDL subclasses (which differ in cholesterol content—small HDL being relatively cholesterol-poor) in opposite directions, an effect which is hidden in conventional lipid profiles. The HbA_{1c} level at which high-risk LDL particle concentrations (>1,400 nmol/l) is reached is ~8%—perhaps appropriately, this coincides with recommendations of the American Diabetes Association for intensified glycemic control (24).

We did not find a significant association between HbA_{1c} levels and susceptibility of isolated LDL lipid or protein to oxidation by copper. The extent of nonenzymatic glycation of apoB in LDL is correlated with HbA_{1c} levels (24). In another study we isolated LDL from type 1 diabetic subjects and separated it by boronate chromatography into relatively glycated and nonglycated subfractions (40). Consistent with our findings in this cross-sectional study, the glycated LDL was not more susceptible to copper oxidation (unpublished observations). The response of LDL to in vitro oxidative stress has been proposed as a measure of the predisposition of LDL to subendothelial oxidative stress in vivo. While earlier (nondiabetic) vascular disease case control studies were positive (41,42), this assay has yet to be found to be strongly associated with, or predictive of, clinical cardiovascular disease.

The correlation coefficients for all lipoprotein-related associations with HbA_{1c} (Table 4) suggest that glycemia generally contributes ≤4% of the variance in lipoprotein values. Small improvements in lipid levels are associated with larger reductions in coronary events. A 1% decrease in LDL cholesterol and a 1% increase in HDL cholesterol are associated with 2 and 3% reductions, respectively, in cardiovascular disease risk in the general population (43,44). In addition to a favorable effect on lipid levels, improved glycemia would also benefit other aspects of lipoprotein composition, such as nonenzymatic glycation, which is increased in diabetes (24), not detectable by NMR, and associated with increased cholesterol accumulation in macrophages in vitro (5). The limitations of our cross-sectional analyses are recognized. Longitudinal studies of improved glycemic control are essential to determine whether the potentially unfavorable aspects of the NMR profile such as the high LDL particle concentration, small LDL, and small HDL can be improved, and to determine putative cardiovascular benefits. Such a study is in progress. Apart from improved glycemic control, other measures such as increased exercise, weight loss, smoking

cessation, lipid-lowering drugs, and hormonal factors may also favorably impact lipids and the lipoprotein subclass profile.

There are published studies that demonstrate associations or predictive power of NMR lipoprotein measures for vascular disease in the general population. In an angiographic study of 158 men, atherogenic characteristics of the NMR profile were associated with coronary artery disease severity (45). In a substudy of the Pravastatin Limitation of Atherosclerosis in the Coronaries (PLAC-I) study, coronary artery lumen diameter was assessed before and after treatment with pravastatin or placebo. NMR profiles enhanced predictive power of the standard lipid profile for disease progression or regression (46). In the Healthy Women Study of postmenopausal women, the strongest lipid-related predictor of coronary artery calcification was the NMR-measured LDL particle concentration (47).

In our studies, cross-sectional, retrospective, and prospective longitudinal analyses of the relationship of the NMR profile to glycemia and the micro- and macrovascular complications in the DCCT/EDIC cohort are in progress. Preliminary results of the cross-sectional studies have been reported elsewhere (48,49). Associations with hard clinical end points and mortality will be examined when sufficient events to provide adequate statistical power have occurred.

National and international lipid levels for cardiovascular risk have been determined by measurement of lipids in serum or plasma in large epidemiologic studies. NMR lipoprotein analysis is being applied to many major observational and interventional studies. It is anticipated that NMR-specific levels for high vascular risk and targets for intervention will be developed, and that results of analysis of the DCCT/EDIC cohort will facilitate guidelines for type 1 diabetes.

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