

# Associations between intensive diabetes therapy and NMR-determined lipoprotein subclass profiles in type 1 diabetes<sup>S</sup>

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**Abstract** Our objective is to define differences in circulating lipoprotein subclasses between intensive versus conventional management of type 1 diabetes during the randomization phase of the Diabetes Control and Complications Trial (DCCT). NMR-determined lipoprotein subclass profiles (NMR-LSPs), which estimate molar subclass concentrations and mean particle diameters, were determined in 1,294 DCCT subjects after a median of 5 years (interquartile range: 4–6 years) of randomization to intensive or conventional diabetes management. In cross-sectional analyses, we compared standard lipids and NMR-LSPs between treatment groups. Standard total, LDL, and HDL cholesterol levels were similar between randomization groups, while triglyceride levels were lower in the intensively treated group. NMR-LSPs showed that intensive therapy was associated with larger LDL diameter (20.7 vs. 20.6 nm,  $P = 0.01$ ) and lower levels of small LDL (median: 465 vs. 552 nmol/l,  $P = 0.007$ ), total IDL/LDL (mean: 1,000 vs. 1,053 nmol/l,  $P = 0.01$ ), and small HDL (mean: 17.3 vs. 18.6  $\mu\text{mol/l}$ ,  $P < 0.0001$ ), the latter accounting for reduced total HDL (mean: 33.8 vs. 34.8  $\mu\text{mol/l}$ ,  $P = 0.01$ ).<sup>¶¶</sup> In conclusion, intensive diabetes therapy was associated with potentially favorable changes in LDL and HDL subclasses in sera. Further research will determine whether these changes contribute to the beneficial effects of intensive diabetes management on vascular complications.—Zhang, Y., A. J. Jenkins, A. Basu, J. A. Stoner, M. F. Lopes-Virella, R. L. Klein, The DCCT/

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**Supplementary key words** intensive diabetes treatment • dyslipidemia • atherosclerosis • clinical trial • high density lipoprotein • low density lipoprotein • nuclear magnetic resonance

The Diabetes Control and Complications Trial (DCCT) was a randomized trial comparing the effects of intensive (multiple daily insulin injections or insulin pump therapy aimed at near-normalization of blood glucose levels) versus conventional therapy (usually twice daily insulin injections) on the development and progression of micro-vascular complications of type 1 diabetes (1). The trial achieved and sustained a reduction of two percentage points in HbA<sub>1c</sub> in the intensive versus conventional therapy groups, and demonstrated conclusively that intensive therapy delays the onset and slows the progression of diabetic retinopathy, nephropathy, and neuropathy (1). During the observational follow-up study of DCCT [known as the Epidemiology of Diabetes Intervention and Complications (EDIC) study (2)], prior intensive therapy was associated with long-term reductions in

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Abbreviations: CAD, coronary artery disease; CBL, Central Biochemistry Laboratory; DCCT, Diabetes Control and Complications Trial; EDIC, Epidemiology of Diabetes Intervention and Complications study; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; NMR-LSP, nuclear magnetic resonance-determined lipoprotein subclass profile.

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CVD (3–5). These substantial clinical benefits were directly attributed to better glycemic control during DCCT, and not to concomitant changes in other traditional cardiovascular risk factors, such as plasma lipid profiles (5–7). In fact, the DCCT revealed no statistically significant effect of intensive diabetes management on LDL cholesterol (LDL-C) or HDL cholesterol (HDL-C) levels (3).

In the standard lipid profile, only the lipid (triglyceride and cholesterol) content of each major lipoprotein class (VLDL, LDL, and HDL) is measured. LDL-C is the cornerstone of lipid management for CVD prevention (8), yet may not provide a complete measure of LDL-associated atherogenicity (9, 10). It is the interaction between the LDL particles and the arterial wall, not the cholesterol transported within circulating LDL particles, that initiates the pathophysiological process of atherosclerosis (11, 12). At the population level, LDL-C predicts CVD well and interventions targeted at LDL-C, such as lifestyle and drugs, reduce CVD rates (13). However, particle size and the amount of cholesterol carried in each LDL particle vary among individuals, and may be influenced by insulin resistance or hyperglycemia (13). While intensive therapy in the DCCT did not affect LDL-C and HDL-C significantly, a previous study by Purnell et al. (14) using density-gradient ultracentrifugation showed that it was associated with decreased lipoprotein (a) and apolipoprotein B levels, and with changes in lipoprotein cholesterol distribution that were considered favorable.

Each major lipoprotein class in the standard lipid profile (VLDL, LDL, and HDL) consists of subclasses that are heterogeneous in size, density, and potentially in atherogenicity (15, 16). Circulating levels of these subclasses can predict CVD events (17–19). LDL particle concentration has demonstrated comparable, but not greater, CVD predictive power to LDL-C in some large studies (20, 21), but these studies do not involve people with type 1 diabetes. It is possible that lipoprotein particle measurements could identify CVD risk in populations with a “discordant phenotype,” e.g., normal LDL-C but increased LDL particle concentration (22). Thus in the DCCT, without concurrent measurements of individual subclasses and LDL particle concentration, LDL-C measurements considered alone could conceal effects of intensive diabetes management on LDL subclasses, and similar considerations apply to HDL. Plasma triglyceride levels were significantly reduced by intensive therapy in the DCCT (3), but again, no studies have addressed effects on specific VLDL subclasses.

The NMR-determined lipoprotein subclass profile (NMR-LSP) provides automated rapid quantification of multiple subclasses of VLDL, LDL, and HDL using whole plasma or serum, i.e., without the need for physical separation of the lipoproteins. It measures distinctive NMR signals emitted by particles according to size, yielding estimates of molar concentrations of each subclass, as well as an average particle diameter of each class. Specifically, subclass particle number is calculated using an algorithm based on studies of size/composition of lipoproteins from healthy normolipidemic individuals, with the assumption that the NMR signal is unaffected by the differing ratios of cholesterol esters to

triglycerides in particle cores (23–25). NMR-LSP results have been shown to correlate highly with lipoprotein particle sizes and concentrations measured by “gold standard” methods [density gradient ultracentrifugation (26) or gradient gel electrophoresis (23)], but are less labor intensive, and also (and as a result) are clinically applicable.

In the present study, we performed NMR-LSP on stored serum samples obtained from DCCT participants close to the end of the randomization phase of the study. Previously, we have published cross-sectional studies using samples obtained later, during the observational EDIC study, i.e., several years after completion of the intervention phase of DCCT: by that time, glycemic control in the two former randomization groups had become similar (15, 27–29), and NMR-LSPs also did not differ (29). The present analysis is the first report of NMR-LSPs during the DCCT randomization phase, when there were major differences in HbA<sub>1c</sub> levels between the randomization groups. We hypothesized that intensive diabetes treatment is associated with alterations in lipoprotein subclasses that are not revealed by the standard lipid profile, and that these alterations differ between men and women, are favorable in the intensive management group, and have implications for the evolution of the micro- and macro-vascular complications of diabetes.

## MATERIALS AND METHODS

### Study participants and sample collection

The DCCT design, methods, and outcomes have been reported previously (1, 28, 30, 31). In summary, 1,441 patients with type 1 diabetes aged 13–39 years were recruited at 29 centers from 1983 to 1989 (27, 31) and randomized to conventional ( $n = 730$ ) or intensive ( $n = 711$ ) diabetes therapy (1, 31). In 1993, after an average of 6.5 years treatment (range: 3–9 years), the DCCT was terminated early because of clear-cut beneficial effects of intensive treatment on the development and progression of diabetic retinopathy, nephropathy, and neuropathy (1). The current study included 1,294 participants (90% of the entire cohort) and used all available serum samples collected approximately 1 year prior to the end of the DCCT (“near close-out” visit), after a median follow-up of 5 years (interquartile range: 4–6 years). DCCT samples at study entry (“baseline”) were available only for a minority of participants ( $n = 580$ ), of whom 524 had NMR-LSP determined both at baseline and near close-out visits.

After an overnight fast, blood samples were collected from DCCT participants before insulin administration (29). Serum was prepared by centrifugation (1,000 g, 20 min) and stored ( $-70^{\circ}\text{C}$ ) until thawed and analyzed by NMR (28). Blood samples taken at the same venipuncture were sent to the DCCT Central Biochemistry Laboratory (CBL) in Fairview University Medical Center, University of Minnesota for determination of the standard lipid profile and HbA<sub>1c</sub> levels (28, 29). The study was approved by the Institutional Review Boards of the Medical University of South Carolina, the University of Oklahoma Health Sciences Center, and all DCCT centers. Written informed consent was obtained from all participants.

### NMR-LSP

As previously reported (27, 28, 32), freshly thawed serum specimens were analyzed using a 400 MHz proton NMR (LipoScience,

Inc., Raleigh, NC). The NMR-LSP provides estimated molar concentrations of three VLDL subclasses (large, 60–200 nm; medium, 35–59 nm; and small, 27–34 nm), IDL (23–27 nm), two LDL subclasses (large, 21.3–23 nm; small, 18.3–21.2 nm), and three HDL subclasses (large, 8.9–13 nm; medium, 8.3–8.8 nm; small, 7.3–8.2 nm). It also estimates total VLDL, HDL, and LDL particle concentrations (29) and average particle diameters for VLDL, LDL, and HDL (28). The methodology depends on the aggregate signals derived from all lipid terminal methyl groups in a lipoprotein particle, and on the assumption that signals derived from methyl groups in cholesterol esters represent the same “contribution to particle size” as each of the three signals from triglyceride molecules (24, 32).

### Standard lipid profile, HbA<sub>1c</sub>, and other clinical measures at DCCT entry and near close-out visits

Total cholesterol, triglyceride, and HDL-C levels were determined at the CBL using previously reported chemical methods (29). LDL-C was estimated according to the Friedewald formula. HbA<sub>1c</sub> was measured by high-performance ion exchange liquid chromatography (33). The urinary albumin excretion rate was also measured in the CBL. Retinopathy status was assessed centrally using seven-field stereoscopic fundus photography according to the Early Treatment Diabetic Retinopathy Study protocol for individual eyes (34). The absence of retinopathy was defined as a grading of level 10 for both eyes, and presence as a grading from 20 to 85 (34).

### Statistical analyses

NMR-LSPs one year prior to the end of the DCCT randomization phase are presented as mean ± SD according to randomized treatment group, unless otherwise indicated. The comparison of mean NMR-LSP measures between intensive and conventional treatment groups was performed using the Student’s *t*-test. Because of their skewed distributions, VLDL subclasses, and small LDL particle concentrations are presented as median (interquartile range) and the Wilcoxon rank sum test was used to compare the distributions of these variables. Sex-stratified analyses were performed because of the difference in NMR-LSPs between men and women in the DCCT cohort (29). Similar statistical analyses

were used for measures from the standard lipid panel (including total cholesterol, LDL-C, and HDL-C, and triglyceride levels).

Baseline demographic and clinical measures, including age, BMI, systolic and diastolic blood pressures, and HbA<sub>1c</sub> are presented as mean ± SD according to DCCT randomization group and gender, with Student’s *t*-test used for comparisons. Variables with skewed distributions (diabetes duration, fasting triglyceride levels) are presented as median (interquartile range) stratified by DCCT treatment group and then by gender; the Wilcoxon rank sum test was used to compare distributions. The proportions of subjects smoking, or with retinopathy or albuminuria (urinary albumin excretion rate ≥30 mg/24 h) are presented by treatment group and gender; the  $\chi^2$  test was used to compare treatment groups. Two-tailed *P* < 0.05 was considered to be statistically significant. Data analysis was generated using SAS/STAT software (Version 9.3; SAS Institute Inc., Cary, NC).

The data analyses were conducted in accordance with the strategies of statistical analysis in a standard clinical trial (35) and previous DCCT publications (1, 14).

## RESULTS

Regarding DCCT baseline characteristics of our subset, key demographic and clinical characteristics did not differ significantly between those subsequently randomized to intensive or conventional diabetes treatment groups (Table 1). Thus baseline standard lipid profiles, HbA<sub>1c</sub>, systolic and diastolic blood pressures, age, diabetes duration, cigarette smoking, retinopathy, and albuminuria status were similar within the subset between the randomization groups. Sex-stratified analyses revealed minor differences: among men, the average baseline BMI was lower in the intensive than conventional treatment group, and among women, those in the intensive treatment group were about 1.5 years older than those in the conventional treatment group (*P* < 0.05). Baseline characteristics were similar between patients with available serum samples (*n* = 1,294) and those

TABLE 1. Comparisons of DCCT baseline demographic and clinical characteristics by intervention groups for 1,294 DCCT participants included in current study

Variables	All (n = 1,294)			Men (n = 692)			Women (n = 602)		
	Conventional (n = 648)	Intensive (n = 646)	<i>P</i> <sup>a</sup>	Conventional (n = 357)	Intensive (n = 335)	<i>P</i> <sup>a</sup>	Conventional (n = 291)	Intensive (n = 311)	<i>P</i> <sup>a</sup>
Age at entry (years)	26.5 ± 7.1	27.0 ± 7.1	0.18	27.4 ± 6.7	27.0 ± 7.0	0.50	25.4 ± 7.5	27.0 ± 7.2	<b>0.008</b>
BMI (kg/m <sup>2</sup> )	23.4 ± 2.9	23.3 ± 2.7	0.30	23.8 ± 2.9	23.3 ± 2.6	<b>0.007</b>	22.9 ± 3.0	23.3 ± 2.8	0.16
Duration of diabetes (years)	4.0 (2.1, 8.6)	4.1 (2.3, 9.4)	0.26	3.8 (1.9, 8.1)	4.3 (2.3, 9.4)	0.07	4.3 (2.2, 9.3)	4.0 (2.3, 9.6)	0.79
Cigarette smoker (%)	21.8	20.4	0.30	23.3	22.1	0.82	19.9	18.7	0.08
Retinopathy (%)	47.4	50.5	0.26	47.1	52.2	0.17	47.8	48.6	0.85
Albuminuria (%)	9.7	11.3	0.35	9.5	11.3	0.43	10.0	11.3	0.61
Serum creatinine (μmol/l)	71.7 ± 13.8	70.7 ± 13.1	0.18	83.2 ± 14.2	83.8 ± 10.9	0.34	63.9 ± 11.1	63.7 ± 11.0	0.84
Blood pressure (mm Hg)									
Systolic	115 ± 12	113 ± 12	0.08	118 ± 11	117 ± 11	0.21	110 ± 11	110 ± 11	0.46
Diastolic	73 ± 9	72 ± 9	0.65	74 ± 9	74 ± 9	0.91	70 ± 8	70 ± 9	0.68
HbA <sub>1c</sub> (%)	8.8 ± 1.6	8.9 ± 1.5	0.51	8.7 ± 1.5	8.7 ± 1.5	0.62	9.0 ± 1.7	9.0 ± 1.6	0.77
Fasting lipids (mmol/l)									
Total cholesterol	4.6 ± 0.9	4.5 ± 0.9	0.66	4.5 ± 0.9	4.5 ± 0.9	0.98	4.6 ± 0.8	4.7 ± 0.8	0.66
Triglycerides	0.8 (0.6, 1.0)	0.8 (0.6, 1.0)	0.58	0.8 (0.6, 1.1)	0.8 (0.6, 1.1)	0.73	0.8 (0.6, 1.0)	0.8 (0.6, 1.0)	0.79
LDL-C	2.8 ± 0.7	2.8 ± 0.7	0.55	2.8 ± 0.8	2.8 ± 0.8	0.80	2.8 ± 0.7	2.9 ± 0.7	0.57
HDL-C	1.3 ± 0.3	1.3 ± 0.3	0.53	1.2 ± 0.3	1.2 ± 0.3	0.90	1.4 ± 0.3	1.4 ± 0.3	0.78

Data are mean ± SD or median (interquartile range) for continuous variables with a skewed distribution. Percentages are used for categorical measures.

<sup>a</sup>Comparison between conventional and intensive treatment groups: *t*-test for difference in means,  $\chi^2$  test for difference in proportions, and Wilcoxon rank sum test for difference of continuous variables with skewed distributions. Bolded numbers indicate *P* < 0.05.

in whom serum samples were not available (n = 147), except that mean diastolic blood pressure, total cholesterol, LDL-C, and HbA<sub>1c</sub> were lower in the sampled cohort (supplementary Table 1). In a minority (n = 524) of our 1,294 subjects, samples were available to measure NMR-LSP at DCCT baseline: again, there were no differences in subclasses between those subsequently randomized to intensive versus standard management (data not shown).

**Table 2** shows the comparison of near close-out standard lipid profiles and other clinical characteristics of our study cohort. Again, mean levels of total cholesterol, LDL-C, and HDL-C were not significantly different between the two intervention groups, but triglyceride levels were lower among those receiving intensive versus conventional treatment, as previously shown for the entire cohort (3). Gender-stratified analysis generated similar results, except in addition, total cholesterol levels among women were significantly lower in the intensive versus conventional treatment group.

**Table 3** shows the comparison of the near close-out NMR-LSP between the two treatment groups. Overall, intensive therapy was associated with lower concentrations of small LDL, total IDL/LDL, small HDL, and total HDL particles when compared with conventional therapy. Intensive diabetes treatment was also associated with larger-diameter LDL particles.

Analyzing men and women separately (Table 3), differences between the randomization groups appeared more prominent among women, in whom intensive therapy was associated with significantly lower large VLDL and chylomicrons, total IDL/LDL, and small HDL particles, and, as in the whole cohort, the decrease of small HDL resulted in a decrease in total HDL particle concentration. Among men, only small HDL particle concentration was significantly lower with intensive versus conventional therapy, however, homogeneity analyses did not reveal a significant difference in responses between the sexes.

In the 524 patients in whom both DCCT baseline and near close-out NMR-LSP measurements were available, we compared the longitudinal changes of NMR-LSP between

intensive and conventional treatment groups. We observed similar trends as those reported in Table 3, but with less statistical power, which is likely due to the smaller sample size and lesser representation of the DCCT as a whole (data not shown).

## DISCUSSION

Analyses of DCCT/EDIC data led to the conclusion that “the long-term benefits of intensive versus conventional therapy are almost completely explained by the differences between the two groups in the mean level of HbA<sub>1c</sub> during the mean of 6.5 years of treatment in the DCCT” (5). This is, however, a statistical and not a pathophysiologic explanation, because the vascular complications are not caused by the glycation of hemoglobin. Highlighting this point, a later analysis of DCCT data revealed that “total glycemic exposure (A1c and duration of diabetes) explains only ~11% of the variation in retinopathy risk in the complete cohort”, implying that other factors are operative (6, 36). The effects of intensive therapy on the development of vascular complications (including the subsequent development of CVD) have proven durable (37, 38), but the underlying mechanisms remain unclear and are the subject of intense investigation. Dyslipoproteinemia is implicated as both marker and mechanism, not only for atherosclerosis and CVD, but also for the microvascular complications of diabetes, and therefore new techniques allowing detailed lipoprotein analyses may facilitate improved understanding. Using stored sera, we assessed the associations of intensive diabetes management with NMR-based subclasses and mean particle size of VLDL, LDL, and HDL.

In this study of DCCT participants near the end of the study’s randomization phase (1991–1993), we observed favorable associations of intensive (vs. conventional) therapy with lipoprotein subclasses. Intensive therapy was associated with lower concentrations of small LDL, total IDL/LDL, and small HDL. While lower levels of total HDL

TABLE 2. Comparisons of standard lipids and some other clinical characteristics at the near close-out DCCT visit (1991–1993), after a median follow-up of 5 years (interquartile range, 4–6 years) conventional or intensive diabetes treatment

Variables	All (n = 1,294)			Men (n = 692)			Women (n = 602)		
	Conventional (n = 648)	Intensive (n = 646)	P <sup>a</sup>	Conventional (n = 357)	Intensive (n = 335)	P <sup>a</sup>	Conventional (n = 291)	Intensive (n = 311)	P <sup>a</sup>
Total cholesterol (mmol/l)	4.7 ± 0.9	4.6 ± 0.8	0.07	4.6 ± 0.9	4.6 ± 0.8	0.51	4.8 ± 0.8	4.7 ± 0.8	<b>0.03</b>
Triglycerides (mmol/l)	0.81 (0.6, 1.1)	0.75 (0.6, 1.0)	<b>0.002</b>	0.83 (0.6, 1.1)	0.76 (0.6, 1.0)	<b>0.04</b>	0.78 (0.6, 1.1)	0.73 (0.6, 1.0)	<b>0.03</b>
LDL-C (mmol/l)	2.8 ± 0.8	2.9 ± 0.7	0.20	2.9 ± 0.8	1.9 ± 0.7	0.81	2.9 ± 0.7	2.8 ± 0.7	0.11
HDL-C (mmol/l)	1.3 ± 0.3	1.3 ± 0.3	0.78	1.2 ± 0.3	1.2 ± 0.3	0.69	1.46 ± 0.3	1.45 ± 0.3	0.60
HbA <sub>1c</sub> (%)	9.1 ± 1.6	7.2 ± 1.1	<b>&lt;0.0001</b>	9.1 ± 1.4	7.2 ± 1.1	<b>&lt;0.0001</b>	9.1 ± 1.7	7.2 ± 1.1	<b>&lt;0.0001</b>
BMI (kg/m <sup>2</sup> )	24.9 ± 3.0	26.3 ± 3.9	<b>&lt;0.0001</b>	25.3 ± 2.8	26.2 ± 3.4	<b>0.0002</b>	24.5 ± 3.2	26.4 ± 4.3	<b>&lt;0.0001</b>
Albuminuria (%)	16.6	10.2	<b>0.0008</b>	17.2	10.8	<b>0.01</b>	16.9	9.7	<b>0.02</b>
Serum creatinine (μmol/l)	75.9 ± 14.8	77.1 ± 12.6	0.12	83.2 ± 14.2	83.8 ± 10.9	0.58	66.8 ± 9.6	69.8 ± 10.2	<b>0.0002</b>

Data are mean ± SD or median (interquartile range) for continuous variables with a skewed distribution. Percentages are used for categorical measures.

<sup>a</sup>Comparison between conventional and intensive treatment groups: *t*-test for difference in means, Wilcoxon rank sum test for difference of continuous variables with skewed distributions, and  $\chi^2$  test for difference of proportions of albuminuria. Bolded numbers indicate *P* < 0.05.

TABLE 3. Comparisons of NMR-LSP at the DCCT near close-out visit (1991–1993), after an average of 5 years (range 2–9 years) conventional or intensive diabetes treatment

NMR Subclasses and Particle Size	All (n = 1,294)			Men (n = 692)			Women (n = 602)		
	Conventional (n = 648)	Intensive (n = 646)	P <sup>a</sup>	Conventional (n = 357)	Intensive (n = 335)	P <sup>a</sup>	Conventional (n = 291)	Intensive (n = 311)	P <sup>a</sup>
Total VLDL and chylomicrons (nmol/l)	29.4 (18.5, 50.3)	28.7 (18.7, 46.7)	0.45	33.7 (19.6, 58.2)	31.5 (19.9, 49.8)	0.38	26.9 (17.0, 41.5)	25.0 (18.2, 42.1)	0.97
Large VLDL and chylomicrons (nmol/l)	1 (0.6, 2)	1 (0.5, 1.7)	0.07	1.1 (0.6, 2.1)	1.1 (0.6, 2.0)	0.74	1.0 (0.6, 1.8)	0.8 (0.5, 1.4)	<b>0.03</b>
Medium VLDL (nmol/l)	10.8 (5.8, 18.8)	10.2 (5.6, 17.4)	0.23	11.6 (6.7, 21.4)	10.9 (6.1, 18.2)	0.28	9.6 (5.3, 16.4)	9.1 (4.8, 16.3)	0.66
Small VLDL (nmol/l)	16.8 (9.3, 29.6)	16.1 (9.1, 28)	0.34	18.8 (10.2, 31.4)	17.4 (9.9, 29.8)	0.32	15.0 (8.5, 25.8)	14.6 (8.2, 25.5)	0.84
Total IDL/LDL (nmol/l)	1,053 ± 381	1,000 ± 353	<b>0.0098</b>	1,059 ± 384	1,025 ± 363	0.24	1,045 ± 379	973 ± 340	<b>0.01</b>
IDL (nmol/l)	141 ± 89	140 ± 86	0.83	130 ± 90	131 ± 87	0.91	155 ± 86	150 ± 84	0.50
Large LDL (nmol/l)	361 ± 268	376 ± 246	0.29	278 ± 227	299 ± 229	0.22	462 ± 278	458 ± 237	0.85
Small LDL (nmol/l)	552 (83, 828)	465 (70, 783)	<b>0.007</b>	640 (348, 889)	622 (103, 883)	0.18	272 (68, 731)	98 (63, 664)	0.06
Total HDL (μmol/l)	34.8 ± 6.9	33.8 ± 6.4	<b>0.009</b>	33.7 ± 6.1	32.9 ± 6.4	0.13	36.2 ± 7.6	34.8 ± 6.3	<b>0.01</b>
Large HDL (μmol/l)	6.5 ± 3.4	6.6 ± 3.4	0.59	5.3 ± 2.7	5.6 ± 3.1	0.22	8.0 ± 3.6	7.7 ± 3.2	0.34
Medium HDL (μmol/l)	9.7 ± 5.6	9.9 ± 5.4	0.46	9.0 ± 5.0	8.9 ± 4.9	0.66	10.6 ± 6.2	11.1 ± 5.7	0.26
Small HDL (μmol/l)	18.6 ± 5.9	17.3 ± 5.6	<b>&lt;0.0001</b>	19.3 ± 5.2	18.5 ± 5.1	<b>0.03</b>	17.6 ± 6.5	15.9 ± 5.9	<b>0.0009</b>
NMR particle diameter (nm)									
VLDL particle size	45.0 ± 5.5	44.8 ± 5.6	0.58	45.3 ± 5.9	45.2 ± 5.9	0.93	44.7 ± 5.0	44.3 ± 5.3	0.45
LDL particle size	20.6 ± 0.7	20.7 ± 0.7	<b>0.01</b>	20.4 ± 0.7	20.5 ± 0.7	0.07	20.8 ± 0.6	20.9 ± 0.6	0.16
HDL particle size	9.3 ± 0.5	9.3 ± 0.6	0.18	9.1 ± 0.5	9.1 ± 0.5	0.23	9.5 ± 0.5	9.5 ± 0.5	0.91

Data are mean ± SD or median (interquartile range) for continuous variables with a skewed distribution. Percentages are used for categorical measures.

<sup>a</sup>Comparison between conventional and intensive treatment groups: *t*-test for difference in means, and Wilcoxon rank sum test for difference of continuous variables with skewed distributions.

Bolded numbers indicate  $P < 0.05$ .

particles were also observed (an apparently adverse association), this was entirely explained by the lower concentrations of small HDL. The association between small HDL particle concentrations and clinical CVD outcome has not been fully elucidated (39), but these particles were related to increased CVD risk in the “Pravastatin Limitation of Atherosclerosis in the Coronary Arteries” clinical trial (40). Consistent with the reduction in small LDL concentrations, we also observed that intensive diabetes therapy was associated with larger average LDL particle diameter. In general, these associations were especially marked among women, though the directions of change for men and women were the same, and overall the responses were not significantly different between the sexes. To the authors’ knowledge, this is the first report of a possible effect of intensive diabetes therapy on NMR-LSP among type 1 diabetes patients. Our results complement and extend a previous DCCT study by Purnell et al. (14): using density gradient ultracentrifugation, they found intensive diabetes therapy to be associated with favorable effects on lipoprotein (a) and apolipoprotein B levels, and on lipoprotein cholesterol distribution.

We did not observe significant differences in VLDL subclasses between the two randomization groups. Because it is well-established that improved glycemic control is associated with a reduction in plasma triglycerides (as was seen in the DCCT as a whole and in our present subset study), reductions in NMR VLDL subclasses might have been expected. It must be noted that NMR-LSP estimates the molar concentrations of lipoprotein particles, and is distinct from biochemical measures such as plasma triglyceride concentration. In our study, plasma triglycerides were 7.4% lower in the intensive versus conventional treatment group at near close-out ( $P < 0.002$ ), while medium and small VLDL (the predominant subclasses of VLDL) were 5.6 and 4.2% lower, respectively (neither reaching statistical significance). Estimated VLDL particle volume (from diameter) was on average 1.3% lower in the intensive versus conventional group. Thus there were consistent “directions of difference” between triglyceride levels and the VLDL particle subclass concentrations, and some of the discrepancies in significance may be the result of shifts in VLDL volume (greatly affected by small changes in diameter) and triglyceride content.

Ideally, we would have analyzed changes in NMR-LSP from baseline to near close-out in all DCCT participants. While we were able to study 1,294 participants at near close-out, we had access to only 40% of these ( $n = 524$ ) at DCCT baseline. In this smaller subset, there were, again, no differences in baseline NMR-LSP between those subsequently randomized to intensive versus conventional therapy, and despite the reduced power, when we examined changes between baseline and near close-out according to randomization group, we reached conclusions similar to those drawn from the cross-sectional analyses in Table 3. We acknowledge that differences in concurrent HbA<sub>1c</sub>, weight gain, and renal dysfunction between the intensive and conventional treatment groups at near close-out

(1, 3) may have contributed to the observed NMR-LSP differences.


In the general population, lipoprotein particle concentrations and sizes are established predictors of future cardiovascular events, and are at least comparable in this regard to the cholesterol content of lipoproteins (18, 19). Smaller LDL and HDL particle diameters, low particle numbers of large HDL, and high particle numbers of large VLDL, small HDL, total LDL, and small LDL have all been associated with CVD risk in large prospective studies in the general population (18, 41, 42). There is a paucity of data regarding the longitudinal association between NMR-LSP and CVD in type 1 diabetes, and we intend to address this important question in coming years, as longer-term “hard” CVD events develop in the now-aging DCCT/EDIC cohort, providing case numbers that will enable more rigorous and strongly powered statistical analysis. In a prospective nested case-control study [59 controls and 59 coronary artery disease (CAD) cases] from the Pittsburgh Epidemiology of Diabetes Complication Study, NMR-LSP improved the prediction of CAD in people with type 1 diabetes (43): CAD cases versus controls had smaller HDL particles, lower large HDL, and higher medium HDL particle concentrations (43).

The current study adds to the literature by providing evidence that intensive diabetes therapy is associated with favorable effects on detailed serum lipoprotein profiles that cannot be discerned by standard lipid profiles, and is consistent with the notion that CVD risk may be lowered through beneficial effects of intensive management on lipoprotein particle sizes and subclass concentrations. We postulate that such beneficial effects, if in force throughout the DCCT randomization phase (i.e., during young adulthood), could extend years into the future by reducing the subclinical burden (e.g., sub-intimal extravasated lipoproteins, fatty streaks, foam cells, plaque) “inherited” upon entering middle age (i.e., postrandomization, upon entry to EDIC). This inheritance, through “compound interest,” may yield a “dividend” of cardiovascular events in later life. These concepts are consistent with the possibility of a “lipemic memory” as a component of the “metabolic memory” that has been postulated in DCCT/EDIC to explain the long persistence of benefits derived from prior intensive management (44). Vascular “memory” has also been demonstrated for lipids and for lipid-lowering drugs (45).

Our study suggests possible gender differences in the associations between intensive diabetes therapy and NMR-LSP profiles. Women who received intensive versus conventional diabetes treatment had lower concentrations of large VLDL and chylomicrons, small LDL, total IDL/LDL, and small HDL particles. Similar trends were observed in men, but did not reach statistical significance, except in the case of small HDL. Given that the average age of menopause in the USA is 51 years (46), it is likely that most of our female subjects (mean age 26.2 years at sample collection) were premenopausal. Premenopausal women usually have a lower risk of CVD compared with men in the same age group (47, 48), but women with type 1 diabetes lose this protection (49–52). The reason for this loss of protection

against CVD among young type 1 diabetic women is not fully understood. Whether beneficial associations of lipoprotein subclass profiles with improved glycemic control will help premenopausal type 1 diabetic women to regain some CVD protection is an important question awaiting an answer (50).

The strengths of this study include the fact that it is focused on type 1 diabetes, it uses clinical data of the highest quality from the DCCT, the sample size in each treatment group is large, and the lipoprotein analyses are comprehensive, including both standard lipid profiles and NMR-LSP. Limitations include its cross-sectional nature, and the presence of some differences between the analyzed cohort of 1,294 patients and the remaining DCCT participants ( $n = 147$ ), i.e., those for whom no appropriate stored serum samples were available. Finally, although our study suggests that intensive diabetes management may improve lipoprotein subclass profiles, it does not define the duration of intervention needed to achieve this effect: subjects were studied at a median of 5 years postrandomization, which is likely long enough for changes to have been induced. Indeed, clinical experience suggests that circulating lipoprotein levels respond within days to a few weeks to an altered environment (e.g., altered glycemia, implementation of lipid-lowering treatments), and therefore we consider it probable that the associations observed were operative throughout the DCCT randomization period, making it more likely they could influence the development of vascular complications.

In summary, in type 1 diabetes patients in the DCCT, intensive diabetes treatment is associated with favorable LDL and HDL subclass characteristics, and thus beneficial effects on serum lipids/lipoproteins extend beyond the previously recognized lowering of triglyceride levels. These NMR-LSP-related associations tended to be more marked among women than men, although this gender difference was not statistically significant. Further studies are needed to define whether these hitherto-unrecognized beneficial effects of intensive glycemic management contribute to improved outcomes for the vascular complications of type 1 diabetes. 

A complete list of participants in the DCCT/EDIC Research Group is provided in the supplementary Appendix.

## REFERENCES

1. The Diabetes Control and Complications Trial Research Group. 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.
2. Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. 1999. Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. *Diabetes Care.* **22**: 99–111.
3. Nathan, D. M., P. A. Cleary, J. Y. Backlund, S. M. Genuth, J. M. Lachin, T. J. Orchard, P. Raskin, and B. Zinman; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. 2005. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. *N. Engl. J. Med.* **353**: 2643–2653.

4. Nathan, D. M., J. Lachin, P. Cleary, T. Orchard, D. J. Brillon, J. Y. Backlund, D. H. O'Leary, and S. Genuth; Diabetes Control and Complications Trial; Epidemiology of Diabetes Interventions and Complications Research Group. 2003. Intensive diabetes therapy and carotid intima-media thickness in type 1 diabetes mellitus. *N. Engl. J. Med.* **348**: 2294–2303.
5. Lachin, J. M., T. J. Orchard, and D. M. Nathan; DCCT/EDIC Research Group. 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.
6. Lachin, J. M., S. Genuth, D. M. Nathan, B. Zinman, and B. N. Rutledge; DCCT/EDIC Research Group. 2008. Effect of glycemic exposure on the risk of microvascular complications in the diabetes control and complications trial—revisited. *Diabetes.* **57**: 995–1001.
7. Writing Team for the DCCT/EDIC Research Group. 2003. Sustained effect of intensive treatment of type 1 diabetes mellitus on development and progression of diabetic nephropathy: the Epidemiology of Diabetes Interventions and Complications (EDIC) study. *JAMA.* **290**: 2159–2167.
8. Stone, N. J., J. G. Robinson, A. H. Lichtenstein, C. N. Bairey Merz, C. B. Blum, R. H. Eckel, A. C. Goldberg, D. Gordon, D. Levy, D. M. Lloyd-Jones, et al.; American College of Cardiology/American Heart Association Task Force on Practice Guidelines. 2014. American College of Cardiology/American Heart Association Task Force on Practice Guidelines: 2013 ACC/AHA guideline on the treatment of blood cholesterol to reduce atherosclerotic cardiovascular risk in adults: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines. *Circulation.* **129**: S1–S45.
9. Sniderman, A. D. 2005. Apolipoprotein B versus non-high-density lipoprotein cholesterol: and the winner is. *Circulation.* **112**: 3366–3367.
10. deGoma, E. M., J. W. Knowles, F. Angeli, M. J. Budoff, and D. J. Rader. 2012. The evolution and refinement of traditional risk factors for cardiovascular disease. *Cardiol. Rev.* **20**: 118–129.
11. Barter, P. J., C. M. Ballantyne, R. Carmena, M. Castro Cabezas, M. J. Chapman, P. Couture, J. de Graaf, P. N. Durrington, O. Faergeman, J. Frohlich, et al. 2006. Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. *J. Intern. Med.* **259**: 247–258.
12. Sniderman, A., K. Williams, and C. Cobbaert. 2009. ApoB versus non-HDL-C: what to do when they disagree. *Curr. Atheroscler. Rep.* **11**: 358–363.
13. Brunzell, J. D., M. Davidson, C. D. Furberg, R. B. Goldberg, B. V. Howard, J. H. Stein, and J. L. Witztum. 2008. Lipoprotein management in patients with cardiometabolic risk: consensus conference report from the American Diabetes Association and the American College of Cardiology Foundation. *J. Am. Coll. Cardiol.* **51**: 1512–1524.
14. Purnell, J. Q., S. M. Marcovina, J. E. Hokanson, H. Kennedy, P. A. Cleary, M. W. Steffes, and J. D. Brunzell. 1995. Levels of lipoprotein(a), apolipoprotein B, and lipoprotein cholesterol distribution in IDDM. Results from follow-up in the Diabetes Control and Complications Trial. *Diabetes.* **44**: 1218–1226.
15. Lyons, T. J., A. J. Jenkins, D. Zheng, D. T. Lackland, D. McGee, W. T. Garvey, and R. L. Klein. 2004. Diabetic retinopathy and serum lipoprotein subclasses in the DCCT/EDIC cohort. *Invest. Ophthalmol. Vis. Sci.* **45**: 910–918.
16. Hoogeveen, R. C., J. W. Gaubatz, W. Sun, R. C. Dodge, J. R. Crosby, J. Jiang, D. Couper, S. S. Virani, S. Kathiresan, E. Boerwinkle, et al. 2014. Small dense low-density lipoprotein-cholesterol concentrations predict risk for coronary heart disease: the Atherosclerosis Risk in Communities (ARIC) study. *Arterioscler. Thromb. Vasc. Biol.* **34**: 1069–1077.
17. Mackey, R. H., P. Greenland, D. C. Goff, Jr., D. Lloyd-Jones, C. T. Sibley, and S. Mora. 2012. High-density lipoprotein cholesterol and particle concentrations, carotid atherosclerosis, and coronary events: MESA (multi-ethnic study of atherosclerosis). *J. Am. Coll. Cardiol.* **60**: 508–516.
18. Mora, S., J. D. Otvos, N. Rifai, R. S. Rosenson, J. E. Buring, and P. M. Ridker. 2009. Lipoprotein particle profiles by nuclear magnetic resonance compared with standard lipids and apolipoproteins in predicting incident cardiovascular disease in women. *Circulation.* **119**: 931–939.
19. Parish, S., A. Offer, R. Clarke, J. C. Hopewell, M. R. Hill, J. D. Otvos, J. Armitage, and R. Collins; Heart Protection Study Collaborative Group. 2012. Lipids and lipoproteins and risk of different vascular events in the MRC/BHF Heart Protection Study. *Circulation.* **125**: 2469–2478.
20. Steffen, B. T., W. Guan, A. T. Remaley, P. Paramsothy, S. R. Heckbert, R. L. McClelland, P. Greenland, E. D. Michos, and M. Y. Tsai. 2015. Use of lipoprotein particle measures for assessing coronary heart disease risk post-American Heart Association/American College of Cardiology guidelines: the Multi-Ethnic Study of Atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **35**: 448–454.
21. Hsia, J., J. D. Otvos, J. E. Rossouw, L. Wu, S. Wassertheil-Smoller, S. L. Hendrix, J. G. Robinson, B. Lund, and L. H. Kuller; Women's Health Initiative Research Group. 2008. Lipoprotein particle concentrations may explain the absence of coronary protection in the women's health initiative hormone trials. *Arterioscler. Thromb. Vasc. Biol.* **28**: 1666–1671.
22. Otvos, J. D., S. Mora, I. Shalurova, P. Greenland, R. H. Mackey, and D. C. Goff, Jr. 2011. Clinical implications of discordance between low-density lipoprotein cholesterol and particle number. *J. Clin. Lipidol.* **5**: 105–113.
23. Jeyarajah, E. J., W. C. Cromwell, and J. D. Otvos. 2006. Lipoprotein particle analysis by nuclear magnetic resonance spectroscopy. *Clin. Lab. Med.* **26**: 847–870.
24. Rifai, N., G. R. Warnick, and M. H. Dominiczak. 2000. Handbook of Lipoprotein Testing. 2nd edition. AACC Press, Washington, DC.
25. Otvos, J. D., E. J. Jeyarajah, D. W. Bennett, and R. M. Krauss. 1992. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma lipoprotein concentrations and subspecies distributions from a single, rapid measurement. *Clin. Chem.* **38**: 1632–1638.
26. Tsai, M. Y., A. Georgopoulos, J. D. Otvos, J. M. Ordovas, N. Q. Hanson, J. M. Peacock, and D. K. Arnett. 2004. Comparison of ultracentrifugation and nuclear magnetic resonance spectroscopy in the quantification of triglyceride-rich lipoproteins after an oral fat load. *Clin. Chem.* **50**: 1201–1204.
27. 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, et al.; DCCT/EDIC Research Group. 2006. Nuclear magnetic resonance-determined lipoprotein subclass profile in the DCCT/EDIC cohort: associations with carotid intima-media thickness. *Diabet. Med.* **23**: 955–966.
28. Jenkins, A. J., T. J. Lyons, D. Zheng, J. D. Otvos, D. T. Lackland, D. McGee, W. T. Garvey, and R. L. Klein; DCCT/EDIC Research Group. 2003. Lipoproteins in the DCCT/EDIC cohort: associations with diabetic nephropathy. *Kidney Int.* **64**: 817–828.
29. Jenkins, A. J., T. J. Lyons, D. Zheng, J. D. Otvos, D. T. Lackland, D. McGee, W. T. Garvey, and R. L. Klein; DCCT/EDIC Research Group. 2003. Serum lipoproteins in the diabetes control and complications trial/epidemiology of diabetes intervention and complications cohort: associations with gender and glycemia. *Diabetes Care.* **26**: 810–818.
30. The DCCT Research Group. 1986. The Diabetes Control and Complications Trial (DCCT). Design and methodologic considerations for the feasibility phase. The DCCT Research Group. *Diabetes.* **35**: 530–545.
31. The DCCT Research Group. 1990. Diabetes Control and Complications Trial (DCCT). Update. *Diabetes Care.* **13**: 427–433.
32. Otvos, J. D. 2002. Measurement of lipoprotein subclass profiles by nuclear magnetic resonance spectroscopy. *Clin. Lab.* **48**: 171–180.
33. The DCCT Research Group. 1987. Feasibility of centralized measurements of glycated hemoglobin in the Diabetes Control and Complications Trial: a multicenter study. *Clin. Chem.* **33**: 2267–2271.
34. 1995. The effect of intensive diabetes treatment on the progression of diabetic retinopathy in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial. *Arch. Ophthalmol.* **113**: 36–51.
35. Friedman, L. M., C. D. Furberg, and D. L. Demets. 2010. Fundamentals of Clinical Trials. Springer Science+Business Media, LLC, New York.
36. 1995. The relationship of glycemic exposure (HbA1c) to the risk of development and progression of retinopathy in the Diabetes Control and Complications Trial. *Diabetes.* **44**: 968–983.
37. Nathan, D. M., P. McGee, M. W. Steffes, and J. M. Lachin; DCCT/EDIC Research Group. 2014. Relationship of glycated albumin to blood glucose and HbA1c values and to retinopathy, nephropathy, and cardiovascular outcomes in the DCCT/EDIC study. *Diabetes.* **63**: 282–290.
38. Lachin, J. M., N. H. White, D. P. Hainsworth, W. Sun, P. A. Cleary, and D. M. Nathan; Diabetes Control and Complications Trial

- (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. 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.
39. Kingwell, B. A., M. J. Chapman, A. Kontush, and N. E. Miller. 2014. HDL-targeted therapies: progress, failures and future. *Nat. Rev. Drug Discov.* **13**: 445–464.
  40. Rosenson, R. S., J. D. Otvos, and D. S. Freedman. 2002. Relations of lipoprotein subclass levels and low-density lipoprotein size to progression of coronary artery disease in the Pravastatin Limitation of Atherosclerosis in the Coronary Arteries (PLAC-I) trial. *Am. J. Cardiol.* **90**: 89–94.
  41. Virani, S. S., D. J. Catellier, L. A. Pompeii, V. Nambi, R. C. Hoogeveen, B. A. Wasserman, J. Coresh, T. H. Mosley, J. D. Otvos, A. R. Sharrett, et al. 2011. Relation of cholesterol and lipoprotein parameters with carotid artery plaque characteristics: the Atherosclerosis Risk in Communities (ARIC) carotid MRI study. *Atherosclerosis*. **219**: 596–602.
  42. Mora, S., M. Szklo, J. D. Otvos, P. Greenland, B. M. Psaty, D. C. Goff, Jr., D. H. O'Leary, M. F. Saad, M. Y. Tsai, and A. R. Sharrett. 2007. LDL particle subclasses, LDL particle size, and carotid atherosclerosis in the Multi-Ethnic Study of Atherosclerosis (MESA). *Atherosclerosis*. **192**: 211–217.
  43. Soedamah-Muthu, S. S., Y. F. Chang, J. Otvos, R. W. Evans, and T. J. Orchard. 2003. Pittsburgh Epidemiology of Diabetes Complications Study: Lipoprotein subclass measurements by nuclear magnetic resonance spectroscopy improve the prediction of coronary artery disease in Type 1 diabetes. A prospective report from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetologia*. **46**: 674–682.
  44. Albers, J. W., W. H. Herman, R. Pop-Busui, E. L. Feldman, C. L. Martin, P. A. Cleary, B. H. Waberski, and J. M. Lachin. 2010. Diabetes Control Complications Trial /Epidemiology of Diabetes Interventions Complications Research Group. Effect of prior intensive insulin treatment during the Diabetes Control and Complications Trial (DCCT) on peripheral neuropathy in type 1 diabetes during the Epidemiology of Diabetes Interventions and Complications (EDIC) Study. *Diabetes Care*. **33**: 1090–1096.
  45. Jermendy, G. 2012. Vascular memory: can we broaden the concept of the metabolic memory? *Cardiovasc. Diabetol.* **11**: 44.
  46. Gold, E. B., J. Bromberger, S. Crawford, S. Samuels, G. A. Greendale, S. D. Harlow, and J. Skurnick. 2001. Factors associated with age at natural menopause in a multiethnic sample of midlife women. *Am. J. Epidemiol.* **153**: 865–874.
  47. Go, A. S., D. Mozaffarian, V. L. Roger, E. J. Benjamin, J. D. Berry, W. B. Borden, D. M. Bravata, S. Dai, E. S. Ford, C. S. Fox, et al. 2013. American Heart Association Statistics, Committee, Stroke Statistics, Subcommittee: Heart disease and stroke statistics–2013 update: a report from the American Heart Association. *Circulation*. **127**: e6–e245.
  48. Zhang, Y. 2010. Cardiovascular diseases in American women. *Nutr. Metab. Cardiovasc. Dis.* **20**: 386–393.
  49. Dabelea, D., G. Kinney, J. K. Snell-Bergeon, J. E. Hokanson, R. H. Eckel, J. Ehrlich, S. Garg, R. F. Hamman, and M. Rewers; Coronary Artery Calcification in Type 1 Diabetes Study Group. 2003. Effect of type 1 diabetes on the gender difference in coronary artery calcification: a role for insulin resistance? The Coronary Artery Calcification in Type 1 Diabetes (CACTI) Study. *Diabetes*. **52**: 2833–2839.
  50. Colhoun, H. M., M. B. Rubens, S. R. Underwood, and J. H. Fuller. 2000. The effect of type 1 diabetes mellitus on the gender difference in coronary artery calcification. *J. Am. Coll. Cardiol.* **36**: 2160–2167.
  51. Lloyd, C. E., L. H. Kuller, D. Ellis, D. J. Becker, R. R. Wing, and T. J. Orchard. 1996. Coronary artery disease in IDDM. Gender differences in risk factors but not risk. *Arterioscler. Thromb. Vasc. Biol.* **16**: 720–726.
  52. 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.