Low-Density Lipoprotein Particle Size and Coronary Artery Disease in a Childhood-Onset Type 1 Diabetes Population

John R. Erbey, David Robbins, Kimberly Y.-Z. Forrest, and Trevor J. Orchard

Low-density lipoprotein (LDL) cholesterol has been widely recognized as a strong predictor of coronary artery disease (CAD). Recently, studies have examined the influence of LDL particle size (an integral part of the insulin resistance syndrome) on the development of CAD in the general population. This report examines the correlates of LDL particle size and its association with CAD in a type 1 diabetes population. We evaluated the interrelationships between LDL particle size and the presence of CAD in a cohort of childhood-onset type 1 diabetic subjects using the Pittsburgh Epidemiology of Diabetes Complications (EDC) study. LDL particle size was measured in 337 subjects (mean age, 35.6 years; mean diabetes duration, 27.2 years) who underwent the 8-year follow-up examination. LDL particle size was determined by vertical polyacrylamide gel (2% to 16%) electrophoresis. Subjects with the small dense LDL particle phenotype (<235.5 nmol/L) had a longer diabetes duration, higher cholesterol, triglyceride, LDL, fibrinogen, waist to hip ratio (WHR), and hemoglobin A_1 (HbA_1), and lower high-density lipoprotein (HDL) cholesterol compared with subjects with the large LDL particle phenotype (>257 nmol/L). Males were also more likely to have an increased body mass index (BMI) and CAD, while females were more likely to have hypertension and a family history of type 2 diabetes (a potential marker of insulin resistance and CAD risk). The odds ratio ([OR] 95% confidence, interval [CI]) using logistic regression analysis for LDL particle size in association with CAD was 0.79 (0.60 to 1.04). Multivariate modeling indicated that the duration of type 1 diabetes, depressive symptomatology, and triglycerides were independently associated with the presence of CAD. We conclude that although small dense LDL particle size is associated with CAD in our type 1 diabetes population, its borderline association can largely be explained by the triglyceride concentration. However, as in the general population, LDL particle size is associated with many elements of the insulin resistance syndrome, including a family history of type 2 diabetes, and is likely an important element in the contribution of insulin resistance to the development of CAD in type 1 diabetes.

THE PRIMARY MODIFIABLE risk factor for coronary artery disease (CAD) in the general population is low-density lipoprotein (LDL) cholesterol.[1-3] In addition to the concentration of LDL particles, which predicts the rate of coronary heart disease (CHD) events both within and across populations,[4] LDL particle size has been increasingly recognized as a potential marker of increased risk for CAD and is relatively rare, as is CHD, in premenopausal women.[5] Along with numerous case-control studies,[6-10] the Quebec Cardiovascular Study has recently provided prospective data confirming the independent association of small dense LDL particles and subsequent development of ischemic heart disease (IHD).[11] LDL particle size is also closely linked to insulin resistance and the triglyceride concentration,[12] and is now considered part of the insulin resistance syndrome[5,12] and a predominant feature of type 2 diabetes.[13] However, little is known about LDL particle size in type 1 diabetes, particularly whether it shows the same association with CHD, shares the same correlates, and is related to glycemic control.

This report examines such associations in type 1 diabetes with a particular focus on markers of insulin resistance, including family history of type 2 diabetes, which we have recently shown to be associated with CAD risk in this population.

SUBJECTS AND METHODS

The Pittsburgh Epidemiology of Diabetes Complications (EDC) study is a 10-year prospective study based on a well-defined cohort of childhood-onset (<17 years) type 1 diabetes subjects. There were 658 eligible subjects (325 women and 333 men) diagnosed between January 1, 1950 and May 30, 1980, who were first evaluated at the baseline examination (1986 to 1988). They have been evaluated biennially thereafter. The design and methods of the study have been previously described.[14]

For this analysis, a cross-sectional design was used for participants attending the 8-year follow-up (1994 to 1996) examination. LDL particle size was determined in 337 subjects using the method of Krauss and Burke.[15] Gradient gels were obtained from IsoLab (Akron, OH). The measurement of the predominant peak size was calibrated using LDL subfractions with molecular diameter determined by analytical ultracentrifugation (courtesy of Dr R. Krauss, Donner Laboratories, Berkeley, CA). The LDL size of the predominant peak was defined as the subject's LDL size.[13]

The presence of CAD was established by clinic physician-diagnosed angina or confirmed myocardial infarction (pathological Q waves or validated hospital records). A 12-lead electrocardiogram was obtained, along with blood pressure measured by a random-zero sphygmomanometer according to the Hypertension Detection Follow-up Program protocol[16] after a 5-minute rest period. Subjects were considered hypertensive if they were on treatment with blood pressure medication and/or if they had blood pressure greater than 140 mm Hg systolic and/or 90 mm Hg diastolic.

Family history information was ascertained on the general medical history questionnaire. A family history of presumed type 2 diabetes was defined as diabetes diagnosed after age 30 years in first-degree relatives of the participant, after further evaluation to validate the diagnosis and type of diabetes.[17] A family history of type 2 diabetes was confirmed if insulin therapy was not initiated within 1 year of diagnosis and there was no history of ketosis. Fuller details are reported elsewhere.[17]

Details regarding the clinical and metabolic evaluations have also

From the Department of Epidemiology, Graduate School of Public Health, University of Pittsburgh, Pittsburgh; Penn Medical Laboratory, Medlantic Research Institute, Washington, DC; and Department of Allied Health, Slippery Rock University, Pittsburgh, PA.

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Address reprint requests to Trevor J. Orchard, MBBch, MMedSci, Rangos Research Center, 5th Floor, 3460 Fifth Ave, Pittsburgh, PA 15213.

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been previously reported.[14,18] Fasting blood samples were taken from each EDC participant for measurement of lipids, lipoproteins, hemoglobin A_1 (HbA_1), and fibrinogen. The Beck Depression Inventory, a quantitative measure of depressive symptomatology,[19] was also included among the medical history and life-style questionnaires.

The nephropathy status was determined based on consistent results from at least two of three (24-hour, overnight, and random timed postclinic urine) timed determinations of the urine albumin excretion rate (AER). Microalbuminuria was defined as an AER between 20 and 200 μg/min. Overt nephropathy was defined as an AER greater than 200 μg/min or end-stage renal disease (renal dialysis or transplant). In the absence of two complete urine collections, a urinary albumin to creatinine ratio (milligram/milligram) greater than 0.31 was used to define overt nephropathy as previously described.[20] In the absence of any urine specimens, a serum creatinine level greater than 2 mg/dL was considered indicative of overt nephropathy. Urinary albumin was determined immunonephelometrically.[20]

Cross-sectional analyses included Student's *t* test for continuous variables and the chi-square test for categorical variables. Corrections for multiple comparisons were not made; instead, exact significance levels are indicated where appropriate, allowing the reader to determine the correction according to preference. Logistic regression was used where the odds ratio (OR) for continuous variables is expressed per standard deviation of the variable. For variables that were highly intercorrelated (ie, age and diabetes duration, total and LDL cholesterol), only one variable was chosen (ie, diabetes duration and LDL cholesterol).

RESULTS

LDL particle size was determined in 337 subjects with a mean age of 35.6 years and a mean type 1 diabetes duration of 27.2 years (176 males and 161 females). Thirty-two subjects were classified as the type B phenotype (small dense, <235.5 nmol/L) and 279 as type A (large buoyant, >257 nmol/L). The remaining 26 were classified as the intermediate pattern. All but 16 of 161 females were premenopausal.

Table 1 shows that subjects of both genders with the type B phenotype had a significantly longer diabetes duration, higher cholesterol, triglycerides, LDL cholesterol, fibrinogen, waist to hip ratio (WHR), and HbA_1, and lower high-density lipoprotein (HDL) cholesterol compared with the type A phenotype. Males with type B phenotype were also more likely to have a longer duration of type 1 diabetes, a higher body mass index (BMI) and HbA_1, and CAD. Premenopausal women with type B phenotype were more likely to have CAD, hypertension, and a family history of type 2 diabetes, as well as borderline elevated HbA_1. Postmenopausal women had a greater frequency of type B phenotype than premenopausal women (11.8% *v* 6.2%) and were more likely to have CAD and a family history of type 2 diabetes, as well as an increased Beck Depression Inventory score. Interestingly, HbA_1 was increased in men and postmenopausal women with type B phenotype but decreased in type B premenopausal women.

Table 2 lists characteristics of the subjects by CAD status. Table 3 presents the OR for the presence of CAD in association with known risk factors in this population. The OR (95% confidence interval [CI]) using logistic regression analysis for LDL particle size, family history of type 2 diabetes, and triglycerides in association with CAD was 0.79 (0.60 to 1.04), 1.88 (1.17 to 3.02), and 5.93 (2.54 to 13.84), respectively. Forward stepwise multiple logistic regression indicated that the duration of type 1 diabetes, triglycerides, and depressive symptomatology were independently associated with the pres-

Table 1. Characteristics of the Subjects by Gender and LDL Particle Size Phenotype

	Males				Females						
					Premenopausal				Postmenopausal		
Characteristic	Type A (n = 131)	Type B (n = 21)	*P*	*r* (n = 176)	Type A (n = 134)	Type B (n = 9)	*P*	*r* (n = 144)	Type A (n = 14)	Type B (n = 2)	*r* (n = 17)
Age (yr)	34.6	37.7	.07	−.10	35.2	32.2	.24	.10	47.7	52.3	.07
Diabetes duration (yr)	26.4	29.8	.047	−.08	26.4	24.7	.49	.04	38.1	40.0	.05
Cholesterol (mg/dL)	179.1	215.0	<.000	−.35†	183.1	255.1	<.000	−.27†	210.8	243.5	−.38
HDL cholesterol (mg/dL)	51.5	40.2	<.000	.39†	61.2	50.4	.033	.36†	65.6	59.8	.17
Triglyceride (mg/dL)	66.7	154.5	<.000	−.53†	70.3	185.7	<.000	−.52†	83.6	248.9	−.63†
LDL cholesterol (mg/dL)	112.3	142.4	<.000	−.37†	106.8	160.5	<.000	−.23†	130.4	130.7	−.15
Fibrinogen (mg/dL)	327.3	442.5	<.000	−.32†	343.3	429.4	.018	−.33†	425.7	340.0	−.02
HbA_1 (%)	10.2	11.2	.026	−.23†	10.4	9.3	.053	.10	10.3	10.8	−.32
Insulin dose (U/kg/d)	0.73	0.78	.32	−.19*	0.64	0.70	.47	−.16	0.60	0.56	.33
BMI (kg/m^2)	24.8	27.5	<.000	−.27†	24.6	27.3	.06	−.16*	24.9	23.5	.01
WHR	0.89	0.94	<.000	−.15	0.79	0.85	.021	−.26†	0.82	0.97	−.51*
Beck Depression Inventory	5.1	4.0	.54	−.03	8.5	11.6	.29	−.06	7.4	10.0	−.18
Smoker (%)	30.4	40.0	.40	−.07	32.6	33.3	.96	−.14	35.7	50.0	.18
CAD (%)	11.4	28.6	.058	−.06	10.4	0.0	.31	−.08	28.6	100.0	−.68†
Hypertension (%)	24.4	38.1	.19	−.09	19.4	55.6	.011	−.24†	35.7	0.0	−.05
Microalbuminuria (%)	48.0	70.0	.07	−.25†	59.5	67.0	.68	−.16	78.6	50.0	−.06
Overt nephropathy (%)	24.4	36.8	.25	−.10	25.2	50.0	.13	−.18*	35.7	50.0	−.37
Family history of type 2 DM (%)	11.4	19.1	.33	−.13	15.7	44.4	.028	−.14	21.4	50.0	−.41
Family history of MI (%)	24.4	28.6	.68	.02	20.9	33.3	.38	−.06	50.0	50.0	.23
LDL particle size (nmol/L)	264.6	248.5	<.000		267.8	247.2	<.000		264.6	251.4	

Abbreviations: DM, diabetes mellitus; MI, myocardial infarction.

*$P < .05$.

†$P < .01$.

Table 2. Characteristics of the Subjects by CAD Status

Characteristic	CAD (n = 44)	Non-CAD (n = 297)	P
Age (yr)	42.2	34.6	<.000
Diabetes duration (yr)	34.5	26.1	<.000
Cholesterol (mg/dL)	205.4	185.1	003
HDL cholesterol (mg/dL)	55.5	54.8	.773
Triglyceride (mg/dL)	111.1	77.2	<.000
LDL cholesterol (mg/dL)	124.9	113.2	.059
Fibrinogen (mg/dL)	415.1	343.9	<.000
HbA_1 (%)	10.7	10.3	.112
Insulin dose (U/kg/d)	0.66	0.69	.386
Beck Depression Inventory	11.2	6.0	<.000
BMI (kg/m^2)	25.6	24.9	.328
WHR	0.89	0.84	.001
LDL particle size (nmol/L)	261.3	264.0	.030
Type B phenotype (%)	17.2	7.9	.047
Family history of MI (%)	47.7	21.9	<.000

Abbreviation: MI, myocardial infarction.

ence of CAD. In this sample, neither a family history of type 2 diabetes nor LDL particle size were related to CAD independently of diabetes duration and triglycerides.

DISCUSSION

The LDL cholesterol concentration has been shown to be a strong independent predictor of CAD in the general population[1] and in diabetes.[4] This risk has been shown to relate to the initiation and promotion of atherosclerosis by high concentrations of LDL cholesterol.[21] These findings have been supported by the results of recent clinical trials in which statin therapy used to reduce LDL cholesterol also reduced the risk for subsequent coronary events,[2,3] including individuals with diabetes.[22] The precise nature of the link between LDL cholesterol and atherosclerosis is unknown. LDL particle size has been proposed as a key determinant of the atherogenic potential of LDL, as it may be associated with a decreased binding affinity for the LDL receptor,[23] more likely to bind to proteoglycans in the arterial wall,[24] and more susceptible to oxidation.[25]

LDL particle size has also been shown to relate to CAD in numerous case-control studies.[6-10] However, this relationship is not fully independent of triglycerides and other risk factors. Our results are therefore consistent with the general literature. However, in a recent analysis of the Quebec Cardiovascular Study, LDL particle size was associated with the development of IHD independently of triglyceride and apolipoprotein B (apo B) concentrations.[11] Indeed, the combination of small LDL particles and a high apo B level showed an OR of 6.2. In the Quebec study, the mean age was 59 years, while the mean age in the present study is less than 36 years. The study design may also contribute to differences in the findings. The Quebec study used a powerful prospective matched case-control design involving more than double the number of IHD events. Prospective follow-up study of the current cohort will hopefully define the role of LDL particle size in type 1 diabetes more clearly.

Table 3. Forward Stepwise Logistic Regression Analysis

Model of Best Fit	OR	95% CI
Duration (8 yr)	3.07	2.07-4.56*
Beck Depression Inventory (7.5)	1.88	1.36-2.58*
Triglycerides (3.6)†	1.82	1.22-2.71*

NOTE. Results are for forward stepwise logistic regression with CAD as the dependent variable. Continuous variables are expressed per standard deviation of the variable.

*$P < .001$.

†Logarithmically transformed; the untransformed standard deviation for triglycerides is 110 mg/dL.

The relationship of LDL size and type 2 diabetes has been studied extensively.[5,6,13,26,27] Two clinic-based studies first noted an increase in small dense LDL in type 2 diabetes.[26,27] Studies i npopulation-based samples, ie, the Framingham Study[6] and Kaiser Women Twins Study,[5] also reported associations with diabetes. Haffner et al[13] reported cross-sectional decreases in LDL particle size in both men and women with type 2 diabetes. The association remained significant in women after adjustments for obesity, body fat distribution, triglyceride, and HDL cholesterol. This may indicate that the differences in LDL particle size may relate to increased atherogenic potential, especially in diabetes, ultimately accounting for the loss of the gender advantage in cardiovascular disease in diabetics. In our study, HbA_1 was inversely related to LDL particle size in men and postmenopausal women, but not in premenopausal women.

Few data are available on LDL density in type 1 diabetes. A report from the Diabetes Control and Complications Trial (DCCT) indicates that intensive therapy, in addition to reducing HbA_1 levels, was associated with more buoyant LDL fractions.[28] While this particular study used density-gradient ultracentrifugation to determine LDL size, the interpretation of the results is the same. The association between intensive therapy and more buoyant LDL fractions supports other reports from the DCCT that intensive therapy is associated with a less atherogenic lipid profile.[29]

The association between LDL particle size and a family history of type 2 diabetes is an important finding, as it may indicate a link to insulin resistance. Our data indicate that a family history of type 2 diabetes is associated with CAD independently of diabetes duration and nephropathy status.[17] In addition to CAD, a family history of type 2 diabetes is also associated with markers of the insulin resistance syndrome, namely triglycerides, and in females, both hypertension and waist circumference.[17] A family history of type 2 diabetes has been shown to be associated with an increased risk of type 2 diabetes, as well as an increased risk of CAD, in normal and diabetic adults.[30,31] This relationship is likely the result of inherited insulin resistance.

In the current analyses, the Beck Depression Inventory score and triglycerides were the major predictors of CAD beyond diabetes duration. The Beck Depression Inventory is a quantitative measure of depressive symptomatology.[21] These results are supported by our prospective analyses.[32]

In conclusion, LDL particle size is not strongly associated with CAD in type 1 diabetes. This borderline association can

largely be explained by the triglyceride concentration. In addition, the association between LDL particle size and a family history of type 2 diabetes is noted in women, which may reflect the manifestation of insulin resistance in a subgroup of patients with type 1 diabetes. Such a subgroup may be at increased risk of CAD, and LDL particle size may be a valuable marker. Such subjects enable a fuller evaluation of the predictive and possible pathogenic role of LDL particle size in CAD in type 1 diabetes.

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