Carotid Artery Intima-Media Thickness in Children With Type 1 Diabetes

Mikko J. Järvisalo,[1,2] Anne Putto-Laurila,[3] Laura Jartti,[1] Terho Lehtimäki,[4] Tiina Solakivi,[4] Tapani Rönnemaa,[5] and Olli T. Raitakari[1,6]

Postmortem studies have shown a relationship between diabetic state and atherosclerotic arterial lesions in adolescents. The aim of the present study was to determine the presence of increased subclinical atherosclerosis (measured as carotid intima-media thickness [IMT]) and its risk factors, including lipoprotein oxidation, in children with type 1 diabetes. We measured carotid IMT using high-resolution ultrasound in 85 children (mean age, 11 ± 2 years): 50 with type 1 diabetes (mean duration, 4.4 ± 3.0 years) and 35 healthy control subjects matched for age, sex, and body size. The susceptibility of LDL to oxidation was determined by measuring the formation of conjugated dienes induced by Cu^{2+} in 42 children (21 with diabetes and 21 control subjects). The mean carotid IMT was increased in children with diabetes (0.47 ± 0.04 vs. 0.42 ± 0.04 mm; $P < 0.0001$). Total cholesterol and LDL cholesterol concentrations were similar between the groups, but the children with diabetes had increased LDL diene formation rate (0.49 ± 0.06 vs. 0.45 ± 0.07 μmol/min; $P < 0.05$), suggesting increased in vitro LDL oxidizability. In a multivariate model for all subjects, the independent correlates for IMT were the diabetic state ($P < 0.001$), LDL cholesterol level ($P < 0.001$), and systolic blood pressure ($P < 0.001$). In children with diabetes but not in control subjects, LDL oxidizability correlated significantly with mean IMT ($r = 0.47$, $P < 0.05$), and this relationship remained significant after controlling for LDL cholesterol level. We conclude that type 1 diabetes is an independent risk factor for increased carotid IMT in children. These data also suggest that increased oxidative modification of LDL may be related to early structural atherosclerotic vascular changes in children with diabetes. *Diabetes* 51:493–498, 2002

From the [1]Department of Clinical Physiology, University of Turku, Turku, Finland; the [2]Research Centre of Applied and Preventive Cardiovascular Medicine, University of Turku, Turku, Finland; the [3]Department of Pediatrics, University of Turku, Turku, Finland; the [4]Laboratory of Atherosclerosis Genetics, Department of Clinical Chemistry, Centre for Laboratory Medicine, Tampere University Hospital and University of Tampere, Medical School, Tampere, Finland; the [5]Department of Medicine, University of Turku, Turku, Finland; and the [6]Turku PET-Centre, University of Turku, Turku, Finland.

Address correspondence and reprint requests to Olli T. Raitakari, MD, Turku PET Centre, Kiinamyllynkatu 4-8, FIN-20500, Turku, Finland. E-mail: olli.raitakari@utu.fi.

Received for publication 23 August 2000 and accepted in revised form 23 October 2001.

IMT, intima-media thickness.

Although the clinical complications of atherosclerosis, such as coronary artery disease and stroke, usually occur in middle and late age, autopsy studies have shown that the atherosclerotic process in the vascular wall begins in childhood and is accelerated in the presence of risk factors (1,2). A noninvasive ultrasound measure of carotid wall intima-media thickness (IMT) is a marker of generalized atherosclerosis that in adults correlates with the extent of coronary artery disease (3–6) and predicts future cardiovascular events (7–9). Previous observations suggest that thickening of arterial IMT occurs in children with familial hypercholesterolemia (10–12). Therefore, carotid IMT may provide an index of atherosclerotic vascular process that can be used to study subclinical atherosclerosis in vivo in children. Type 1 diabetes has been shown to be associated with increased carotid IMT in adults (13–17), although the results are partly controversial (14,15,18). The role of diabetes in the development of atherosclerosis in childhood, however, has received less attention. Study of diabetic children may provide unique data on early vascular changes that are relatively unobscured by other diseases or lifestyle habits.

Individuals with type 1 diabetes have a two- to fourfold increased risk of developing atherosclerotic diseases, which is inadequately explained by differences in the levels of traditional vascular risk factors, such as dyslipidemia, hypertension, or smoking (19). Therefore, other risk factors may be operational in diabetes. Diabetic state is characterized by alterations in serum lipoproteins that may enhance their susceptibility to oxidation (20–22), e.g., changes in the fatty acid composition (20,23,24), lipoprotein density (25), and increased glycosylation (20–22). It has been suggested that the increased risk of atherosclerotic disease in individuals with diabetes may be due to enhanced foam cell formation following greater susceptibility of LDL to oxidation (20). It is not known, however, whether LDL oxidation has a primary role in the pathogenesis of atherosclerosis in diabetes or is merely an indicator of oxidative stress associated with end-stage tissue damage.

The present study was undertaken to evaluate the presence of early atherosclerosis and its determinants in diabetes. We measured arterial wall IMT in the common carotid arteries in young children with type 1 diabetes and in healthy control subjects matched for age, sex, and body size and assessed the effects of vascular risk factors, including the susceptibility of LDL to oxidation, on arterial IMT.

TABLE 1
Characteristics of the study groups

	Children with diabetes	Control children	*P*
Number of subjects (boys)	50 (34)	35 (22)	—
Age (years)	11 ± 2	11 ± 1	0.38
Duration of diabetes (years)	4.4 ± 3.0	—	—
BMI (kg/m^2)	19.1 ± 2.4	19.4 ± 4.1	0.65
Ponderal index (kg/m^3)	12.6 ± 1.2	13.1 ± 2.4	0.35
Systolic blood pressure (mmHg)	110 ± 9	113 ± 8	0.19
Diastolic blood pressure (mmHg)	65 ± 7	67 ± 7	0.24
Total cholesterol (mmol/l)	4.3 ± 0.8	4.3 ± 1.0	0.95
LDL cholesterol (mmol/l)	2.3 ± 0.7	2.5 ± 0.9	0.42
HDL cholesterol (mmol/l)	1.63 ± 0.36	1.42 ± 0.40	0.02
Triglycerides (mmol/l)	0.70 ± 0.50	0.85 ± 0.43	0.04
HbA_{1c} (%)	8.9 ± 1.4	5.3 ± 0.2	0.001
Carotid artery diameter (mm)	4.91 ± 0.47	5.12 ± 0.55	0.20

Data are means ± SD.

RESEARCH DESIGN AND METHODS

Children. We studied 50 children with type 1 diabetes (aged 11 ± 2 years) and 35 healthy control subjects. The groups were matched for age, sex, and body size. None of the children had hypercholesterolemia, as judged by the reference values for Finnish children, i.e., all children had total cholesterol and LDL cholesterol values less than the age- and sex-specific 90th percentile (26). The clinical characteristics of the study groups are shown in Table 1.

The patients with diabetes were recruited from the outpatient clinic of the Department of Pediatrics, Turku University Central Hospital. The inclusion criteria were age 7 to 14 years, diabetes duration >6 months, normotensive, nonsmoker, and no chronic diseases other than type 1 diabetes. We studied 50 (41%) consecutively seen consenting outpatient children of the total 123 eligible children with diabetes (meeting the inclusion criteria) who were treated at our clinic. The study subjects were representative of the total eligible children with diabetes, as age (11 ± 2 vs. 11 ± 2 years; $P = 0.27$), duration of diabetes (4.4 ± 3.0 vs. 5.2 ± 2.3 years), HbA_{1c} (8.9 ± 1.4 vs. 9.1 ± 1.5%), LDL cholesterol (2.3 ± 0.7 vs. 2.4 ± 0.5 mmol/l), and triglycerides (0.70 ± 0.50 vs. 0.76 ± 0.33 mmol/l) were similar in those who did not participate (all comparisons $P > 0.10$). The mean duration of diabetes was 4.4 ± 3.0 years (range, 6 months to 12 years). None of the children were taking regular medications other than daily insulin. The daily insulin dose was 0.95 ± 0.24 IU/kg (range, 0.62–1.53 IU/kg). None of the patients with diabetes had evidence of microvascular complications, such as diabetic retinopathy, neuropathy, or microalbuminuria. In the diabetic group, the mean HbA_{1c} level was 8.9 ± 1.4% (range, 6.2–12.7%; reference range, 4.2–6.0%). The healthy control children included in the study were friends and relatives of the children with diabetes studied or children of the staff members of the Turku University or Turku University Central Hospital. None of the control children had chronic diseases or were taking regular medications, and all were nonsmokers. Tanner staging was not systematically performed in all children. In unclear cases (not clearly prepubertal), puberty stage was evaluated by a pediatrician. Two girls and two boys, both in the diabetic group and in the control group, turned out to be pubertal (defined as menarche in girls and/or advanced pubarche and breaking of voice and/or advanced pubarche in boys). Written informed consent was acquired from the legal guardians of the children, and they were also encouraged to get approval from the child. The study was conducted according to the declaration of Helsinki, and the study protocol had been approved by the Joint Commission on Ethics of the Turku University and the Turku University Central Hospital.

Ultrasound studies. All studies were performed using an Acuson Sequoia 512 mainframe (Acuson, Mountain View, CA) and a 13.0-MHz linear array transducer. All ultrasound scans were performed by an experienced vascular operator who was unaware of children's clinical details. The studies were performed in the morning between 7:30 and 9:30 A.M. after the children had fasted overnight. Blood pressure was measured from the brachial artery three times during the ultrasound study using a standard mercury sphygmomanometer.

Carotid artery studies. All studies were done following a predetermined, standardized scanning protocol for the right and left carotid arteries, using images of the far wall of the distal common carotid arteries, as described previously (27). The place of measurement was anatomically standardized in every study by identifying the proximal part of the carotid bulb and then scanning the common carotid artery (28). The bulb region was first scanned carefully in many interrogation angles to identify the beginning of the bulb. The scan was focused on the posterior (far) wall, and resolution box function was used to magnify the arterial far wall. Several images of common carotid far wall segment from 10 to 20 mm proximal to the carotid bulb (a far wall segment of 10 mm in width) were acquired. Images of the common carotid segment were acquired by using two interrogation angles in each case; anterior oblique (30° from midline) and lateral (100° from midline). To justify the use of these two interrogation angles, we performed IMT measurements in 10 children (5 control subjects and 5 diabetic children) using 15 different interrogation angles covering ~120° of the carotid far wall circumference. The measurement of common carotid IMT, using either a mean of the two interrogation angles (anterior oblique and lateral) or a mean of 15 interrogation angles, yielded similar results (intraclass correlation coefficient, $r = 0.98$, coefficient of variation = 1.1%; mean difference, 0.006 ± 0.004 mm).

All scans were digitally stored on the ultrasound system internal hard disk for subsequent off-line analysis. Two end-diastolic frames were selected and analyzed for mean IMT and maximum IMT, and the average reading from these two frames was calculated for both right and left carotid arteries. Several measurements of IMT were taken covering the entire width of the common carotid segment in every subject by two independent and experienced readers who were blinded to the children's clinical details. Average values of the two readers were used in the analyses.

The far wall of the common carotid artery and the carotid bulb region were also scanned for the presence of atherosclerotic plaques, defined as a distinct area of the vessel protruding >50% of the adjacent parts of the intima-media layer (10). For all measurements, the between-observer intraclass correlation coefficient for mean IMT was $r = 0.86$, with a mean between observer error of 0.018 ± 0.017 mm (range, 0–0.09 mm) and a coefficient of variation of 3%. The within-subject repeatability of mean carotid IMT measurements was studied in 22 children who were studied twice 5–8 days apart. The within-subject intraclass correlation coefficient was $r = 0.94$, with a mean absolute within-subject error of 0.041 ± 0.025 mm (range, 0.0–0.09 mm) and a coefficient of variation of 4%.

Serum lipoproteins, LDL oxidizability, and HbA_{1c}. Venous blood samples were taken in the morning, after an overnight fast (10–12 h). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured using standard enzymatic methods with the use of Boehringer Mannheim reagents, with a fully automated analyzer (Hitachi 917; Hitachi, Tokyo, Japan). LDL cholesterol concentration was calculated using Friedewald's equation (29). HbA_{1c} was measured with high-performance liquid chromatography (Variant Analyzer, Bio-Rad, CA).

The susceptibility of LDL to oxidation was determined in a subgroup of 42 children (21 children with diabetes and 21 matched control subjects) by monitoring the formation of conjugated dienes induced by copper. This subgroup was representative of all study children, as age, sex distribution, body size, LDL cholesterol (2.4 ± 0.8 vs. 2.2 ± 0.6 mol/l; $P = 0.42$), and the duration of diabetes (4.6 ± 2.9 vs. 4.2 ± 3.2 years; $P = 0.60$) and glycemic control (8.8 ± 1.5 vs. 8.9 ± 1.4%; $P = 0.90$) in children with diabetes were comparable to the entire study group.

The copper-induced LDL oxidation method used in this study has been described previously (30). In brief, LDL was isolated by single-step ultracentrifugation for 30 min at 338,000*g* (Beckman TL-100) with a TLV-100 rotor. A 1-ml sample of desalted and in-gel–filtered LDL solution was standardized to 0.05 mg protein/ml with PBS. The sample was oxidized at 37°C as described previously (31). The final concentration of $CuSO_4$ in the mixture was 1.67 μmol/l. Ultraviolet absorbance at 234 nm was monitored every minute for 300

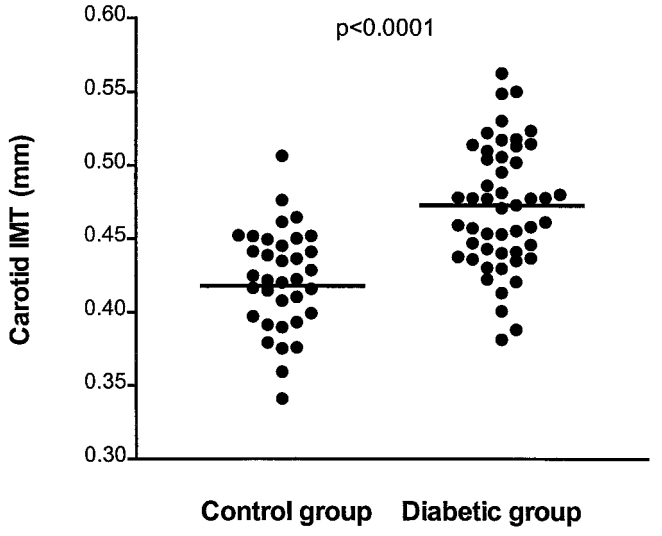

FIG. 1. Carotid IMT in control children and children with diabetes. The figure shows the actual values and the mean values.

min with the use of a Perkin Elmer Lambda Bio 10 spectrometer. Lag time to the start of the propagation phase of diene formation was defined as the intersection of the tangents of the initial phase (first 5 min) and maximal propagation.

Statistical methods. Results are expressed as means ± SD. Data on serum triglycerides were skewed toward high values and were included as their logarithms in the analyses. Comparisons between the groups were conducted by Student's *t* test. Univariate associations between the study variables were analyzed by calculating the Pearson's correlation coefficients. Multivariate analyses were done using linear regression technique. All statistical analyses were performed using SAS (SAS Institute, Cary, NC).

RESULTS

The characteristics of the children are shown in Table 1. The groups were matched for age, sex, and BMI. The diabetic group had lower serum triglycerides and higher HDL cholesterol concentration compared with control subjects. There were no differences in serum total cholesterol, LDL cholesterol, blood pressure, or carotid artery baseline diameter between the study groups (Table 1).

The rate of diene formation was greater in the children with diabetes (0.49 ± 0.06 vs. 0.45 ± 0.07 μmol/min; $P = 0.05$). Also, the total amount of conjugated dienes produced tended to be greater in the diabetic group (521 ± 50 vs. 499 ± 45 μmol; $P = 0.14$). The lag time did not differ between the groups (74.1 ± 6.6 vs. 73.1 ± 5.5 min; $P = 0.58$).

The mean carotid IMT was significantly increased in children with diabetes compared with healthy control subjects (0.47 ± 0.04 vs. 0.42 ± 0.04 mm; $P < 0.001$) (Fig. 1). The maximal IMT was also significantly higher in children with diabetes (0.53 ± 0.05 vs. 0.48 ± 0.04 mm; $P < 0.001$), but no plaque formations (10) were observed in any of the children studied. Mean IMT was 0.47 ± 0.05 vs. 0.42 ± 0.04 mm ($P = 0.02$) in girls with diabetes and control girls, respectively, and 0.47 ± 0.04 vs. 0.42 ± 0.03 mm ($P < 0.001$) in boys with diabetes and control boys, respectively. Thus, the difference in IMT between children with diabetes and control subjects was similarly seen in boys and in girls. The difference in IMT remained highly significant when children who were considered to be pubertal (4 + 4) were excluded from the analysis (0.47 ± 0.04 vs. 0.42 ± 0.04 mm; $P < 0.0001$).

TABLE 2
Correlation coefficients between carotid IMT risk factors

	Diabetic group	Control group
Age		
Mean IMT	0.10	−0.12
Max IMT	0.09	−0.08
BMI		
Mean IMT	0.28*	0.30
Max IMT	0.18	0.27
Ponderal index		
Mean IMT	0.21	0.29
Max IMT	0.07	0.27
Duration of diabetes		
Mean IMT	0.32*	—
Max IMT	0.28*	—
HbA_{1c}		
Mean IMT	−0.04	−0.01
Max IMT	−0.09	−0.03
Total cholesterol		
Mean IMT	0.32*	0.37*
Max IMT	0.26	0.36*
LDL cholesterol		
Mean IMT	0.40†	0.36*
Max IMT	0.33*	0.40*
HDL cholesterol		
Mean IMT	−0.19	−0.26
Max IMT	−0.17	−0.34*
HDL/total cholesterol		
Mean IMT	−0.21	−0.26
Max IMT	−0.17	−0.34*
Triglycerides		
Mean IMT	−0.15	0.22
Max IMT	−0.21	0.19
Systolic blood pressure		
Mean IMT	0.30*	0.18
Max IMT	0.39†	0.30‡
Diastolic blood pressure		
Mean IMT	0.28*	0.19
Max IMT	0.31*	0.31†

*$P \leq 0.05$; †$P \leq 0.01$; ‡$P = 0.07$. Max, maximum.

The correlations between risk factors and carotid IMT are shown in Table 2, separately for children with diabetes and control subjects. In children with diabetes, carotid IMT correlated significantly with BMI, diabetes duration, total cholesterol, LDL cholesterol, and blood pressure. In control subjects, mean carotid IMT correlated with total cholesterol and LDL cholesterol, and maximum IMT also correlated with systolic and diastolic blood pressure and HDL cholesterol/total cholesterol ratio (Table 2). The scatter plots between mean carotid IMT and LDL cholesterol are shown in Fig. 2, separately for children with diabetes and control subjects. Susceptibility of LDL to oxidation, measured as the rate of diene formation, was significantly correlated with mean carotid IMT in children with diabetes ($r = 0.47$, $P = 0.04$) (Fig. 3) but not in control children ($r = -0.29$, NS).

In a multivariate regression model for all study subjects, including age, sex, BMI, study group, HbA_{1c}, LDL cholesterol, log-transformed triglycerides, and systolic blood pressure as independent variables, the significant correlates for mean carotid IMT were the study group ($\beta = 0.054$, $P < 0.001$), LDL cholesterol ($\beta = 0.018$, $P < 0.001$), and systolic blood pressure ($\beta = 0.0010$, $P < 0.01$), the total variance explained being 47%.

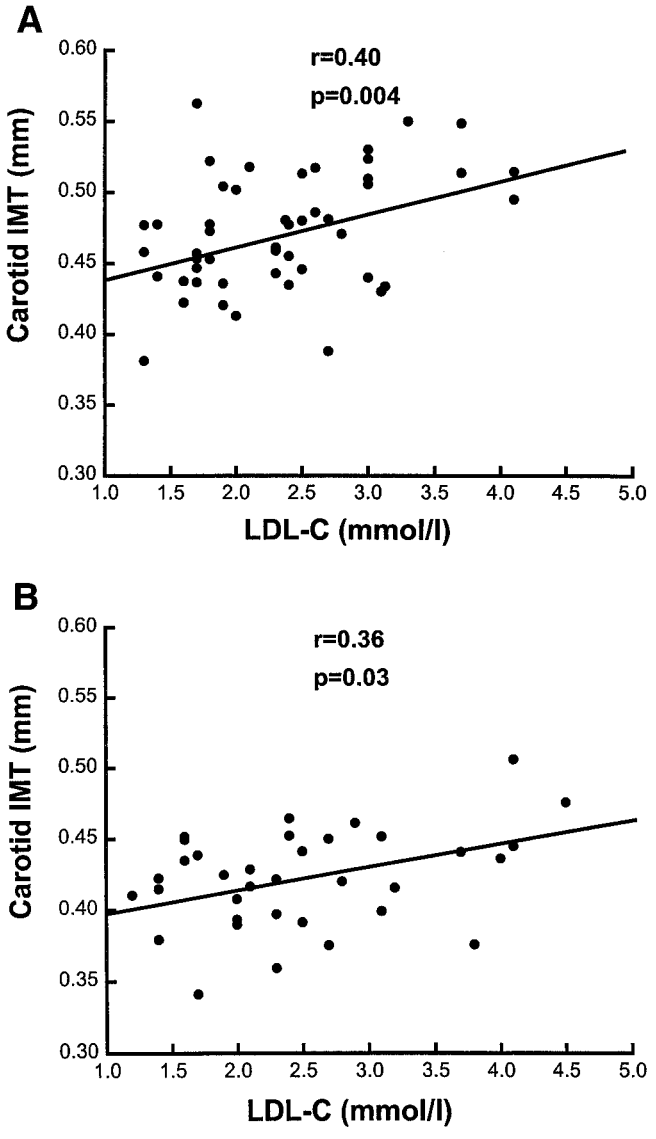

FIG. 2. Relationship between mean carotid IMT and LDL cholesterol. *A*: Children with diabetes. *B*: Control children.

In a multivariate regression model restricted to those children with data on LDL oxidation ($n = 42$), including study group, LDL cholesterol, the rate of diene formation, and systolic blood pressure as independent variables, the significant correlates for mean carotid IMT were the study group ($\beta = 0.060$, $P < 0.001$) and LDL cholesterol ($\beta = 0.024$, $P < 0.001$), the total variance explained being 56%. To study whether other risk factors could account for the effect of diabetic status, the regression analysis was performed without the group variable in the model. In this model, the correlates of carotid IMT were LDL cholesterol ($P < 0.05$) and the rate of diene formation ($P = 0.25$), with the total variance explained being only 17%, indicating that the effect of diabetic status was not fully explained by other risk factors.

To examine whether the correlation between diene formation rate and carotid IMT in children with diabetes remains significant after controlling for the effect of LDL cholesterol, we included diene formation rate and LDL cholesterol as independent variables in the model for carotid IMT. Both of these variables emerged as independent correlates for mean IMT in children with diabetes: LDL cholesterol ($\beta = 0.033$, $P < 0.01$) and the rate of diene formation ($\beta = 0.28$, $P < 0.05$), the total variance explained being 48%.

DISCUSSION

The present study shows that young children with type 1 diabetes have significantly increased carotid artery IMT compared with healthy control children. These findings extend to observations of postmortem studies that have indicated a relation between early atherosclerotic vascular lesions and diabetic state (32), by demonstrating in vivo that diabetes predisposes to increased subclinical atherosclerosis at a very early age. Several previous studies demonstrated that carotid IMT is increased in adults with type 1 diabetes (13–17). The results of the recent Epidemiology of Diabetes Interventions and Complications study (18), however, were somewhat contradictory, showing increased IMT only in male subjects in the internal carotid arteries but not in the common carotid. Differences in methodology and study population may offer an explanation for the discrepancy. In our experience, the image quality of carotid scans in children is superior to the scans of adult subjects. Furthermore, in the present study, we used the latest digital ultrasound technology and 13-MHz scanning frequency, which yielded very high-resolution images.

The earliest histologic atherosclerotic vascular changes, i.e., fatty streaks, are commonly found in the arteries of adolescents, whereas the development of raised lesions mainly occurs after the age of 20 years (33). According to these postmortem findings and consistent correlations between lipid risk factors and IMT seen in the present study, it may be suggested that the diffusely increased carotid artery wall thickness in the children with diabetes reflects intimal changes related to early atherogenesis. Consistent with previous studies, blood pressure was an independent predictor of IMT in the present study. The relationship between increased IMT and blood pressure suggests that smooth muscle proliferation also plays a role in the early diffuse thickening of the arterial wall. It is not clear, however, whether increased carotid IMT without focal plaques reflects subclinical atherosclerosis or merely represents a preatherosclerotic change that predisposes to atherosclerosis. To address this issue, the relationship between the histologic prevalence of carotid atherosclerosis and ultrasonographically measured wall thickness would need to be studied in pediatric subjects.

Increased carotid IMT has previously been demonstrated in children with familial hypercholesterolemia (10–12). These studies, however, have not been able to show a significant relationship between carotid IMT and serum LDL cholesterol concentration within the normocholesterolemic range. In the present study, all children had normal LDL cholesterol levels (26). Despite this, LDL cholesterol concentration was significantly related to increased IMT, both in children with diabetes and in control subjects. Moreover, in multivariate analysis including all children, LDL cholesterol emerged as an independent correlate for IMT, together with the diabetic state and systolic blood pressure. Our observations thus suggest that serum LDL cholesterol concentration, even within a

normal range, is an important determinant of structural arterial changes already in childhood. In line with this, autopsy studies in children have also shown a significant relationship between serum cholesterol concentration and early atherosclerotic lesions (1,2).

Increased IMT in children with diabetes was not explained by the levels of conventional risk factors, as these were similar between the study groups. Instead, the children with diabetes showed an increased rate of LDL diene formation in response to copper-induced oxidation, indicating increased in vitro susceptibility of LDL to oxidation. Postsecretory modifications of LDL, such as oxidation, have been suggested to increase its atherogenicity. LDL particles with increased susceptibility to oxidation may become more easily oxidatively modified within the arterial wall and then recognized by the scavenger receptor, leading to increased foam cell formation and accelerated atherogenesis (34). Studies in adults have shown that LDL oxidation is increased in diabetes (35–38) and may explain some of the enhanced cardiovascular risk in type 1 diabetes (39). In the present study, differences in LDL oxidizability between the study groups did not fully explain the effect of diabetic state on IMT, as the regression model without the group variable as an independent variable resulted in a low explanatory R^2 value for carotid IMT. This suggests that other unmeasured factors may also account for the higher IMT in individuals with diabetes. In the multivariate analysis restricted to children with diabetes, however, LDL oxidizability remained significantly associated with IMT, independent of LDL cholesterol level. Therefore, these data support the idea that oxidative modification of LDL may have a role in the development of early structural atherosclerotic vascular changes in children with diabetes.

Epidemiologic and clinical evidence has emphasized the role of hyperglycemia in explaining the increased cardiovascular morbidity and mortality in diabetes (40,41). Chronic state of hyperglycemia may induce atherogenesis by increasing oxidative stress (42), leading to increased LDL oxidation (43,44) and decreased nitric oxide bioavailability, including endothelial dysfunction (45,46). In the present study, the HbA_{1c} levels in the children with diabetes were comparable to those reported previously in a population-based sample of children and adolescents (47). We were unable to show a relationship between HbA_{1c} and carotid IMT in children with diabetes. These data suggest that LDL cholesterol concentration and/or postsecretory LDL modification may be more important correlates of carotid IMT in children with diabetes than measures of chronic hyperglycemia. Alternatively, hyperglycemia may exert its deleterious effects by leading to glycosylation of LDL, which may increase its atherogenicity (48,49).

The present study examined the relationships between type 1 diabetes and arterial IMT using a cross-sectional setting. An ideal approach would be a longitudinal study of diabetic subjects to investigate the progression/regression of atherosclerotic vascular changes in response to therapy. The study included a relatively small number of participants, especially regarding subjects with data on LDL oxidizability. This may have increased the risk of selection bias. However, children with diabetes who participated in the present study were representative of the total number of eligible children with diabetes treated in our clinic with regard to age, disease duration, glycemic control, and lipid levels. Moreover, the subgroup of children with LDL oxidation measures were representative of all children studied. LDL oxidizability was assessed by using an assay that is based on measuring the formation of conjugated dienes in LDL lipids in response to copper-induced oxidation in vitro (31). Increased susceptibility of LDL to in vitro oxidation, however, reflects only one aspect of LDL oxidizability and has still-uncertain physiologic significance in vivo.

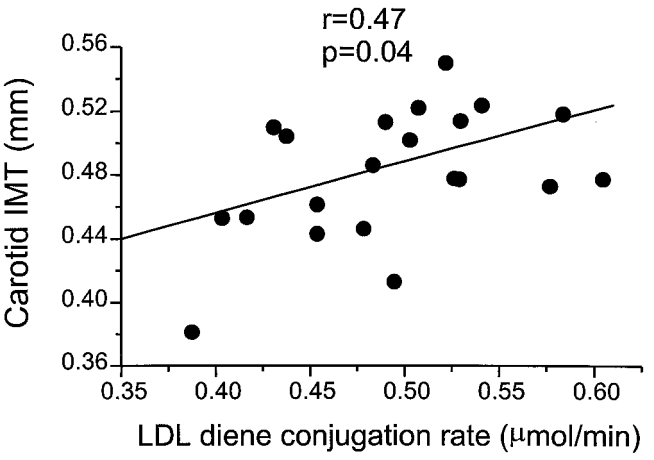

FIG. 3. Relationship between mean carotid IMT and LDL oxidizability (diene formation rate during copper induced oxidation) in children with diabetes.

These data may have implications in the treatment of pediatric patients with diabetes. Our results emphasize the importance of early detection and control of vascular risk factors in young children with diabetes. As diabetes is a chronic disease and cardiovascular morbidity is very high among individuals with diabetes, noninvasive methods for monitoring vascular changes might be useful in clinical practice. Prospective studies are needed to evaluate the usefulness of IMT measurement as an index of atherosclerosis in the treatment of children with diabetes.

ACKNOWLEDGMENTS

This study was financially supported by the Finnish Medical Foundation, the Medical Society Duodecim in Turku, the Research Foundation of Orion Corporation, the Turku University Foundation, the Lydia Maria Julin Foundation, the Wäinö Edward Miettinen Foundation, the Academy of Finland, the Juho Vainio Foundation, the Finnish Foundation of Cardiovascular Research, and the Medical Research Fund of the Tampere University Hospital.

We thank Mia Laakkonen, Tuula Laukkanen, Nina Peltonen, and Marita Koli for skillful technical assistance.

REFERENCES

1. Wissler RW, Strong JP: Risk factors and progression of atherosclerosis in youth: PDAY Research Group: Pathological Determinants of Atherosclerosis in Youth. *Am J Pathol* 153:1023–1033, 1998
2. Malcom GT, Oalmann MC, Strong JP: Risk factors for atherosclerosis in young subjects: the PDAY Study: Pathobiological Determinants of Atherosclerosis in Youth. *Ann N Y Acad Sci* 817:179–188, 1997
3. Craven TE, Ryu JE, Espeland MA, Kahl FR, McKinney WM, Toole JF, McMahan MR, Thompson CJ, Heiss G, Crouse JR III: Evaluation of the associations between carotid artery atherosclerosis and coronary artery stenosis: a case-control study. *Circulation* 82:1230–1242, 1990

4. Wofford JL, Kahl FR, Howard GR, McKinney WM, Toole JF, Crouse JR III: Relation of extent of extracranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of coronary atherosclerosis. *Arterioscler Thromb* 11:1786–1794, 1991
5. Adams MR, Nakagomi A, Keech A, Robinson J, McCredie R, Bailey BP, Freedman SB, Celermajer DS: Carotid intima-media thickness is only weakly correlated with the extent and severity of coronary artery disease. *Circulation* 92:2127–2134, 1995
6. Hodis HN, Mack WJ, LaBree L, Selzer RH, Liu CR, Liu CH, Azen SP: The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med* 128:262–269, 1998
7. Salonen JT, Salonen R: Ultrasound B-mode imaging in observational studies of atherosclerotic progression. *Circulation* 87:II56–II65, 1993
8. Bots ML, Hoes AW, Koudstaal PJ, Hofman A, Grobbee DE: Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 96:1432–1437, 1997
9. O'Leary DH, Polak JF, Kronmal RA, Manolio TA, Burke GL, Wolfson SK Jr: Carotid-artery intima and media thickness as a risk factor for myocardial infarction and stroke in older adults: Cardiovascular Health Study Collaborative Research Group. *N Engl J Med* 340:14–22, 1999
10. Tonstad S, Joakimsen O, Stensland-Bugge E, Leren TP, Ose L, Russell D, Bonaa KH: Risk factors related to carotid intima-media thickness and plaque in children with familial hypercholesterolemia and control subjects. *Arterioscler Thromb Vasc Biol* 16:984–991, 1996
11. Virkola K, Pesonen E, Akerblom HK, Siimes MA: Cholesterol and carotid artery wall in children and adolescents with familial hypercholesterolaemia: a controlled study by ultrasound. *Acta Paediatr* 86:1203–1207, 1997
12. Pauciullo P, Iannuzzi A, Sartorio R, Irace C, Covetti G, Di Costanzo A, Rubba P: Increased intima-media thickness of the common carotid artery in hypercholesterolemic children. *Arterioscler Thromb* 14:1075–1079, 1994
13. Yamasaki Y, Kawamori R, Matsushima H, Nishizawa H, Kodama M, Kajimoto Y, Morishima T, Kamada T: Atherosclerosis in carotid artery of young IDDM patients monitored by ultrasound high-resolution B-mode imaging. *Diabetes* 43:634–639, 1994
14. Peppa-Patrikiou M, Scordili M, Antoniou A, Giannaki M, Dracopoulou M, Dacou-Voutetakis C: Carotid atherosclerosis in adolescents and young adults with IDDM: relation to urinary endothelin, albumin, free cortisol, and other factors. *Diabetes Care* 21:1004–1007, 1998
15. Frost D, Beischer W: Determinants of carotid artery wall thickening in young patients with type 1 diabetes mellitus. *Diabetes Med* 15:851–857, 1998
16. Mohan V, Ravikumar R, Shanthi RS, Deepa R: Intimal medial thickness of the carotid artery in South Indian diabetic and non-diabetic subjects: the Chennai Urban Population Study (CUPS). *Diabetologia* 43:494–499, 2000
17. Yokoyama H, Yoshitake E, Otani T, Uchigata Y, Kawagoe M, Kasahara T, Omori Y: Carotid atherosclerosis in young-aged IDDM associated with diabetic retinopathy and diastolic blood pressure. *Diabetes Res Clin Pract* 21:155–159, 1993
18. Effect of intensive diabetes treatment on carotid artery wall thickness in the epidemiology of diabetes interventions and complications: Epidemiology of Diabetes Interventions and Complications (EDIC) Research Group. *Diabetes* 48:383–390, 1999
19. Pyorala K, Laakso M, Uusitupa M: Diabetes and atherosclerosis: an epidemiologic view. *Diabetes Metab Rev* 3:463–524, 1987
20. Dimitriadis E, Griffin M, Owens D, Johnson A, Collins P, Tomkin GH: Oxidation of low-density lipoprotein in NIDDM: its relationship to fatty acid composition. *Diabetologia* 38:1300–1306, 1995
21. Chait A, Brazg RL, Tribble DL, Krauss RM: Susceptibility of small, dense, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. *Am J Med* 94:350–356, 1993
22. Kreisberg RA: Diabetic dyslipidemia. *Am J Cardiol* 82:67U–73U, 1998
23. Pelikanova T, Kohout M, Valek J, Base J, Stefka Z: Fatty acid composition of serum lipids and erythrocyte membranes in type 2 (non-insulin-dependent) diabetic men. *Metabolism* 40:175–180, 1991
24. Salomaa V, Ahola I, Tuomilehto J, Aro A, Pietinen P, Korhonen HJ, Penttila I: Fatty acid composition of serum cholesterol esters in different degrees of glucose intolerance: a population-based study. *Metabolism* 39:1285–1291, 1990
25. Makimattila S, Liu ML, Vakkilainen J, Schlenzka A, Lahdenpera S, Syvanne M, Mantysaari M, Summanen P, Bergholm R, Taskinen MR, Yki-Jarvinen H: Impaired endothelium-dependent vasodilation in type 2 diabetes: relation to LDL size, oxidized LDL, and antioxidants. *Diabetes Care* 22:973–981, 1999
26. Porkka KV, Viikari JS, Ronnemaa T, Marniemi J, Akerblom HK: Age and gender specific serum lipid and apolipoprotein fractiles of Finnish children and young adults: the Cardiovascular Risk in Young Finns Study. *Acta Paediatr* 83:838–848, 1994
27. Toikka JO, Laine H, Ahotupa M, Haapanen A, Viikari JS, Hartiala JJ, Raitakari OT: Increased arterial intima-media thickness and in vivo LDL oxidation in young men with borderline hypertension. *Hypertension* 36:929–933, 2000
28. Wikstrand J, Wendelhag I: Methodological considerations of ultrasound investigation of intima-media thickness and lumen diameter. *J Intern Med* 236:555–559, 1994
29. Friedewald WT, Levy RI, Fredrickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 18:499–502, 1972
30. Palomaki A, Malminiemi K, Solakivi T, Malminiemi O: Ubiquinone supplementation during lovastatin treatment: effect on LDL oxidation ex vivo. *J Lipid Res* 39:1430–1437, 1998
31. Esterbauer H, Striegl G, Puhl H, Rotheneder M: Continuous monitoring of in vitro oxidation of human low density lipoprotein. *Free Radic Res Commun* 6:67–75, 1989
32. McGill HC Jr, McMahan CA, Malcom GT, Oalmann MC, Strong JP: Relation of glycohemoglobin and adiposity to atherosclerosis in youth: Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Research Group. *Arterioscler Thromb Vasc Biol* 15:431–440, 1995
33. McGill HC, McMahan CA, Herderick EE, Tracy RE, Malcom GT, Zieske AW, Strong JP: Effects of coronary heart disease risk factors on atherosclerosis of selected regions of the aorta and right coronary artery: PDAY Research Group: Pathobiological Determinants of Atherosclerosis in Youth. *Arterioscler Thromb Vasc Biol* 20:836–845, 2000
34. Ross R: The pathogenesis of atherosclerosis—an update. *N Engl J Med* 314:488–500, 1986
35. Bowie A, Owens D, Collins P, Johnson A, Tomkin GH: Glycosylated low density lipoprotein is more sensitive to oxidation: implications for the diabetic patient? *Atherosclerosis* 102:63–67, 1993
36. Beaudeux JL, Guillausseau PJ, Peynet J, Flourie F, Assayag M, Tielmans D, Warnet A, Rousselet F: Enhanced susceptibility of low-density lipoprotein to in vitro oxidation in type 1 and type 2 diabetic patients. *Clin Chim Acta* 239:131–141, 1995
37. Babiy AV, Gebicki JM, Sullivan DR, Willey K: Increased oxidizability of plasma lipoproteins in diabetic patients can be decreased by probucol therapy and is not due to glycation. *Biochem Pharmacol* 43:995–1000, 1992
38. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, Manto A, Ghirlanda G: Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. *Diabetes* 46:1853–1858, 1997
39. Orchard TJ, Virella G, Forrest KY, Evans RW, Becker DJ, Lopes-Virella MF: Antibodies to oxidized LDL predict coronary artery disease in type 1 diabetes: a nested case-control study from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes* 48:1454–1458, 1999
40. Singer DE, Nathan DM, Anderson KM, Wilson PW, Evans JC: Association of HbA_{1c} with prevalent cardiovascular disease in the original cohort of the Framingham Heart Study. *Diabetes* 41:202–208, 1992
41. Quatraro A, Giugliano D, De Rosa N, Minei A, Ettorre M, Donzella C, Saccomanno F, Ceriello A: Is a family history of diabetes associated with an increased level of cardiovascular risk factors? Studies in healthy people and in subjects with different degree of glucose intolerance. *Diabetes Metab* 19:230–238, 1993
42. Hunt JV, Dean RT, Wolff SP: Hydroxyl radical production and autoxidative glycosylation. Glucose autoxidation as the cause of protein damage in the experimental glycation model of diabetes mellitus and ageing. *Biochem J* 256:205–212, 1988
43. Hiramatsu K, Rosen H, Heinecke JW, Wolfbauer G, Chait A: Superoxide initiates oxidation of low density lipoprotein by human monocytes. *Arteriosclerosis* 7:55–60, 1987
44. Heinecke JW, Baker L, Rosen H, Chait A: Superoxide-mediated modification of low density lipoprotein by arterial smooth muscle cells. *J Clin Invest* 77:757–761, 1986
45. Gryglewski RJ, Palmer RM, Moncada S: Superoxide anion is involved in the breakdown of endothelium-derived vascular relaxing factor. *Nature* 320:454–456, 1986
46. Tesfamariam B, Cohen RA: Free radicals mediate endothelial cell dysfunction caused by elevated glucose. *Am J Physiol* 263:H321–H326, 1992
47. Donaghue KC, Fairchild JM, Chan A, Hing SJ, Howard NJ, Silink M: Diabetes complication screening in 937 children and adolescents. *J Pediatr Endocrinol Metab* 12:185–192, 1999
48. Bucala R, Makita Z, Koschinsky T, Cerami A, Vlassara H: Lipid advanced glycosylation: pathway for lipid oxidation in vivo. *Proc Natl Acad Sci U S A* 90:6434–6438, 1993
49. Ravandi A, Kuksis A, Shaikh NA: Glycosylated glycerophosphoethanolamines are the major LDL glycation products and increase LDL susceptibility to oxidation: evidence of their presence in atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 20:467–477, 2000