Evidence for Association Between Insulin Resistance and Premature Carotid Atherosclerosis in Childhood Obesity

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ABSTRACT: The present study was undertaken to determine the presence and predictors of the subclinical atherosclerosis in obese children. Fifty obese children [mean age: 11.7 ± 2.5 y, mean body mass index (BMI): 28.2 ± 4.0 kg/m^2] and 50 age- and sex-matched healthy nonobese controls (mean age: 11.4 ± 3.73 y, mean BMI: 17.6 ± 3.0 kg/m^2) were enrolled in the present study. Oral glucose tolerance test was performed to all obese subjects. Common carotid artery intima-media thickness (IMT) was measured by high-resolution B-mode ultrasonography. Carotid artery IMT was significantly increased (0.0476 ± 0.007 *versus* 0.033 ± 0.011 cm; $p < 0.001$) in the obese group. There were significant relations between carotid artery IMT and insulin sensitivity indexes derived from fasting samples (fasting glucose to insulin ratio (FGIR; $p = 0.004$, $r = -0.404$), quantitative insulin-sensitivity check index (QUICK-I; $p = 0.002$, $r = -0.401$) and homeostasis model assessment of insulin resistance (HOMA-IR; $p = 0.034$, $r = 0.300$) in the obese group. In a multivariate regression model, QUICK-I emerged as independent correlates for mean IMT in obese children with the total variance explained being 20.7% ($\beta = -0.58$, $p < 0.001$). We concluded that insulin resistance is an independent risk factor for increased carotid artery IMT in obese children. (*Pediatr Res* **61:** 345–349, 2007)

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). Clustering of cardiovascular risk factors is seen in children and adolescents with the highest degree of insulin resistance suggesting that adult cardiovascular disease is more likely to develop in these young people (3,4). Type 2 diabetes mellitus and metabolic syndrome prevalences among obese adolescents are quite high in the urban area of Konya, central Anatolia. In our previous study, we found that the prevalence of metabolic syndrome was 27.2% with a significantly higher rate among obese adolescents aged 12–18 y (37.6%) than among obese children aged 7–11 y (20%) (5).

Increased common carotid artery IMT is significantly related to known cardiovascular risk factors and to carotid plaque, a more advanced atherosclerotic lesion (6). Measuring carotid artery IMT with ultrasonography correlates well with pathologic measurements and is reproducible. Increased carotid artery IMT is correlated with cardiovascular risk factors

and the severity of coronary atherosclerosis and predicts cardiovascular events in population groups (7,8). Carotid artery IMT as a marker of early atherosclerosis has been studied using vascular ultrasonography in children with familial hypercholesterolemia (9), diabetes (10,11), hypertension (12), and childhood obesity (13,14). In a recent study, Zhu *et al.* found a significant thickening of the intima-media layer in the carotid artery of obese Chinese school children, compared with nonobese controls (15). However, they have not extensively investigated the predictors of increased carotid artery IMT.

Although insulin resistance is a major risk factor for cardiovascular events, only one study has been conducted for a relation between insulin sensitivity indexes and carotid artery IMT in obese children (16). In the present study, we investigated the relationships between carotid artery IMT and insulin sensitivity indices based on fasting samples and OGTT in children with obesity.

METHODS

Subjects. Fifty children (25 girls and 25 boys, mean age: 11.7 ± 2.5 y, mean body mass index (BMI): 28.2 ± 4.0) were recruited from obese children who attended the outpatient clinic of Pediatric Endocrinology Unit of Selcuk University Hospital in Konya, Turkey. Control subjects (25 girls and 25 boys, mean age: 11.4 ± 3.73, mean BMI: 17.6 ± 3.0) were enrolled the study through nonobese healthy children who attended the hospital for minor illness such as common cold, conjunctivitis, short stature, and constipation. After managed in the primary care setting, we collected the blood samples of the control group at the time of the control examination. Obese children were included if they were 8–18 y age with BMI $\geq$95th percentile for age and gender based on the standards of the Centers for Disease Control and Prevention. Children were excluded if they had prior major illness including type 1 or type 2 diabetes, took medications, or had a condition known to influence body composition, insulin action, or insulin secretion (*e.g.* glucocorticoid therapy, hypothyroidism, Cushing's disease). All subjects were in good health and had normal thyroid function. The study was approved by the local ethics committee of the University of Selcuk. Signed informed consent was obtained for each subject over 12 y of age, and informed parental consent was also obtained for all children regardless of age (Fig. 1).

Each child underwent a complete physical examination, including anthropometric measures. Height and weight were measured in postabsorptive conditions and with an empty bladder. Height was measured to the nearest 0.5 cm on a standard height board, and weight was determined to the nearest 0.1 kg on a standard physician's beam scale with the subject dressed only in light underwear and no shoes. The BMI was calculated as weight (in kilograms) divided by height (in meters) squared. The degree of obesity was quantified

Abbreviations: BMI-SDS, body mass index–standard deviation score; **FGIR,** fasting glucose-to-insulin ratio; **HOMA-IR,** homeostasis model assessment of insulin resistance; **IMT,** intima-media thickness; **ISI,** insulin sensitivity index; **OGTT,** Oral glucose tolerance test; **QUICK-I,** quantitative insulin-sensitivity check index
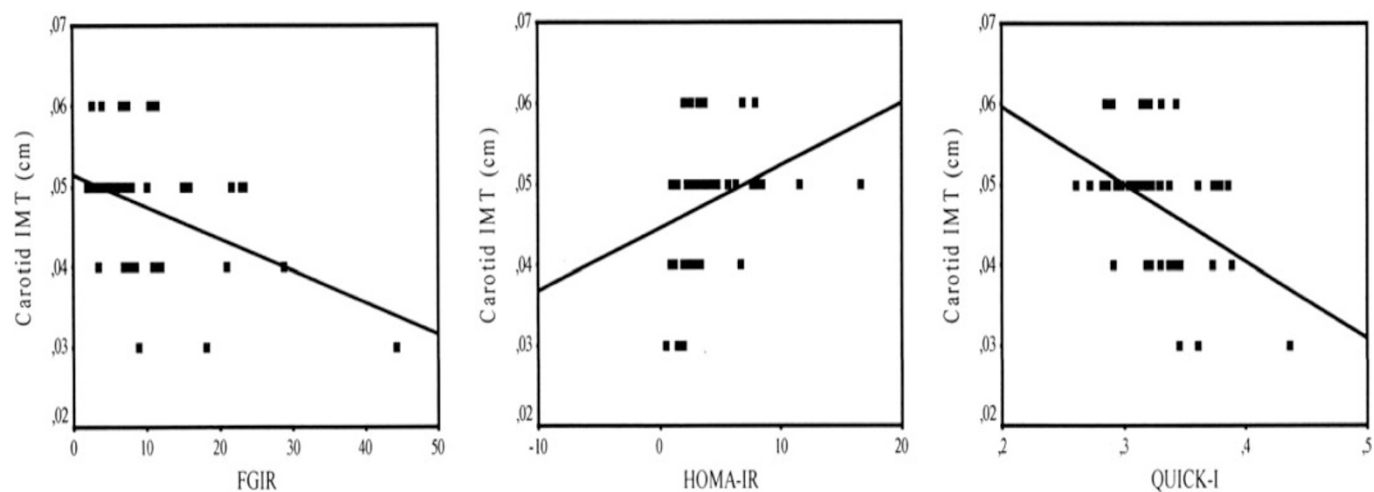
Figure 1. Relationship between carotid artery IMT and insulin sensitivity indexes. FGIR: $r = -0.404$, $p = 0.004$; HOMA-IR: $r = 0.3$, $p = 0.034$; QUICK-I: $r = -0.421$, $p = 0.002$).

using Cole's least mean-square method, which normalizes BMI-skewed distribution and expresses BMI as an SD score (BMI-SDS). This measure gives age- and sex-specific estimates of the distribution median, the coefficient of variation and the degree of skewness by a maximum-likelihood fitting technique (17). Blood pressure was measured with a standard mercury sphygmomanometer after the subjects had rested at least 10 min. Systolic blood pressure (systolic BP) was recorded at the appearance of sounds, and the diastolic blood pressure (diastolic BP) was recorded at the disappearance of sounds.

Blood samples and OGTT. The studies were performed in the morning between 0730 and 0930 h after the children had fasted overnight. After a 3-d, high-carbohydrate diet (300 g/d) and an overnight fast, a standard OGTT (1.75 g/kg or a maximum of 75 g of glucose) was performed for all subjects except for control group because of the ethical reasons. Venous blood samples were obtained at 0, 30, 60, 90, and 120 min to measure plasma glucose and insulin levels in the morning by venipuncture after an overnight fasting. After clotting, the serum was separated and immediately explored for analyses. Glucose was determined by the glucose oxidase method. Plasma concentrations of total cholesterol, HDL cholesterol, LDL cholesterol, and triglycerides were measured using a routine enzymatic methods with Olympus 2700 Analyzer (Olympus Diagnostica GmbH, Hamburg, Germany). Insulin levels were measured by an Immulite immunoassay (Diagnostic Products, Los Angeles, CA). Fasting total homocysteine concentrations were determined in EDTA plasma with competitive immunoassay. Samples were separated from the cells, and matched samples were spiked with total homocysteine and then assayed by Immulite 2000 Analyzer (Diagnostic Products). Results were expressed as micromoles per liter. Normal concentrations were 5 and 15 μM (18).

Insulin indexes derived from fasting blood samples and OGTT. The HOMA-IR, QUICK-I, and FGIR were derived as estimates of insulin resistance. FGIR was calculated as fasting insulin concentration (μU/mL)/fasting glucose concentration (mg/dL). HOMA-IR was calculated as fasting insulin concentration (μU/mL) × fasting glucose concentration (mmol/L)/22.5 (19). QUICK-I was calculated as 1/[(log fasting insulin concentration (μU/mL) + log fasting glucose concentration (mg/dL)] (20).

The total plasma glucose response and insulin secretion were evaluated from the area under the response curve (AUC) estimated by the trapezoid rule. The insulin sensitivity index (ISI) proposed by Matsuda and De-Fronzowas calculated as follows: ISI-composite = 10.000/square root of [(mean plasma insulin × mean plasma glucose during OGTT) × (fasting plasma glucose × fasting plasma insulin)] (21).

Ultrasound imaging. The same sonographer, who was blinded to the participant's laboratory values and risk factor levels, did all examinations. Scans were obtained at rest; the subjects were laid quietly for 10 min before the first scan. High-resolution B-mode ultrasonography of the right common carotid artery was performed with a LOGIQ 9 (GE Healthcare, Milwaukee, WI) with a linear 10-MHz linear transducer. The participants were examined in the supine position with the head turned slightly to the left. Longitudinal images of the common carotid artery were obtained by combined B-mode and color Doppler ultrasound examinations. The IMT of the common carotid artery far wall was measured with the electronic calipers of the machines, as described by Pignoli *et al.* (22). On a longitudinal, two-dimensional ultrasound image of the carotid artery, the posterior (far) wall of the carotid artery

was displayed as two bright white lines separated by a hypoechogenic space. The distance between the leading edge of the first bright line of the far wall and the leading edge of the second bright line indicated the carotid artery IMT. The carotid artery IMT was measured during end diastole. The carotid artery IMT measurements were performed on-line. The mean carotid artery IMT was calculated for each children as the average of three consecutive measurements of maximum far wall thickness obtained from the common carotid artery, 20 mm below the carotid bulb. The coefficients of variation of the measurements were less than 3%.

Statistical methods. Data were expressed as mean ± SD. The Kolmogorov–Smirnov test was applied separately for boys and girls to check the normality of the variables. Differences in the means of variables were tested using both parametric and nonparametric tests depending on the distribution of the variables. Any variables that were not normally distributed were log-transformed before data analysis. Statistical correlation was assessed using the Pearson test (r). Separate relationships between IMT and insulin sensitivity indices (HOMA-IR, FGIR, QUICK-I, and ISI) were also examined after adjustment for age, sex, BMI, BMI-SDS, systolic and diastolic blood pressure, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and total homocysteine using general linear regression models (backward analysis). Statistical significance was taken as $p < 0.05$. All statistical analysis was performed using the Statistical Package for Social Sciences (SPSS/Windows version 11.0, SPSS Inc., Chicago, IL).

RESULTS

The characteristics of the study population are shown in Table 1. Both the obesity group and control group showed no significant difference in terms of gender composition, age, and body height. Subjects in the obese group had a significantly higher body weight, BMI, BMI-SDS and blood pressure. Total cholesterol, LDL cholesterol, and triglyceride levels were significantly elevated in obese children, whereas HDL was only slightly lower than the controls. The carotid artery IMT of obese children and controls ranged from 0.04 to 0.06 cm and 0.02 to 0.05 cm, respectively. The obese group had significantly higher carotid artery IMT than the controls (0.0476 ± 0.007 *versus* 0.033 ± 0.011 cm; $p < 0.001$). The values of total homocysteine in obese group, which were all within normal ranges, were slightly higher than those in controls, but not significantly so ($p = 0.053$).

Table 2 shows the correlations between carotid artery IMT and other measurements in obese children. When IMT was considered as a continuous variable in the whole population of obese children, it was found to be positively correlated in

Table 1. *Characteristics of the study population*

	Children with obesity	Control children	p value
Number of subjects (f/m)	50 (25/25)	50 (25/25)	—
Age (y)	11.7 ± 2.5	11.4 ± 3.73	0.721
Weight (kg)	64.1 ± 21.6	38.4 ± 14.8	<0.001
Height (cm)	148.8 ± 16.1	143.8 ± 19.8	0.174
BMI (kg/m^2)	28.2 ± 4.0	17.6 ± 3.0	<0.001
BMI-SDS	2.03 ± 0.34	0.42 ± 0.4	<0.001
Systolic blood pressure (mm Hg)	120.8 ± 17.6	106.1 ± 11.8	<0.001
Diastolic blood pressure (mm Hg)	79.3 ± 11.8	66.4 ± 9.0	<0.001
Lipids			
Total cholesterol (mg/dL)†	180.2 ± 27.6	154.3 ± 26.3	<0.001
Triglycerides (mg/dL)‡*	133.6 ± 84.1	81.2 ± 31.7	<0.001
LDL cholesterol (mg/dL)†	109.6 ± 29.3	92.5 ± 24.3	0.002
HDL cholesterol (mg/dL)†	47.3 ± 13.5	48.5 ± 10.2	0.626
Total homocysteine (μmol/L)	9.8 ± 3.9	8.3 ± 3.6	0.053
Carotid artery IMT (cm)	0.0476 ± 0.007	0.033 ± 0.011	<0.001
Fasting glucose level (mg/dL)§	97.5 ± 8.5	88.9 ± 8.7	<0.001
Fasting insulin level (μU/mL)¶*	16.0 ± 11.0	5.8 ± 4.6	<0.001
FGIR	9.6 ± 7.8	32.5 ± 46.3	0.001
HOMA-IR	3.92 ± 3.0	1.32 ± 0.95	<0.001
QUICK-I	0.32 ± 0.03	0.39 ± 0.08	<0.001
OGTT derived indexes			
AUC$_{glucose}$ (mg/dL × 120 min)	11754 ± 1431	—	—
AUC$_{insulin}$ (μU/mL ×120 min)	6504 ± 2689	—	—
ISI	4.35 ± 2.52	—	—

Data are given as mean ± SD.
† To convert to mmol/L, multiply by 0.0259.
‡ To convert to mmol/L, multiply by 0.0113.
§ To convert to mmol/L, divide by 18.
¶ To convert to pmol/L, multiply by 6.945.
* Statistical analysis was undertaken on log-transformed variables.

Table 2. *Relationship between carotid artery intima media thickness, insulin sensitivity indexes, and the other cardiovascular risk factors in obese children*

Variables	r	p
Weight	0.159	0.202
Height	0.227	0.174
BMI-SDS	0.20	0.893
Systolic blood pressure	0.172	0.233
Diastolic blood pressure	0.052	0.718
Total cholesterol	0.085	0.558
Triglycerides	0.076	0.600
LDL cholesterol	0.002	0.988
HDL cholesterol	−0.120	0.405
Total homocysteine	0.105	0.469
Fasting glucose level	0.167	0.247
Fasting insulin level	0.315	0.026
FGIR	−0.404	0.004
HOMA-IR	0.300	0.034
QUICK-I	−0.421	0.002
AUC$_{glucose}$	0.04	0.782
AUC$_{insulin}$	0.029	0.843
ISI	−0.252	0.078

univariate analysis with fasting insulin level (r = 0.315, p = 0.026), HOMA-IR (r = 0.300, p = 0.034) and negatively correlated with FGIR (r = −0.404, p = 0.004), QUICK-I (r = −0.421, p = 0.002), but there was no association with weight (r = 0.202, p = 0.159), height (r = 0.174, p = 0.227), BMI

Table 3. *Variable independently associated, in an age-, sex-, and other risk factor-adjusted backward multiple linear regression analysis, with the dependent variable carotid artery IMT in obese children*

	Carotid artery IMT		
	r^2	β	p
QUICK-I	0.207	−0.58	<0.001
AUC$_{insulin}$		−0.29	0.057

(r = 0.205, p = 0.153), BMI-SDS (r = 0.2, p = 0.893), systolic (r = 0.172, p = 0.233) and diastolic blood pressure (r = 0.052, p = 0.718), total cholesterol (r = 0.085, p = 0.558), triglycerides (r = 0.076, p = 0.600), LDL cholesterol (r = 0.002, p = 0.988), total homocysteine (r = 0.105, p = 0.469), fasting glucose level (r = 0.167, p = 0.247), AUC$_{glucose}$ (r = 0.04, p = 0.782), AUC$_{insulin}$ (r = 0.029, p = 0.843) and ISI (r = −0.252, p = 0.078). There were no significant relationships between carotid artery IMT and clinical and laboratory parameters in controls (data not shown).

In the regression analysis, QUICK-I was negatively correlated with increased IMT (β = −0.58, p < 0.001) even after adjusting for age, sex, BMI, BMI-SDS, systolic/diastolic blood pressure, total cholesterol, triglycerides, LDL cholesterol, HDL cholesterol, and total homocysteine as co-factors with the total variance explained being 20.7% (Table 3). HOMA-IR, FGIR, and ISI were not significantly correlated with IMT after adjusting for atherosclerotic risk factors.

DISCUSSION

In this study, we demonstrated the effect of insulin resistance on carotid IMT in obese children. There has been considerable interest in the insulin resistance and cardiovascular disease. Central obesity and the attendant insulin resistance/hyperinsulinemia occurring in childhood are the driving force of the multiple metabolic syndrome X, insulin resistance syndrome, or the "deadly quartet" (23). Therefore, we decided to measure the IMT of common carotid artery in obese children, since an increased IMT is known to be a parameter of atherosclerotic changes and to be detected without obvious abnormalities of the classic vascular risk factors. In the present study, the number of risk factors identified in obese children was associated with increased carotid artery IMT.

Autopsy studies in adolescents and young adults document that atherosclerosis begins in adolescence and that the traditional risk factors are associated with its development (24). Studies on early atherosclerotic changes in children have mainly focused on dyslipidemia, hypertension, obesity, and diabetes as risk factors but not on insulin resistance or importance of the insulin sensitivity indexes. The central role of insulin in the background of the clustering of some cardiovascular risk factors was first suggested by the reports on endogenous hyperinsulinemia and insulin resistance in essential hypertension (25). However, obese children exhibit glucose intolerance, which is strongly associated with evidence of both insulin resistance and impaired insulin secretion (26). Insulin-

resistant individuals who can compensate by hyperinsulinemia may escape diabetes, but are still prone to other complications, such as early atherosclerosis, progression of obesity (especially central type), acanthosis nigricans, increased skin tags, hypertension, dyslipidemia, hypercoagulation, polycystic ovary syndrome, fatty liver infiltration, focal segmental glomerulosclerosis, as well as increased cancer rate (27). However, few research reports have studied the relations between carotid artery IMT and insulin resistance indexes.

In the present study, fasting glucose was not associated with carotid artery IMT, although fasting insulin was significantly associated with carotid artery IMT in children with obesity. Therefore, these data supported the idea that insulin resistance may have a role in the development of early structural atherosclerotic vascular changes in children with obesity. The ISI and AUC$_{insulin}$, derived from the OGTT appear to be useful outcome measures for clinical trials in obese children and adolescents in terms of improving insulin sensitivity and glucose tolerance (28). We did not find a relation between insulin sensitivity indexes (ISI and AUC) derived from OGTT measurements and carotid artery IMT in obese children. Balletshore *et al.* (16) have also reported that glucose-clamp assessed insulin sensitivity was not correlated with IMT. However, in the present study, conducted in a group of obese children, we showed that insulin sensitivity indexes derived from fasting samples and elevated basal insulin levels were significantly associated with increased carotid artery IMT. We found FGIR and QUICK-I are more significantly related with carotid artery IMT than HOMA-IR.

Carotid artery IMT is affected by many factors including serum lipids. Autopsy studies in children have also shown a significant relationship between serum cholesterol concentration and early atherosclerotic lesions (1,2). Increased carotid artery IMT has previously been demonstrated in children with familial hypercholesterolemia. Case-control studies of children and young adults demonstrate that familial hypercholesterolemia and borderline hypertension are associated with greater IMT (11–13). In the present study, serum lipid levels in obese children were significantly higher than those of healthy subjects. However, we did not find a significant relationship between carotid artery IMT and serum lipids. The reason for this negative result might be owing to fact that our subjects had not very high lipid levels as did in familial hypercholesterolemia. Although, reduction in BMI slows the yearly rate of increase in carotid-wall thickness (29), the thickness of the arterial wall increasing with proportional to BMI is still a matter of debate. In the present study, we also did not find a significant relationship between anthropometric data and carotid artery IMT. The number of subjects is relatively small which might explain these non significant results.

Retrospective and prospective studies have demonstrated that hyperhomocysteinemia is the other risk factor for premature cardiovascular disease independent of other classic risk factors, such as smoking, hypercholesterolemia, arterial hypertension, and diabetes (30–33). Elevated plasma homocysteine has been shown to be a significant risk factor for higher carotid artery IMT (34). However, the other studies from Davis *et al.* (35) and Mahoney *et al.* (36), in the same cohort, did not find plasma homocysteine as a risk factor. These observations suggest that elevated homocysteine levels and lipids may not affect carotid artery IMT at a younger age. In the present study, we demonstrated that homocysteine levels in obese children were slightly higher than healthy subjects and also homocysteine levels was significantly associated with basal insulin levels (data not shown), although we were unable to show a relationship between fasting plasma homocysteine levels and carotid artery IMT. Our data support that plasma homocysteine levels might be a characteristic for early atherosclerosis in obese children.

Childhood obesity seems to contribute to the development and progression of early atherosclerosis, particularly in combination with insulin resistance. Our observations suggest that high insulin levels may have an important role in the pathogenesis of early atherosclerosis. According to our findings, the insulin indices derived from basal samples seem to be more useful for assessing subclinical atherosclerosis than indices obtained from OGTT, FSIVGTT, and clamp studies none of which are feasible in the pediatric age group.

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