

ASSOCIATIONS BETWEEN CAROTID INTIMA-MEDIA THICKNESS AND CARDIOVASCULAR RISK MARKERS IN OBESE CHILDREN

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Abstract

Excessive amounts of adipose tissue in children of school age (6-18 years) is widely accepted as coronary heart disease risk factors, such as hypertension and dyslipidemia. Exposure to these cardiovascular risk factors may induce atherogenic changes in the arteries. Measurement of the intima-media thickness (IMT) of the common carotid artery is a non-invasive marker for early atherosclerotic changes. The aim of this study was to investigate the risk factors which have an impact on common carotid artery intima media thickness (C-IMT) in obese children.

A total of 50 obese children of school age and 50 healthy children and adolescents with normal weight were enrolled. Correlations between C-IMT and cardiovascular risk factors (dyslipidemia, hypertension, obesity, resistance to insulin, hyperuricemia) were evaluated by the Pearson test.

C-IMT (0.53mm) was significantly increased in obese children ($p < 0.001$) versus the control group (0.41mm). C-IMT was correlated with body weight, body mass index, waist circumference, hip circumference, systolic and diastolic blood pressure, fasting C peptide, low-density lipoprotein-cholesterol, total cholesterol, uric acid and fatty liver.

Childhood obesity, especially abdominal obesity seems to contribute to the development and progression of early atherosclerosis. Routine measurement of carotid IMT may be used in order to prevent coronary atherosclerosis and other cardiovascular complications.

Rezumat

Excesul de țesut adipos la copii cu vârste între 6 și 18 ani este considerat un factor de risc în boala coronariană, datorită dislipidemiei și hipertensiunii arteriale.

Grosimea arterei coronare, este un marker important pentru diagnosticarea precoce a modificărilor aterosclerotice.

Scopul prezentului studiu a fost investigarea factorilor de risc ce au un impact major asupra grosimii arterei coronare la copiii obezi.

Keywords: obesity, intima-media thickness, obese children, atherosclerosis, cardiovascular risk factors

Introduction

In the past decade the prevalence of overweight and obesity has increased dramatically [1]. Obesity tracks from childhood to adulthood, and childhood adiposity is a strong predictor of obesity, insulin resistance and abnormal lipids in adulthood. Moreover, the rate of increase in adiposity during childhood was significantly related to the development of cardiovascular risk in young adults [2].

Recent studies in children indicate that the process of atherosclerosis starts at an early age and is linked to visceral obesity [3]. Severely obese vs. normal-weight children had lower brachial artery compliance, lower distensibility, increased wall stress, increased stiffness, impaired endothelial function and increased insulin resistance [2].

The common carotid artery intima-media thickness (C-IMT) measured by ultrasound imaging is a marker of preclinical atherosclerosis. C-IMT relates to the severity and extent of coronary artery disease and predicts the likelihood of cardiovascular events in adults. C-IMT was increased in children with type 1 diabetes mellitus [2]. Also, increased IMT was reported in children with obesity, familial hypercholesterolemia and nonalcoholic fatty liver disease (NAFLD) compared with control children [4-6].

There is increasing evidence that obesity in humans is associated with peripheral [7] and coronary [8] endothelial dysfunction. Although the association between obesity and atherosclerosis is well documented, the exact nature of this relation remains unclear. Endothelial dysfunction may explain this link. Endothelial dysfunction is characterized by an imbalance between vasodilating and vasoconstricting substances, with an impairment of vasodilators such as nitric oxide (NO) and prostacyclin (PGI₂) and a predominance of vasoconstrictors such as endothelin-1 (ET-1) and angiotensin II (Ang II) [9, 28].

Endothelial dysfunction is seen early in subjects with severe hypercholesterolaemia and has been described in young children with familial hypercholesterolaemia. These effects may be mediated, in part by oxidation products of LDL. High HDL-cholesterol plasma levels protect against adverse effects of hypercholesterolaemia on endothelium-dependent vasodilatation and inhibit LDL oxidation. The antioxidant action of HDL may be related to its protein components which bind transition metals and also to two intrinsic antioxidative enzyme systems, platelet activating factor

acetylhydrolase and paraoxonase. Triglyceride-rich lipoproteins and particularly their postprandial remnants are cytotoxic to cultured endothelial cells and can also cross the endothelial barrier and enter the arterial wall, placing them in a position to promote endothelial damage [10].

Insulin resistance is associated with endothelial dysfunction and impaired insulin-mediated nitric oxide-dependent vasodilation [9]. It was demonstrated that impaired endothelial function may be a common denominator of pathogenesis of microvascular complications and atherosclerosis in type 2 diabetes mellitus [11].

In the last years, it was demonstrated that uric acid induces endothelial dysfunction and vascular inflammation, decreases endothelial nitric oxide production and becomes prooxidant in the vascular environment of diabetic patients (the urate redox shuttle) [12]. In turn, hyperuricemia leads to an increased risk of atherosclerotic cardiovascular disease [13].

The aim of this study was to investigate the risk factors (dyslipidemia, hypertension, obesity, insulin resistance, hyperuricemia) which have an impact on common carotid artery intima media thickness (IMT) in children of school age.

Materials and methods

Subjects

Two groups of children, obese and non-obese were enrolled. Each group was formed by 50 children (21 boys and 29 girls) with a mean age of 12.6 ± 3.1 years. Children under medication or those with chronic disease (endocrine disease, hereditary disease, or systemic inflammation) were excluded.

The study protocol was approved by the Ethical Commission of "Carol Davila" University of Medicine, Bucharest and a written informed consent was obtained from each child's parent or tutor.

Clinical characteristics

Anthropometric measurements: body mass index (BMI), waist/hip ratio (WHR), waist circumference (WC), hip circumference (HC), were assessed. BMI was calculated as body weight (kg) divided by square height (m^2). Overweight is defined as a BMI at or above the 85th percentile and lower than the 95th percentile. Obesity is defined as a BMI at or above the 95th percentile for children of the same age and sex [14]. The obese group was formed by overweight and obese children. Waist circumference (WC) was measured at the midway between the lower rib and the iliac crest and hip circumference was measured at the widest part at the gluteal region.

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice at the right arm after a 10-minute rest, in the supine position using an automated sphygmomanometer.

Biochemical measurements

Blood samples were taken after an overnight fast. Metabolic markers: fasting serum glucose, serum lipids, serum uric acid and enzymes activities for alanine aminotransferase (ALT) and for γ -Glutamyl transferase (γ -GT) were performed by standard methods using an automatic analyzer HITACHI and kits from Diasys (Germany). Glycated hemoglobin HbA_{1c} was measured by the turbidimetric immunoassay and C peptide by using Human C-Peptide ELISA Kit. The ALT was evaluated as an indicator of fatty liver due to obesity.

IMT measurement

IMT was measured by B-mode ultrasound using a 10-MHz linear transducer (ecograph Chison 600 M) in terms of fasting for 8 hours. IMT was measured at the common carotid artery near the bifurcation, during end diastole, following a standardized protocol. The maximal thicknesses of the intima-media width were measured to give three readings and the mean value was used for statistical purposes.

Statistical analysis

The source of variation between the control group and the obese subjects was assessed by the unpaired Student t-test. The associations of variables were examined by Pearson correlation and multiple stepwise linear regression analysis was used. The value $p < 0.05$ was considered statistically significant.

Results and Discussion

Biochemical and clinical characteristics for the two studied groups of subjects are shown in Table I. No difference in age and gender was observed between the control and the obese groups. The obese group demonstrated increased values for: body weight, BMI, WC, HC, WHR, glycemia, HbA_{1c}, uric acid, SBP, DBP, TG, LDL-cholesterol, ALT and γ -GT levels, decreased HDL-cholesterol levels and higher prevalence of fatty liver ($p < 0.01$). The obese children also demonstrated significantly increased mean carotid IMT values as compared with non-obese children matched for age and sex. Thus, obese children and adolescents had enhanced concentrations of markers of future cardiovascular disease, and an increased carotid IMT, in agreement with their degree of obesity.

Table I
Clinical and biochemical characteristics of the studied groups.

Parameters	Obese group	Control group	P
Age (6-18 years)	12.6 ± 3.1	12.6 ± 3.1	NS
Sex (F/M)	29/21	29/21	NS
Weight (kg)	69.61 ± 15.1	44.82 ± 10.33	<0.001
Height (m)	1.52 ± 0.12	1.52 ± 0.12	1.00
BMI (kg/m ²)	29.34 ± 2.4	18.81 ± 1.7	<0.001
WC (cm)	85.86 ± 9.2	61.31 ± 6.6	<0.001
HC (cm)	94.57 ± 10.5	76.37 ± 7.1	<0.001
WHR	0.92 ± 0.05	0.8 ± 0.07	<0.001
Uric Acid (mg/dL)	5.16 ± 0.85	3.3 ± 0.5	<0.001
γ-GT (U/l)	16.53 ± 1.5	15.29 ± 1.3	0.06
Glycemia (mg/dL)	86.82 ± 5.8	72.27 ± 3.4	<0.001
HbA _{1c} (%)	5.04 ± 0.08	4.87 ± 0.05	<0.001
C peptid (ng/ml)	3.05 ± 0.42	2.59 ± 0.24	0.007
ALT (U/l)	22.04 ± 4.3	17.65 ± 2.19	<0.01
Total cholesterol (mg/dL)	172.8 ± 14.2	142.8 ± 9.9	<0.001
HDL-C (mg/dL)	43.4 ± 4.01	40.68 ± 3.9	0.07
LDL-C (mg/dL)	102.5 ± 12.3	84.7 ± 7.7	<0.01
Triglycerides (mg/dL)	117.9 ± 35.76	60.55 ± 11.4	<0.001
SBP (mmHg)	108.7 ± 5.08	96.59 ± 5.2	<0.001
DBP (mmHg)	66.8 ± 4.06	59.25 ± 2.6	<0.001
Mean carotide IMT (mm)	0.53 ± 0.08	0.41 ± 0.03	<0.001
Fatty liver(ultrasound)	Present in all subjects	0.00	<0.001

Data are expressed as means ± SD. BMI = body mass index; WC = waist circumference; HC=hip circumference; WHR = waist to hip ratio; ALT = alanine aminotransferase; γ-Glutamyl transferase (γ-GT); HDL-C = high-density lipoprotein -cholesterol; LDL-C = low-density lipoprotein-cholesterol; SBP = systolic blood pressure; DBP = diastolic blood pressure. NS = not significant

To determine the predictive factors for increased carotid IMT in the obese population, we performed correlations between the mean of the IMT values and the variables presented above (Table II). In our obese population, univariate analysis showed a statistically significant positive correlation of IMT values with: weight, height, BMI, WC (Fig.1), HC, total and LDL cholesterol (Fig.2), the degree of systolic and diastolic hypertension, fasting C peptide levels (Fig.3). No correlation was observed between carotid IMT and other classical cardiovascular risk factors such as: WHR, glycemia, HbA_{1c}, g-GT, HDL-cholesterol and triglycerides levels.

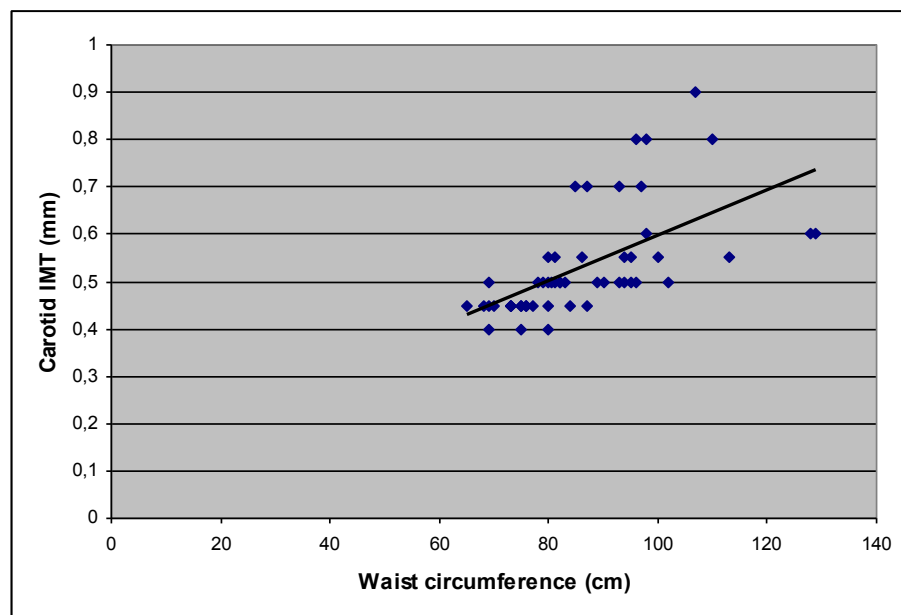
A strong significant positive correlation was observed between g-GT activity and: anthropometric data, uric acid, glycemia, HbA_{1c}, ALT activity, cholesterol and systolic and diastolic blood pressure.

A positive correlation between BMI and ALT activity was calculated, which is an indicator of fatty liver due to obesity ($r=0.52$; $p<0.05$).

Table II

The Pearson correlations ($p < 0.05$) between the studied parameters. All values have statistical significance.

	BMI (kg/m ²)	Uric acid (mg/dL)	Glycemia (mg/dL)	g-GT (UI)	Cholesterol (mg/dL)	LDL-C (mg/dL)	SBP (mmHg)	DBP (mmHg)	C-IMT (mm)
Age (6-18 years)	0.65	0.52		0.51	0.55	0.6	0.47	0.37	0.65
Weight (kg)	0.82	0.71		0.58	0.43	0.43	0.58	0.50	0.59
Height (m)	0.61	0.62		0.4	0.37	0.38	0.46	0.30	0.56
BMI (kg/m ²)	1	0.58		0.57	0.36	0.28	0.52	0.54	0.49
WC (cm)	0.85	0.69	0.35	0.69	0.43	0.43	0.64	0.64	0.59
HC (cm)	0.82	0.61		0.58	0.43	0.46	0.59	0.55	0.58
Uric acid (mg/dL)	0.58	1	0.49	0.64	0.42		0.56	0.52	0.35
Glycemia (mg/dL)		0.49	1	0.72			0.47	0.62	
HbA1c (%)	0.49	0.60	0.88	0.82	0.31		0.51	0.70	
C peptide (ng/mL)	0.49	0.81	0.54	0.61	0.37	0.30	0.54	0.49	0.41
g-GT (UI)	0.57	0.64	0.72	1	0.36	0.30	0.49	0.68	
ALT (UI)	0.52	0.42	0.39	0.64			0.45	0.44	0.28
Cholesterol (mg/dL)	0.36	0.42		0.36	1	0.85	0.40	0.33	0.55
LDL cholesterol (mg/dL)	0.28			0.30	0.85	1	0.38	0.32	0.63
Triglycerides (mg/dL)		0.52	0.29				0.36		
SBP (mmHg)	0.52	0.56	0.47	0.49	0.40	0.38	1	0.80	0.51
DBP (mmHg)	0.54	0.52	0.62	0.68	0.33	0.32	0.80	1	0.41
C-IMT (mm)	0.49	0.35			0.55	0.63	0.51	0.41	1

**Figure 1**

Correlation between Carotid IMT and Waist circumference

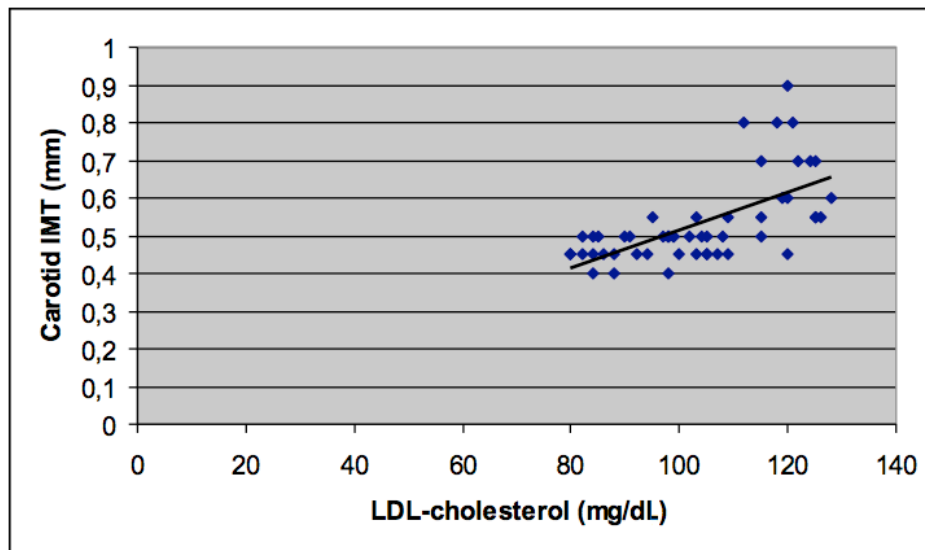


Figure 2
Correlation between Carotid IMT and LDL-cholesterol

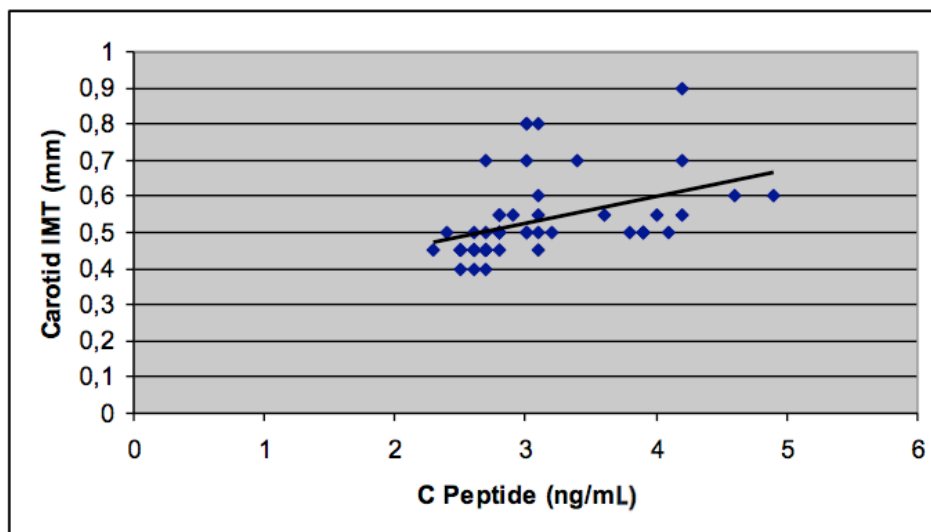


Figure 3
Correlation between Carotid IMT and C Peptide

Childhood obesity is associated with elevated blood pressure, higher total cholesterol levels, higher low-density lipoprotein cholesterol levels, high triglyceride levels, low HDL cholesterol levels, abnormal glucose metabolism, insulin resistance and inflammation [15].

In this study, we showed that these biomarkers of an increased risk of cardiovascular disease are already altered in obese children of school age. The association of these markers with waist circumference and also with carotid IMT values may explain the link between obesity and early atherosclerosis.

Recent evidence in children shows that waist circumference is more often associated with visceral fat, whereas BMI is associated more with subcutaneous fat. Similarly, only visceral fat (as measured by magnetic resonance imaging), not BMI or waist-hip ratio, was associated with fasting insulin and triglycerides in obese adolescent girls [2]. In table II it can be noticed that IMT is positively correlated with anthropometric markers and the strongest association is with the waist circumference.

The relationship between waist circumference-measured abdominal obesity and health outcomes appears to be explained by its strong association with visceral adipose tissue, an independent predictor of metabolic and cardiovascular diseases [2]. Recently, waist circumference in children was found to be an independent predictor of insulin resistance [16]. Visceral adiposity is more easily mobilised than peripheral fat and so, causes higher plasma values for free fatty acids; as a consequence DAG (diacylglycerol) will accumulate in skeletal muscle and will activate the phosphoinositide-dependent kinase (PKC), which will inhibit PI-3K (phosphoinositide-3-kinase), an enzyme important for glucose transport inside the cell under the action of insulin. Visceral fat is associated with high plasma levels for inflammatory markers which determine triglyceride deposits formation in non-adipose tissues (ectopic places) [10].

Insulin resistance promotes free fatty acid release from adipocytes, which are taken up by the liver and which, if not processed immediately, precipitate into lipid droplets, termed "hepatic steatosis." This condition may evolve into nonalcoholic steatohepatitis and ultimately cirrhosis. The prevalence of nonalcoholic fatty liver disease is difficult to estimate in children, because the diagnosis is confirmed only by liver biopsy. A recent study of autopsy specimens suggested the prevalence in 13% of children and 38% of obese children [17]. Alanine transaminase elevations, along with abdominal ultrasound, may be useful in the diagnosis; however, only 40% of patients will have elevated liver enzymes, and the degree of elevation does not always correlate with the degree of obesity [2]. In this study, the average for ALT activity measured in obese children is in normal range, but increased compared to values from the control group. Abdominal ultrasound revealed hepatic steatosis or fatty pancreas in all investigated obese children.

High C-peptide levels may reveal insulin resistance. In our study we measured C peptide level which was increased in obese children and correlated with: carotid IMT, BMI, glycemia, uric acid, γ -GT activity, blood pressure, total cholesterol and LDL-cholesterol. All these correlations reveal the link between visceral obesity (characterised by insulin resistance and chronic inflammation) and atherosclerosis. Scientific literature data offer arguments for each observed correlation as it follows:

- decreased resting brachial distensibility (not flow mediated) was associated with insulin resistance, and a graded relation was found between a number of metabolic syndrome components and worsening brachial artery function [2];

- both insulin and leptin appear to have a direct effect on sympathetic nervous system activity and increased sympathetic tone has been associated with obesity in adolescents; fasting insulin, used as an estimate of insulin resistance, has been significantly correlated with blood pressure in children and adolescents [18];

- in adults and more recently in children, a high prevalence of small, dense LDL particles was demonstrated in association with abdominal obesity, visceral fat, and insulin resistance. Small dense LDL particles may have increased atherogenic potential, and the mechanisms proposed for this association are their low affinity to LDL receptors, propensity to undergo oxidative stress, prolonged plasma half-life, and high penetration of the intima. However, it is currently unknown whether insulin resistance induces dyslipidemia or whether insulin resistance and dyslipidemia are associated via an underlying cause [19].

Uric acid and γ -GT are also important factors implicated in early atherosclerosis and need more comments. Uric acid, despite being a major antioxidant in the human plasma, both correlates and predicts development of obesity, hypertension, and cardiovascular disease, conditions associated with oxidative stress [13, 12, 20, 21].

Several possible pathologic mechanisms linking serum uric acid to cardiovascular disease have been proposed, including deleterious effects on endothelial function, oxidative metabolism, platelet adhesiveness, haemorheology and aggregation [22].

Conclusions

In our study, uric acid was significantly higher in the obese group than in lean control subjects and was correlated with: anthropometric data, γ -GT activity, total cholesterol, triglycerides, systolic and diastolic blood pressure and IMT.

Evidence from epidemiological studies suggests that higher serum γ -GT is associated with hepatic steatosis [23] and development of cardiovascular disease risk factors, including: diabetes, hypertension, dyslipidemia, and the metabolic syndrome [24]. γ -GT can modulate the redox status of protein thiols at the cell surface, leading to production of reactive oxygen species and can catalyze oxidation of circulating low-density lipoprotein cholesterol (LDL) [25]. It is expressed in the atheromatous core of coronary plaques, where it colocalizes with oxidized LDL and foam cells [26]. γ -GT may have a proinflammatory effect because it mediates interconversion of the glutathione-containing inflammatory mediator leukotriene C₄ into leukotriene D₄ [27]. In our study γ -GT levels are not correlated with IMT but are associated with a lot of cardiovascular risk factors such as: BMI, waist circumference, uric acid, LDL-cholesterol, total cholesterol, glycemia, HbA1c, C peptide, systolic and diastolic blood pressure.

Increased carotid IMT values in obese children are arguments for early atherosclerosis. The mechanisms by which adiposity is linked to early arterial disease remain uncertain. However, the positive correlations of IMT with the cluster of cardiovascular risk factors may demonstrate the synergic effect of dyslipidemia, high blood pressure, insuline resistance, uric acid and γ -GT on atherosclerosis.

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