Original Article

Effect of Obesity on Endothelial Function and Subclinical Atherosclerosis in Children

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ABSTRACT

We aimed to measure flow-mediated dilation (FMD), carotid intima-media thickness (cIMT), and to evaluate the effects of waist circumference (WC), and body mass index Z (BMI-Z) score on these parameters in obese children. This case-control cross-sectional study included 70 obese and 40 non-obese children aged 7-14 years who presented with various complaints and had no concomitant diseases. FMD and cIMT were measured in all subjects and correlated with anthropometric and biochemical factors. WC, BMI-Z score, systolic and diastolic blood pressure (BP), triglyceride (TG) and insulin concentrations, and homeostatic model assessment (HOMA) index were significantly higher, whereas high density lipoprotein (HDL) -cholesterol concentration was significantly lower in the obese than in the non-obese group. FMD values were significantly lower, whereas cIMT values were significantly higher in obese than in non-obese subjects. FMD negatively correlated with WC, BMI-Z score, serum insulin level, HOMA, systolic BP, triglyceride but positively with HDL-cholesterol. cIMT positively correlated with WC, BMI-Z score, serum insulin level, HOMA, systolic BP, triglyceride but negatively with HDL-cholesterol. Increased WC, BMI-Z score, serum insulin level, HOMA, systolic BP, triglyceride and decreased HDL-cholesterol in obese children contribute to endothelial dysfunction and early subclinical atherosclerosis compared to their normal weight peers.

Key words: Children, carotid intima-media thickness, flow mediated- dilatation, obesity

Çocuklarda Obezitenin Endotel Fonksiyonu ve Subklinik Ateroskleroz Üzerine Etkisi

ÖZET

Obez çocuklarda akım aracılı dilatasyonu (AAD), karotis intima-media kalınlığını (kİMK) ölçmeyi ve bel çevresi (BÇ) ile beden-kitle indeksi Z (BKİ-Z) skorunun bu parametrelere etkisini değerlendirmeyi amaçladık. Bu vaka kontrollü karşılaştırmalı kesitsel çalışma 7-14 yaşlarında, değişik şikayetlerle pediatri polikliniğine gelen ve eşlik eden hastalığı olmayan, 70 obez ve 40 obez olmayan çocuğu kapsamaktaydı. AAD ve kİMK tüm bireylerde ölçüldü ve antropometrik, biyokimyasal faktörlerle ilişkisi araştırıldı. Obez grupta yüksek dansiteli lipoprotein (YYL) kolesterol konsantrasyonu obez olmayan gruptan belirgin derecede düşük iken; BÇ, BKİ-Z skoru, sistolik ve diyastolik kan basıncı (KB), trigliserit (TG), insülin konsantrasyonları ve HOMA (homeostatic model assessment) indeksi belirgin derecede yüksekti. Obez grupta kİMK değerleri obez olmayan gruptan belirgin derecede yüksek iken, AAD değerleri belirgin derecede düşüktü. AAD değerleri; BÇ, BKİ-Z skoru, serum insulin seviyesi, HOMA, sistolik KB, TG ile negatif; HDL kolesterol ile pozitif ilişkiliydi. kİMK değerleri; BÇ, BKİ-Z skoru, serum insulin seviyesi, HOMA, sistolik KB, TG ile pozitif; HDL kolesterol ile negatif ilişkiliydi. Normal kilolu yaşıtlarıyla karşılaştırıldığında, obez çocuklarda, artmış BÇ, BKİ-Z skoru, serum insulin seviyesi, HOMA, sistolik KB, TG ve azalmış HDL kolesterol, endotel disfonksiyonu ve erken subklinik ateroskleroza katkıda bulunmaktadır

Anahtar kelimeler: Çocuklar, karotis intima-media kalınlığı, akım aracılı dilatasyon, obezite

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INTRODUCTION

The global prevalence and severity of childhood over-weight and obesity have increased considerably in recent years, with the worldwide prevalence predicted to approach 9.1% in 2020.(1) This increase is considered an indicator for increased incidence of future cardiovascular diseases (CVD) (2).

Children with risk factors for atherosclerotic progression have been found more likely to develop atherosclerosis during adulthood (3). Therefore, primary prevention of CVD should begin in childhood and CVD risk factors should be closely monitored particularly in overweight and obese children (4,5). In the Bogalusa Heart Study, the majority of the children with three risk factors for coronary artery disease were obese (6). The prevalence of metabolic syndrome is higher in obese than in normal-weight children (7).

Endothelial dysfunction is an early event in the development of atherosclerosis. Flow mediated dilation (FMD), is an indicator of endothelial dysfunction, provides direct information on the regulation of vascular tone and,(8) Assessment of cIMT is important in the evaluation of subclinical atherosclerosis (9) and better predictor for early atherosclerosis than FMD. Premature development of abnormal endothelial cell dysfunction and arterial IMT have been reported in obese children (10).

In the present study, we aimed to measure carotid intima-media thickness (cIMT), flow-mediated dilation (FMD), and to evaluate the effects of body mass index Z (BMI-Z) score and waist circumference (WC), on these parameters in obese children.

MATERIALS AND METHODS

Study design

The study included children aged between 6 and 15 years who presented to the pediatric outpatient clinic at our institution with various complaints and had no concomitant diseases. Informed consent was obtained from the parents of the children. Children with congenital cardiac disease, rheumatic heart disease and arrhythmia on electrocardiogram (ECG) were excluded. This case–control study included 70 obese children, 30 (42.8%) girls and 40 (57.2%) boys, mean age 10.6±2.1 years; and 40 non-obese children, 17 (42.5%) girls and 23 (57.5%) boys, mean age 10.7±1.9. years. All children

were weighed and their heights were measured and BMI Z score were calculated. Childhood obesity was defined as a BMI equal to or greater than the 95th percentile for children of the same age and gender (Z score ≥ 1.645). (11) The study groups were matched for age. The protocol of the study was approved by the Institutional Ethics Committee.

Brachial Artery Flow-Mediated Dilation

Brachial artery FMD was measured by ultrasonography. Subjects were asked to refrain from caffeinated beverages 12 hours before the procedure and from exercise immediately before FMD was measured. The procedure was performed at a room temperature of 21- 23°C after an 8-12 hour fast. Before FMD measurement, systolic and diastolic blood pressures (BPs) were measured after a 10-minute rest. Each subject was placed in a comfortable supine position and the brachial artery was palpated immediately above the antecubital fossae in the longitudinal plane. Ultrasonography was performed using an Esaote MyLab 50 (Genoa, Italy) device with a 7.0 MHz transducer. The transducer was placed on the right brachial artery trace; the brachial artery was visualized longitudinally in the area where the best image was obtained, and the image was magnified using the magnifying and focusing characteristics of the ultrasonography device. A segment between the lumen and vascular wall, with the anterior and posterior intimal surfaces specified exactly, was chosen for two-dimensional imaging. The diameter of the brachial artery (intima-to-intima) was measured three times and averaged. Measurements were performed at the end of diastole according to ECG monitoring. The cuff of the sphygmomanometer was placed on the upper part of the right antecubital fossa. After recording baseline measurements, the cuff pressure was increased to 50 mmHg over the systolic BP of the patient to stop arterial blood flow completely and this cuff pressure was maintained for 5 minutes. Ischemia was established by stopping the antegrade blood flow. After the cuff was deflated, a two-dimensional image of the brachial artery for 60 seconds was obtained in the longitudinal plane. The mean of three measurements was recorded as the post-flow diameter of the brachial artery lumen (endothelium-dependent vasodilator response (EDVR)). FMD was defined as the percent increase relative to baseline vascular diameter (BD). Endothelium-dependent vasodilation was calculated as FMD= ((EDVR-BD) /BD) x 100.

Measurement of Carotid Intima-Media Thickness

Subjects were placed in the supine position with their heads tilted backwards, and their right and left carotid arteries were visualized by an ultrasonography device (Esaote MyLab 50, Genoa, Italy) with a 7.0 MHz linear probe. A 1-cm segment was specified within the first 2 cm from the bulbus of the main carotid artery. For ultrasonographic analysis, only the posterior wall was examined within a 1-cm area by benefiting from the characteristic echogenicity of lumen-intima and media-adventitia interfaces, and intima-media thickness was measured. Each carotid artery was measured three times, and the averaged cIMT was calculated after right and left cIMT values divided to 2. Calculations were performed manually by a single operator. Intraobserver variations were minimized.

The FMD and cIMT measurements of 20 children (10 obese, 10 normal) were re-examined after 10 days to determine the coefficients of variation between visits. These were 3.4% and 3.2% for FMD and cIMT respectively.

Statistical Analysis

Data were analyzed using Predictive Analysis Software (PASW) Statistics 18 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were expressed as mean ± standard deviation or median (interquartile range). Normally distributed variables were compared using independent sample t-tests, and non-normally distributed variables were compared using Mann-Whitney U-tests. A p value <0.05 was considered statistically significant. Spearman correlation tests were performed to test the relationships of brachial artery FMD and cIMT, with age, WC, BMI Z score, serum insulin level, HOMA index, systolic and diastolic BPs, triglyceride and HDL-C concentrations.

RESULTS

The obese group comprised 70 children, 30 (42.8%) girls and 40 (57.2%) boys, mean age 10.5±1.7 years and mean obesity duration of 6.5±1.5 years; whereas the non-obese group consisted of 40 children, 17 (42.5%) girls and 23 (57.5%) boys, mean age 10.6±1.6 years (Table 1). WC, BMI, BMI Z score, systolic and diastolic BPs were significantly higher in the obese than in the non-obese group (p<0.001 each). Fasting blood glucose concentrations were similar in the two groups, whereas serum insulin concentration and HOMA index were significantly higher in the obese group (p<0.001 each). Triglyceride concentration was significantly higher (p= 0.001), whereas HDL-C concentration was significantly lower (p= 0.003) in the obese than in the non-obese group, although total cholesterol and low density lipoprotein cholesterol (LDL-C) concentrations were similar in the two groups (p>0.05 each).

Table 1. Anthropometric and biochemical characteristics of obese and non-obese children

Variables	Obese group (n=70)	Non-obese group (n=40)	p vaule*
Age (years)	10.6±2.0	11.3±2.2	0.873
Gender, male (n,%)	40 (57.2)	23 (57.5)	0.871
Weight (kg)	67 (42-91)	36 (22-47)	<0.001
Height, cm	135.5 (110-170)	135 (115-140)	0.966
BMI (kg/m2)	35 (31-38.8)	23 (15-24.9)	<0.001
BMI-Z score	2.2 (1.75-2.7)	0.5 (0.4-0.7)	<0.001
Waist circumference (cm)	85.5 (65-99)	64 (51-81)	<0.001
Obesity duration (years)	7 (5-10)	-	-
Systolic BP (mmHg)	120 (90-140)	105(90-120)	<0.001
Diastolic BP (mmHg)	80 (60-90)	70 (50-85)	<0.001
Glucose (mg/dL)	95 (65-110)	93 (68-96)	0.635
HOMA index	3.3 (0.4-8.1)	0.8 (0.2-4.5)	<0.001
Insulin (mIU/L)	16.5(1.75-35)	3.0 (1.9-16.7)	<0.001
Total cholesterol (mg/dL)	165 (130-240)	162 (110-214)	0.362
Triglyceride (mg/dL)	95 (75-250)	77 (57-250)	0.001
LDL-cholesterol (mg/dL)	103(75-190)	105 (55-185)	0.643
HDL-cholesterol (mg/dL)	43 (35-75)	48 (27-65)	0.003

*Data are presented as mean ± standard deviation, median (minimum - maximum) or n(%). Abbreviations: BMI, body mass index; BP, blood pressure; CRP, C-reactive protein; HDL, high-density lipoprotein; HOMA (Homeostatic model assessment) index = (fasting glucose x fasting insulin concentration x0.0555)/22.5; IU, international unit; LDL, low-density lipoprotein; * by independent sample t-test or Mann-Whitney U-test, as appropriate.*

Table 2. *Carotid intima-media thickness and flow-mediated dilation of obese and non-obese children*

Variables	Obese group (n:70)	Non-obese group (n:40)	p value*
Flow-mediated dilatation (%)	*5.75 (5.1-19.8)*	*9.7 (7.8-26.7)*	*<0.001*
Right carotid intima-media thickness (mm)	*0.59 (0.41-0.68)*	*0.45 (0.32-0.58)*	*<0.001*
Left carotid intima-media thickness (mm)	*0.59 (0.44-0.70*	*0.44 (0.32-0.60)*	*<0.001*
Mean carotid intima-media thickness (mm)	*0.60 (0.43-0.68)*	*0.43 (0.33-0.59)*	*<0.001*

Data are presented as mean ± standard deviation, median (minimum – maximum) or n (%). by Mann-Whitney U test*

Flow-mediated dilation values

The brachial artery FMD (%) (5.75 (5.1 –19.8) vs 9.7 (7.8 –26.7)) response was significantly lower in the obese group than in the non-obese group (p<0.001, Table 2).

Carotid intima-media thickness

The right cIMT (0.59 (0.41 –0.68) vs 0.45 (0.32 –0.58) mm), the left cIMT (0.59 (0.44 -0.70) vs 0.44 (0.32 –0.60) mm) and the mean cIMT values (0.60 (0.43 -0.68) vs 0.43 (0.33 –0.59) mm) were significantly higher, in the obese group than in the non-obese group (p <0.001 each, Table 2).

Correlation analysis between FMD, and cIMT parameters with anthropometric and biochemical variables

FMD was negatively correlated with age (r= -0.273; p= 0.022), WC (r= -0.404; p< 0.001, Figure 1), BMI -Z score (r= -0.275; p= 0.021), serum insulin level (r= -0.400; p= 0.001, Figure 2). HOMA index (r= -0.403; p= 0.001), systolic BP (r= -0.353; p= 0.003), .triglyceride (r= -0.370; p= 0.002).and positively correlated with HDL-cholesterol (r= 0.371; p= 0.002) (Table 3).

Mean cIMT positively correlated with age (r= 0.340; p= 0.004), WC (r= 0.343; p= 0.004, figure 3), BMI -Z score (r= 0.241; p= 0.044), serum insulin level (r= 0.521; p<0.001, Figure 4). HOMA index (r= 0.515; p< 0.001), systolic BP (r= 0.309; p= 0.009), .triglyceride (r= 0.331; p= 0.005). and negatively correlated with HDL-cholesterol (r= -0.304; p= 0.011) (Table 3).

DISCUSSION

The rate of childhood obesity has increased dramatically in recent years. Obesity is associated with significant social and economic burdens by increasing morbidity and mortality rates (12). Since childhood overweight has been found to correlate with overweight and cardiovascular risks in adulthood (13), it is important to identify children at risk for obesity and to implement early preventive measures to reduce modifiable risk factors. Assessment of endothelial function and cIMT has been recently used to evaluate early atherosclerosis in obese individuals. We, therefore, compared FMD and cIMT measurement in groups of obese and non-obese chil-
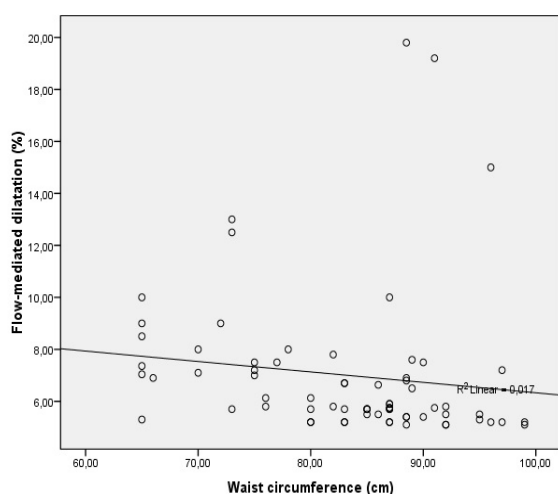

Figure 1. *Correlation graphics between waist circumference and flow mediated dilatation in obese children*
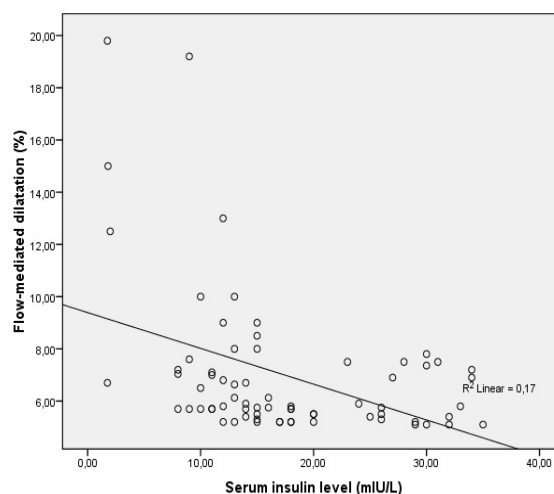

Figure 2. *Correlation graphics between flow mediated dilatation and serum insulin level obese children*
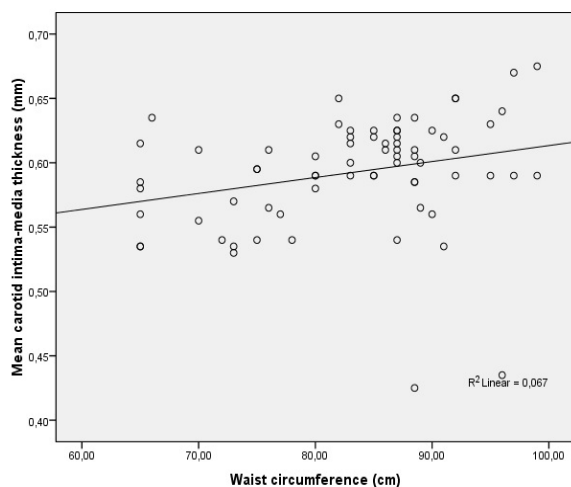
Figure 3. Correlation graphics between waist circumference and mean carotid intima-media thickness in obese children
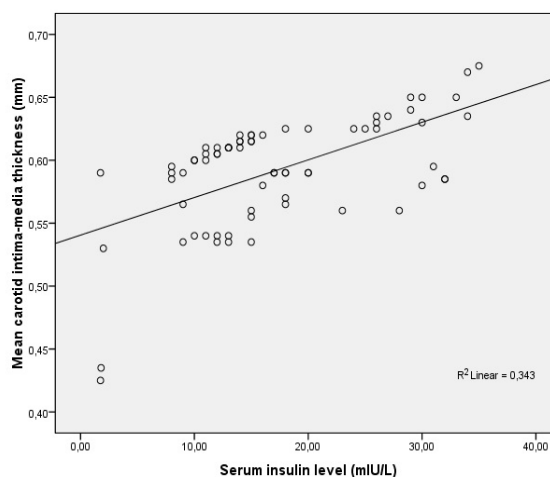

Figure 4. Correlation graphics between mean carotid intima-media thickness and serum insulin level in obese children

dren. In agreement with previous findings (14-20), we found that WC, BMI, BMI Z score, systolic and diastolic BPs were significantly higher in the obese group than in the non-obese group. In addition, HOMA index, insulin and triglyceride concentrations were significantly higher, and HDL-cholesterol concentrations were significantly lower in the obese group.

Endothelial function is affected early during the development of atherosclerosis (21). Similar to previous results (17,18,22), we found that FMD levels were significantly lower in the obese group than in the non-obese group, indicating that endothelial function was impaired in obese children. It has been well known that FMD is correlated with various risk factors such as WC, (17), extent

of obesity, arterial hypertension, fibrinogen, C-reactive protein,(18), BMI,(22) uric acid (23). We found that FMD was significantly correlated with age, WC, BMI Z score, serum insulin level, HOMA index, systolic BP, triglyceride and HDL-cholesterol. These findings suggest that obesity is often associated with endothelial dysfunction.

cIMT measurements are used to detect subclinical atherosclerosis (24) and increased cIMT values have been observed in overweight and obese children (14-27). Not surprisingly, we found that cIMT values were significantly higher in the obese compared with non-obese group,(14,16,19) and that cIMT values were significantly correlated with WC, BMI Z score, serum insulin level, HOMA index, systolic BP, triglyceride and HDL-

Table 3. Spearman rank correlation analysis:of flow mediated dilation (FMD) and carotid intima-media thickness (CIMT), with anthropometric and biochemical variables

Variable	FMD		CIMT	
	r	p value	r	p value
Age	-0.273	0.022	0.340	0.004
Obesity duration	-0.105	0.389	0.076	0.534
Waist circumference	-0.404	0.001	0.343	0.004
BMI Z-score	-0.275	0.021	0.241	0.044
Insulin	-0.400	0.001	0.521	<0.001
HOMA index	-0.403	0.001	0.515	<0.001
Systolic BP	-0.353	0.003	0.309	0.009
Diastolic BP	-0.106	0.420	0.223	0.063
Triglyceride	-0.370	0.002	0.331	0.005
HDL-Cholesterol	0.371	0.002	-0.304	0.011

cholesterol (14, 16-21,27). Number of studies have been proved that cIMT is related to some parameters such as WC and diastolic BP (14,15,22), BMI (15,16,20,22), fat mass percentage, glucose, and high-sensitivity CRP levels (22), age (16), triglyceride (17). These findings suggest that obesity is associated with early subclinical atherosclerosis in childhood.

According to our results, there was a moderate correlation between cIMT and FMD, insulin and waist circumference. Insulin resistance leads to increase in low density lipoproteins, hipertriglyceridemia, and an increase in blood pressure by activation of the sympathetic system (1,28) Hence, insulin resistance is the most important factor predisposing to atherosclerosis in obesity. We speculated that excess of insulin and waist circumference, and other obesity related risk parameters such as BP, HDL-C and triglyceride may lead to structural and functional changes in the vascular wall by accelerating atherosclerosis in obese children.

The main limitation of this study was its small sample size. In addition, we were unable to investigate the brachial artery nitrate-induced endothelium-independent vasodilation response in these subjects. We did not evaluate serum adiponectin levels and obesity type such as central, abdominal or visceral. These variables would be further validated our study.

Childhood obesity is related to endothelial dysfunction and subclinical atherosclerosis. There is a relationship between endothelial dysfunction, early atherosclerosis and obesity as well as insulin resistance. Further studies with longer follow-up periods in larger populations are needed to observe the effects of these changes from childhood to adulthood.

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