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Association of Changes in Oxidative and Proinflammatory States with Changes in Vascular Function after a Lifestyle Modification Trial Among Obese Children

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BACKGROUND: The association of changes in oxidative and proinflammatory states with vascular function after diet and exercise intervention among obese children has not been previously explored.

METHODS: In this 6-week diet and exercise intervention study in 35 obese children, age 12 to 18 years, we evaluated the relationship between changes in anthropometric indices, measures of insulin resistance, C-reactive protein (CRP), oxidized LDL (ox-LDL), and oxidative stress markers with changes in carotid intima-media thickness (C-IMT) and flow mediated dilation (FMD) of the brachial artery.

RESULTS: At the end of the study, body mass index (BMI), waist circumference, and percentage body fat were decreased ($P < 0.05$), but participants remained overweight (BMI $\geq$ 95th percentile). Although FMD improved ($P < 0.05$), the improvement in C-IMT did not reach statistical significance. The changes in BMI, waist circumference, fat mass, ox-LDL, malondialdehyde (MDA), CRP, insulin, and homeostasis model assessment for insulin resistance (HOMA-IR) had an inverse correlation with the changes in mean FMD after adjustment for age and sex, with the highest correlations documented for ox-LDL, CRP, and WC. The age- and sex-adjusted changes in ox-LDL, waist circumference, CRP, MDA, and body fat mass had the highest correlations with changes in C-IMT.

CONCLUSIONS: Our findings suggest that a common inflammatory stress condition associated with childhood obesity, notably with abdominal fat deposition, may play a role in the development of the earliest stages of proatherosclerotic inflammatory processes and subsequent vascular dysfunction. These changes might be partially reversible by short-term diet and exercise intervention, even if patients do not reach ideal body weight.

The prevalence of childhood obesity and its consequences are increasing rapidly in low- and middle-income countries *(1)*. It is well documented that childhood obesity tracks into adulthood. Of special concern are the cardiometabolic consequences of obesity, especially hypertension, dyslipidemia, and impaired glucose metabolism, that are already present in young children of different ethnicities *(2, 3)*. It is now established that the origins of the metabolic syndrome trace back to early life *(4)*. Underlying genetic tendency or early-life adverse events may contribute to the metabolic syndrome and its related complications, notably in non-European populations *(5)*. In Iranian children, we found that based on criteria analogous to the Adult Treatment Panel III *(6)*, the metabolic syndrome was present in 14.1% of children 6 to 18 years old *(7)*.

Inflammation associated with childhood obesity appears central to the development of insulin resistance and atherosclerosis and may be important in the pathogenesis of other comorbid conditions *(8)*. We have previously documented an increase in serum C-reactive protein (CRP)[5] and oxidative stress markers in children with abdominal obesity, suggesting that ox-

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[5] Nonstandard abbreviations: CRP, C-reactive protein; FMD, flow-mediated dilation; C-IMT, carotid intima-media thickness; BMI, body mass index; MDA, malondialdehyde; HOMA-IR, homeostasis model assessment of insulin resistance; IMT, intima-media thickness.

idative stress and CRP may be associated with the early inflammatory processes of atherosclerosis *(7, 9)*.

The proinflammatory state of obese children is accompanied by changes in the flow-mediated dilation (FMD) of the brachial artery, which shows early stages of endothelial dysfunction *(10)*. Longitudinal studies have shown that childhood obesity is associated with carotid intima-media thickness (C-IMT) in adulthood *(11)*.

Programs that attempt to modify lifestyle have been the mainstay of controlling childhood obesity and have proved to be effective in reducing the traditional and novel risk factors of atherosclerosis, as well as inflammatory markers, oxidative stress, and insulin resistance *(12)*. Such programs have also been successful in amelioration of obesity-related vascular changes among children *(13, 14)*.

In the current study, we evaluated the association of changes in obesity-related oxidative and proinflammatory states with vascular function among obese children participating in a lifestyle modification program consisting of diet and exercise components.

Materials and Methods

STUDY DESIGN AND PARTICIPANTS

This study was conducted in 2005–2006 among 35 children (19 boys and 16 girls) selected randomly from among 79 children who were referred in December 2005 from healthcare centers and schools to the Obesity and Metabolic Syndrome Research Clinic of the Preventive Pediatric Cardiology Department, Isfahan Cardiovascular Research Center (ICRC), a collaborating center of the WHO.

The study was conducted according to the Declaration of Helsinki and was approved by Ethics Committee of ICRC (NIH code FWA 0000t8578). After providing detailed oral information to children and parents, we obtained written informed consent from the parents of all eligible study participants, and participants were then randomly allocated to participate in the current study or to get standard care at the obesity clinic. The allocation was conducted by computer-generated random numbers using the children's record numbers in our clinic.

Eligibility criteria for participation included age between 12 and 18 years and body mass index (BMI) equal to or higher than the age- and sex-specific 95th percentile according to the revised Centers for Disease Control and Prevention (CDC) growth charts *(15)*. Individuals with syndromal obesity, endocrine disorders, any physical disability, and/or history of chronic medication use or smoking were excluded from the survey. None of the participants had a history or symptoms of infection during the 2 weeks before blood sampling for the study.

ANTHROPOMETRIC MEASUREMENT AND CLINICAL EXAMINATION

The same team of pediatricians, general physicians, and nurses examined all study participants. Using calibrated instruments following standard protocol *(16)*, we made anthropometric measurements including weight, height, and waist and hip circumferences and calculated BMI and BMI SD score on the basis of CDC growth charts *(15)*. We measured blood pressure using a mercury sphygmomanometers under standard protocol; readings at the 1st and the 5th Korotkoff phase were taken as systolic and diastolic blood pressure, respectively. The mean of the 2 blood pressure measurements was recorded and included in the analysis *(17)*.

LABORATORY METHODS

We instructed participants to fast for 12 h before blood collection and determined compliance with fasting by interview on the morning of examination. Blood samples were taken from the antecubital vein between 0800 and 0900, without the use of anticoagulant.

Fasting venous blood samples were centrifuged for 10 min at 906*g* within 30 min of collection. Sera were analyzed in the central laboratory at Isfahan Cardiovascular Research Center, which meets the standards of the National Reference Laboratory (WHO-Collaborating Center) and is under the quality control of the CDC and the Department of Epidemiology, St. Rafael University, Leuven, Belgium. Fasting blood sugar (FBS), total cholesterol, HDL cholesterol, and triglycerides were measured enzymatically (Pars Azmoun) on Elan2000 autoanalyzers (Eppendorf). HDL was measured after dextran sulfate-magnesium chloride precipitation of non-HDL. LDL cholesterol was calculated in serum samples with triglycerides $<$4.52 mmol/L, according to the Friedewald equation *(18)*.

We have previously described the methods used for measurement of CRP and stress oxidants, i.e., malondialdehyde (MDA) and conjugated diene (CDE) *(10)*. In brief, we measured CRP with the same autoanalyzer by use of assays (Pars Azmoun). The interassay imprecision reflected by CV was 1.0% and 1.5%, respectively, at a concentration of 1 mg/L. We measured the concentration of ox-LDL in plasma with a sandwich ELISA procedure using the murine monoclonal antibody mAb-4E6 as capture antibody (bound to microtitration wells) and a peroxidase-conjugated antiapolipoprotein B antibody recognizing ox-LDL bound to the solid phase (Mercodia AB). The detection limit was $<$1 mU/L; the intraassay and interassay CVs were 4.0% and 4.2%, respectively.

We measured plasma insulin by RIA (Linco Research) that was 100% specific for human insulin with $<$0.2% cross-reactivity with human proinsulin and no cross-reactivity with C-peptide or insulin-like growth

factor. The reagent set had an accuracy of 93% to 100%, and the intraassay and interassay imprecision values were 2.2% to 4.4% and 2.9% to 6.0%, respectively. The linearity of dilution was 86% to 108%.

We calculated insulin resistance on the basis of 2 indices, the homeostasis model assessment of IR [HOMA-IR, (fasting insulin in mU/L × fasting glucose in mmol/L)/22.5] *(19)* and quantitative insulin sensitivity check index [QUICKI, 1/(log fasting insulin in mU/L + log fasting glucose in mg/dL)] *(20)*.

Because no universally accepted definition of the metabolic syndrome exists for children, we used a definition similar to that used by de Ferranti et al. *(6)*, which is defined as 3 or more of the following: fasting triglycerides ≥1.1 mmol/L, HDL <1.3 mmol/L (except in boys age 15–19 years, for whom the cutoff was <1.17 mmol/L), waist circumference >75th percentile for age and sex in the population studied *(21)*; systolic/diastolic blood pressure >90th percentile for sex, age, and height from the National Heart, Lung, and Blood Institute recommended cutoff point *(17)*; and FBS >5.6 mmol/L. It should be noted that de Ferranti et al. *(6)* used the cutoff of FBS >6.1 mmol/L, but as in our previous studies *(8, 10)*, we used the most recent recommendation of the American Diabetes Association, a cutoff value of 5.6 mmol/L *(21, 22)*.

ARTERIAL REACTIVITY AND INTIMA-MEDIA THICKNESS STUDIES

The same cardiologist conducted the studies for measurement of brachial arterial reactivity and intima-media thickness (IMT) for the 2 time points in the individual participants. Studies were conducted in a quiet environment, and no significant changes were observed in participant heart rate and blood pressure.

Using methods previously described, we measured the diameter of the brachial artery from high-resolution B-mode ultrasound images (ATL 5000 system, L10–5 transducer) at rest. Basal brachial dimension was measured 30 and 90 s after cuff deflation in response to reactive hyperemia. Endothelium-dependent dilation, or FMD, was measured at rest and 3 to 4 min after sublingual nitroglycerin (400 μg) given to produce endothelium-independent dilation *(14, 23)*.

We measured C-IMT with high-resolution carotid ultrasonography as described *(24, 25)*. The protocol involved scanning of the far wall of the right and left common carotid arteries in the distal 1.0 cm. The crest at the origin of the bifurcation was used as an anatomical landmark to identify the segment to be visualized. In each exam, the cardiologist used different scanning angles (anterior and lateroposterior) to record the greatest IMT. On a longitudinal B-mode image, the far wall of the common carotid artery appears as 2 bright, parallel lines separated by a hypoechoic space. The inner line arises from the lumen–intima interface, whereas the outer line arises from the media–adventitia interface. The distance between the lumen–intima and media–adventitia interfaces represented the IMT. For the purpose of measurement, the cardiologist selected (for each side) the 3 frames that contained the thickest IMT in the whole distal 1.0 cm of the common carotid artery. We averaged the C-IMT measurements to give the mean common carotid IMT for each side.

The CVs of intra- and interobserver variability were 4.7% and 5.2%, respectively, for brachial diameter and 5.2% and 6.1% for C-IMT.

INTERVENTIONAL PROGRAM

Participants performed supervised aerobic physical activity of moderate to vigorous intensity for 60 min, 3 days a week for 6 consecutive weeks. Each week, a 15-min didactic session to inform participants of the importance of being active was held before the beginning of the exercise. The 60-min sessions of physical training included 30 min of fitness-oriented activities and 30 min of playing games and running.

In addition, participants received dietary advice from a registered dietitian. The recommended diet was based on the optimized mixed diet, containing 30% energy derived from fat, 15% from protein, and 55% from carbohydrate, with energy content based on the calorie requirement for height *(26)*. Participants were asked to use unrefined carbohydrate, dietary fiber primarily in the form of high-fiber whole grains (5 servings per day), vegetables (3 servings per day), fruits (2 servings per day), protein-based foods (2 to 3 servings per day), and low-fat dairy foods (2 to 3 servings per day). Families were advised not to use hydrogenated fat, which is the most commonly used fat in our community. Participants were asked to record their daily food intake, and these records were verified by a nutritionist.

Physical and biochemical examinations, as well as sonographic studies, were repeated at the end of the study. This program was free of charge and all participants completed the study.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS for Windows (version 13:00). Descriptive data are expressed as mean (SD). We verified gaussian distribution of variables with a Kolmogorov-Smirnov test and performed statistical analysis of triglycerides, CRP, HOMA-IR, and QUICKI using log-transformed values because the distribution was skewed. We used paired *t* tests or Mann-Whitney *U* tests to analyze the changes from baseline to end-point measurements and linear regression analysis to assess the influence of baseline anthropometric and biochemical parameters on mean flow-mediated dilation 90 s after cuff deflation

Table 1. Clinical, metabolic and vascular variables before and after a lifestyle modification trial on obese children.

Parameter	Baseline	After Intervention	P value
Weight, kg	57.1 (10.2)	54.7 (9.8)	0.02
BMI, kg/m^2	25.3 (4.06)	24.1 (3.9)	0.04
BMI SD score	2.37 (0.04)	2.31 (0.05)	0.02
Waist circumference, cm	81.4 (4.7)	79.2 (4.1)	0.001
Waist-to-hip ratio	0.91 (0.1)	0.86 (0.2)	0.04
Subcutaneous fat, mm	17.2 (1.5)	16.8 (1.4)	NS
Body fat mass, %	29.7 (2.2)	28.1 (3.1)	0.04
Total cholesterol, mmol/L	4.8 (0.4)	3.7 (0.5)	0.04
LDL cholesterol, mmol/L	3.1 (0.5)	2.7 (0.4)	0.03
HDL cholesterol, mmol/L	1.02 (0.05)	1.1 (0.06)	NS
Triglycerides, mmol/L	1.7 (0.2)	1.4 (0.1)	0.02
FBS, mmol/L	4.6 (0.8)	4.5 (0.7)	NS
Insulin, mU/L	24.8 (3.4)	22.1 (3.5)	0.03
HOMA-IR	5.4 (0.8)	4.2 (0.9)	0.03
QUICKI	0.3 (0.01)	0.3 (0.02)	NS
CRP, mg/L	1.3 (0.02)	1.01 (0.02)	0.04
Oxidized LDL, U/L	73.2 (18.1)	69.9 (17.5)	0.02
MDA, μmol/L	0.9 (0.01)	0.7 (0.02)	0.04
Conjugated diene, μmol/L	2.6 (0.8)	1.8 (0.6)	0.04
Systolic blood pressure, mmHg	128.4 (27.1)	121.5 (27.2)	NS
Diastolic blood pressure, mmHg	75.2 (18.4)	77.1 (20.2)	NS
Brachial artery diameter, mm	3.3 (0.3)	3.4 (0.3)	0.005
FMD90	3.3 (0.3)	3.4 (0.3)	0.005
C-IMT, mm	0.34 (0.05)	0.32 (0.06)	NS

Data are mean (SD). FMD90, flow-mediated dilation 90 s after cuff deflation.

(FMD90) and C-IMT changes, as well as for the association between changes in mean FMD90 and C-IMT and changes in other clinical and biochemical parameters.

Results

The mean (SD) age of participants was 14.1 (2.2) years. Table 1 presents the changes in anthropometric and metabolic parameters of obese adolescents before and after undergoing the interventional program. Because we did not find significant difference between boys and girls in variables studied, the results are presented for the whole population studied. After lifestyle modification, body weight, BMI, BMI SD score, waist circumference, and percentage body fat were decreased; however, participants remained overweight (BMI $\geq$95th percentile). Other than HDL, all serum lipids and fasting insulin and measures of insulin resistance improved, and serum CRP, ox-LDL, and MDA decreased. At the end of the study, FMD improved significantly ($P < 0.005$); although C-IMT improved, the change did not reach statistical significance. At the end of the study, the prevalence of children classified as having the metabolic syndrome decreased from 34.2% to 14.2% ($P = 0.02$).

Baseline BMI, WC, fat mass, LDL, insulin, HOMA-IR, CRP, ox-LDL, and oxidative stress markers showed an inverse association with changes in mean FMD and a direct association with changes in mean C-IMT (Table 2).

After adjustment for age and sex, changes in BMI, WC, fat mass, ox-LDL, MDA, CRP, insulin, and HOMA-IR had an inverse correlation with the changes in mean FMD, with the highest correlations documented for ox-LDL, CRP, and WC. The age and sex-adjusted changes in ox-LDL, WC, CRP, MDA, and body fat mass had the highest correlations with changes in C-IMT (see Supplemental Data).

Table 2. Multiple linear regression analyses with changes in FMD of the brachial artery and C-IMT as dependent variables and baseline characteristics as independent variables.

	Standardized regression coefficient	
	FMD	C-IMT
BMI, kg/m^2	−0.31[a]	0.29[a]
Waist circumference, cm	−0.52[b]	0.41[b]
Subcutaneous fat, mm	−0.15	0.21
Body fat mass, %	−0.46[b]	0.42[b]
Total cholesterol, mmol/L	−0.22	0.24
LDL cholesterol, mmol/L	−0.34[a]	0.37[a]
HDL cholesterol, mmol/L	0.29	−0.22
Triglycerides, mmol/L	−0.21	0.24
FBS, mmol/L	−0.18	0.21
Insulin, mU/L	−0.31[a]	0.35[a]
HOMA-IR	−0.28[a]	0.27[a]
QUICKI	0.32[a]	−0.29[a]
CRP, mg/L	−0.41[b]	0.42[a]
Oxidized LDL, U/L	−0.52[b]	0.49[b]
MDA, μmol/L	−0.38[a]	0.31[a]
Conjugated diene, μmol/L	−0.32[a]	0.34[a]
Systolic blood pressure, mmHg	−0.21	0.25
Diastolic blood pressure, mmHg	−0.22	0.24

Age and sex-adjusted regression analysis. [a] $P < 0.05$, [b] $P < 0.0001$.

Discussion

This study demonstrates that increased ox-LDL and oxidative stress markers and proinflammatory state are associated with vascular function of childhood obesity. Even if ideal body weight was not reached, these changes were partially reversible by a short program of lifestyle modification that included diet and exercise.

Oxidative stress plays a key role in the development and progression of atherosclerosis and increases in childhood obesity *(27)*. Studies in adults have shown reductions in plasma markers of oxidative stress after weight loss induced by diet *(28, 29)*, pharmacologic therapy *(30)*, and surgery *(31)*. Such data are limited among children. Although no previous study has assessed the changes in ox-LDL concentrations after weight loss in obese children, a study of prepubertal severely obese children (n = 13) documented that serum MDA concentration decreased after a 6-month dietary restriction weight loss program *(32)*. Our study showed a decrease in ox-LDL and MDA; this finding confirms that the obesity-related oxidative status is reversible with a short-term weight loss program in children. Furthermore, markers of oxidant status normalized with weight loss, although all of the children remained overweight at the end of the study.

Compelling evidence indicates that atherosclerosis is an inflammatory process *(33)*. Oxidative stress may be a determinant of CRP concentrations and may promote the proatherosclerotic inflammatory process *(34)*. We have previously demonstrated a correlation between CRP and oxidative stress markers (MDA, CDE) in healthy children and adolescents, as well as between CRP, MDA, and CDE with abdominal obesity, but not with generalized obesity *(10)*. In the current study, CRP and oxidative stress markers decreased after weight loss. Previous studies demonstrated that dietary modification improves endothelial function and affects biomarkers of oxidative stress and inflammation in hyperlipidemic children *(35)*. These findings confirm that lifestyle modification, notably dietary intervention, may be effective in decreasing the obesity-related oxidative stress and proinflammatory state during childhood.

The endothelium is directly involved in the development and progression of chronic diseases and is suggested to be a biological determinant of disease prevention and health-promotion strategies *(36)*. Severe obesity in children is associated with arterial-wall stiffness and endothelial dysfunction, and might have a role in early events in the genesis of atheroma *(37)*. In our study, conduit vessel FMD, a validated surrogate measure of endothelial function and early atherosclerosis, was impaired in obese adolescents but improved as a result of a short-term diet and exercise intervention. This finding is in line with some previous studies in showing that obesity-related vascular dysfunction in children is partially reversible with short-term lifestyle modification *(14, 38)*.

We did not document any significant regression of carotid IMT, which was studied as a noninvasive marker for early atherosclerotic changes. This result may be attributable to the brevity of the intervention period in our study; other similar trials demonstrated that lifestyle modification, notably regular exercise, improves carotid IMT after 6 *(14, 39)* to 12 *(14, 40)* months.

This study has some potential limitations. First, all FMD and C-IMT measurements were performed by only 1 investigator; therefore, the reproducibility of these measurements by multiple investigators must be studied for clinical applicability. Second, we could not perform insulin-clamp studies, the best method to assess insulin resistance; however, previous studies documented that the HOMA model correlates with clamp studies and is an acceptable clinical alternative. Third,

FMD and C-IMT in adolescents are potentially influenced by other variables such as homocysteine and fibrinogen, which were not examined in this study. In addition, we did not compare our findings with a control group, and did not examine adipokines such as adiponectin and leptin.

In conclusion, our findings revealed that ox-LDL, CRP, and oxidative stress markers, as well as measures of insulin resistance, influenced the changes in obesity-related vascular function in children. In addition, our findings underscore the potential role of diet and exercise in reversing the interrelated enhanced oxidative and proinflammatory state and impaired vascular function in their early stages. These observations emphasize the importance of population-wide strategies to use lifestyle modification to decrease adiposity in children. Such approaches are particularly important for developing countries facing an epidemic of chronic diseases in the near future and therefore warrant further validation.

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