CLINICAL STUDY

Increased carotid intima-media thickness in pre-pubertal children with constitutional leanness and severe obesity: the speculative role of insulin sensitivity, oxidant status, and chronic inflammation

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Abstract

Design: In order to characterize whether different degrees of adipose tissue storage may be associated with markers of early atherosclerosis, we evaluated oxidant–antioxidant status and inflammatory markers and determined carotid intima-media thickness (cIMT) in healthy constitutional lean and obese pre-pubertal children.

Methods: Eighty healthy pre-pubertal lean and obese children were recruited and compared with 40 age, gender, and pubertal stage-matched normal controls. Anthropometric measurements, oxidant (urinary isoprostanes (PGF-2 α), lag phase, and malondialdehyde (MDA)) and antioxidant status (vitamin E), inflammatory markers (high sensitive C-reactive protein (hs-CRP)), and insulin sensitivity (fasting glucose–insulin ratio, homeostasis model assessment of insulin resistance (HOMA-IR)) were investigated. Furthermore, cIMT was measured by high-resolution ultrasound.

Results: hs-CRP was not different between lean and control subjects ($P=0.45$), while higher values were found in obese compared with lean and control children ($P<0.001$ and $P<0.001$ respectively). PGF-2 α and MDA were higher while lag phase shorter in lean and obese subjects compared with controls (lean $P<0.001$; $P<0.001$; $P<0.001$ and obese $P<0.001$; $P<0.001$; $P<0.001$ respectively), while no differences were documented between lean and obese subjects ($P=0.78$, $P=0.019$, and $P=0.53$ respectively). Compared with controls, cIMT was increased in lean and in obese subjects ($P=0.001$; $P=0.004$), while no differences were documented between obese and lean subjects ($P=0.1$). In a multiple stepwise linear regression analysis, cIMT was related with PGF-2 α ($\beta=0.641$, $P<0.001$) and HOMA-IR ($\beta=0.307$; $P<0.001$).

Conclusions: Pre-pubertal lean and obese children present increased oxidative stress and impaired inflammation and insulin sensitivity, which in turn seem to result in similar impaired endothelial dysfunction and early signs of atherosclerosis, already in childhood.

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Introduction

During the past decades, several studies have established an important role of adipose tissue on the development of cardiovascular diseases (1–4). A number of epidemiological studies have demonstrated an increased morbidity and mortality for cardiovascular disease that appears to be associated with extremely obese as well as lean subjects (1–4). Subjects with body mass index (BMI) of 22.2 kg/m² have the lowest morbidity compared with those who have both higher and lower values suggesting an important role of adipose tissue on cardiovascular events (2).

Adipose tissue has been considered a proper endocrine tissue able to synthesize several molecules, which induce impaired metabolic status or determine

important antiatherogenic, anti-inflammatory, and antidiabetic effects (5). The exposure during childhood to these metabolic alterations may induce changes in the arteries contributing to the development of atherosclerosis in adulthood (6). High-resolution B-mode ultrasound measurements of the carotid intima-media thickness (cIMT) are a feasible, direct, and non-invasive method able to evaluate and detect preclinical atherosclerosis. Studies in childhood showed significant increased cIMT in obese children (7–9), while, to the best of our knowledge, no studies have been performed in lean children.

There are substantial evidences that obesity in childhood lays the metabolic groundwork for adult cardiovascular disease (6, 10–12) where the obese related increase in adipose tissue appears to be the first

step in promoting the initial abnormalities in glucose metabolism and in the development of insulin resistance (IR). The induction of IR appears to sustain an increase in different inflammatory markers (13) associated with impaired oxidative stress, which in turn seem to play the pivotal role in the early stages of atherogenesis, including impairment of endothelial functions and the formation of fatty streaks and plaques (9, 14, 15). Vice versa in lean adult subjects, similar pro-atherosclerotic alterations consisting of increased IR and impaired oxidant/antioxidant status have been demonstrated (16–20). As this was also associated with impaired endothelial function (20), it might be argued that the lack of adipose tissue represents a trigger for the initiation of an atherosclerotic process by inducing peripheral IR, which seems to be secondary to different mechanisms (16–19) such as aberrant storage of triglycerides (TGs) in other organs (i.e. liver and muscles); increased free fatty acid flux leading to altered glucose uptake by the skeletal muscle and increased hepatic glucose output; as well as impaired adipocytokines production (especially leptin and adiponectin).

The present study aimed to verify whether constitutional lean pre-pubertal children present impaired oxidant/antioxidant status, IR, and chronic inflammation resulting in differences in cIMT compared with obese and control subjects.

Materials and methods

Study population

Forty lean pre-pubertal children who had been referred to the Endocrine Clinic of the Department of Paediatrics, University of Chieti, Italy, were recruited according to BMI values. Only subjects with BMI lower than the 2 SDS for the mean age and gender and with constitutional leanness were eligible, while those with secondary leanness were excluded (defined by thyroid dysfunction, celiac disease, chronic diarrhoea, GH/insulin-like growth factor-1 axis alteration, and anorexia). Furthermore, 40 obese pre-pubertal children were recruited according to BMI values. All obese subjects were affected by severe essential obesity (BMI higher than 2 SDS, for the mean age and gender) (21). Eligible obese and lean subjects were appropriate for gestational age at birth and did not have other chronic diseases (diabetes mellitus, celiac disease, thyroid dysfunction, endocrine disorders, hereditary disease, or systemic or bowel inflammatory disease, and malabsorption) as well as not taking any medication. Subjects with severe dyslipidemia (TGs > 600 mg/dl or cholesterol > 300 mg/dl) and ingestion of vitamin E during the previous 4 months were excluded. As a control group, we recruited 40 healthy pre-pubertal children comparable for age, gender, and pubertal stage with a BMI between -2 and $+2$ SDS, who were admitted to the

Department of Paediatrics of the University of Chieti for minor diseases. Blood and urinary sample, anthropometric and instrumental measurements were taken only after complete recovery of those diseases.

A complete physical examination was performed, including anthropometric parameters (height and weight), and staging of puberty on the basis of the breast development in girls and genital development in boys according to the Tanner's criteria. We analyzed also fatness indices (BMI, BMI-SDS, waist and hip circumference (WC and HC), waist-to-hip ratio (WHR), and skin-fold thicknesses) and basal blood pressure.

Venous fasting blood samples to evaluate inflammatory markers (high sensitive C-reactive protein (hs-CRP)), lipid profile (total cholesterol, low density lipoprotein (LDL)-cholesterol, and TGs) and plasma glucose and insulin. Homeostasis model assessment of IR (HOMA-IR) and fasting glucose–insulin ratio (G/I) were calculated as markers of insulin sensitivity.

Urinary isoprostanes (PGF-2 α) as well as lag phase and malondialdehyde (MDA) were analyzed as markers of oxidative stress, while plasma vitamin E content was evaluated in order to characterize the antioxidant status. Furthermore, both right and left cIMT was measured by a high B-mode ultrasonography.

This study was approved by Ethical Committee of University of Chieti. Written informed consent was obtained from all parents and oral consent from all children.

Anthropometric measurements

Body weight was determined to the nearest 0.1 kg, and height was measured in triplicate with a Harpenden stadiometer to the nearest 0.1 cm.

As fatness indices, we used BMI (the weight in kilograms divided by the square of the height in meters), and the BMI-SDS for age and gender was calculated (22). The distribution of fat mass was valued with WHR (WC was measured at its smallest point between iliac crest and rib cage while HC at its largest width over the greater trochanters). Additionally, we used fat mass %, estimated from four skin-fold thickness (made over the triceps and biceps, at subscapular and abdominal, of the left side of the body) with a Holtain plicometer according to Brook's equation, skin-fold measurements being more feasible in small children. Skin-fold thicknesses were measured as previously described (23).

Laboratory procedures

Biochemical analysis Fasting plasma glucose level was determined by using the glucose oxidase method, and plasma insulin was measured with two-site immunoassay (AIA-PACK IRI; Tosoh, Tokyo, Japan). For insulin, the limit of detection was 0.5 μ U/ml with intra- and interassay coefficients of

variation <7% for quality control. We used the following indices for determination of IR: baseline G/I; HOMA-IR calculated with the formula: fasting insulin (mU/l) $\times$ fasting glucose (mmol/l)/22.5.

Lipid analysis Fasting serum total cholesterol and TGs concentrations were determined by colorimetric enzymatic method. LDL-cholesterol was measured by a commercially available enzymatic reagent (CHOD-PAP, MPR1, Boeringer Mannheim).

LDL isolation and oxidation

Fasting venous blood was taken from subjects and placed in tubes containing EDTA (2.7 mmol/l), and plasma was immediately separated by centrifugation. The LDL fraction was isolated from freshly drawn plasma as previously described (24). To protect LDL against oxidative modification during isolation, EDTA (2.7 mmol/l) was added to density solutions. Oxidation of LDL (fresh preparations at a concentration of 0.2 mg LDL cholesterol/ml) was triggered by the addition of 5 μ mol/l CuSO₄ in PBS, pH 7.4, at 37 °C and continuously monitored spectrophotometrically at 234 nm to evaluate the formation of conjugated dienes. Oxidation of LDL was calculated as the measurement of the duration of the phase before the maximum oxidation. The oxidation curve is characterized by the lag phase, the propagation phase, and the decomposition phase (25). In particular, lag phase is the time required by the reaction to gain the maximum velocity of $V_{\max}$ during propagation phase (25). Lag phase and propagation rate were calculated as previously described (25).

Peroxidation of LDL

LDL peroxidation was evaluated spectrophotometrically by the measurement of the MDA using the thiobarbituric acid-reacting substance assay as previously described (26). Results were expressed as MDA (nmol)/LDL protein (mg) (26).

Urinary isoprostanes

Multiple aliquot of early morning urine samples was added with the antioxidant 4-hydroxy-tempo (Sigma Chemical Co.) and stored at -80°C . PGF-2 α were evaluated in triplicate by immunoenzymatic methods (ELISA, Oxford Biomedical Research, Enzyme Immunoassay for urinary isoprostane) (27).

Vitamin E determination

Plasma vitamin E, expressed in μ mol/l, was measured in duplicate with HPLC using a Kontrol System 450 (Milan, Italy) equipped with an u.v.-visible spectrophotometer (Kontrol Detector 430) at different wavelengths. Procedures were as previously reported (28).

High sensitive C-reactive protein

Multiple plasma sample aliquots were collected and stored at -80°C until analysis. hs-CRP was measured by latex-enhanced nephelometry (N High Sensitivity CRP assay) on a BN nephelometer (Dade Behring Inc, Marburg, Germany). The lower limit of detection of this assay was 0.1 mg/l (29).

Instrumental procedures

Blood pressure The blood pressure was measured in children by one investigator using a validation protocol. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured twice according to the previously reported guideline. Hypertension was defined as blood pressure values above the 95th percentile for height, age, and gender (30).

Carotid ultrasonography High-resolution B-mode ultrasonography of the right and left carotid artery was performed with a linear 14 MHz transducer (Philips Sonos). The subjects were examined in the supine position with the head turned slightly to the left and right. The common, internal, and external carotid arteries were identified by combined B-mode and color-doppler ultrasound examinations. A careful search was performed to obtain an optimal visualization of the vessel wall demonstrating the typical double lines representing the intima-media layer. Intima-media thickness was defined as the distance between the leading edge interface of the far wall and the leading edge of the median adventitia interface of the far wall with an automated edge-tracking method, as previously described (31). Three determinations of the right and the left carotid artery were performed and these three determinations were averaged; furthermore, the right and the left carotid artery measurements were averaged and used for analysis (mean cIMT). During the ultrasound, scanning images were digitally frozen and printed. The measurements were performed by the same operator who was blinded to the study design. For quality control assessment, 10% of the subjects were re-examined and these repeated measurements gave a mean difference of 0.01 ± 0.01 mm and a coefficient of variation of 1%.

Statistical analysis

All values were expressed as mean and s.d. Differences in gender variable were analyzed by χ^2 test. Differences between the three groups were tested by one-way ANOVA with Bonferroni's test for *post hoc* analysis. The statistical significance level was $P < 0.05$. Furthermore, adjustment for potential confounding factors (age, gender, BMI-SDS, blood pressure, and LDL-cholesterol) was performed using analysis of covariance.

Within lean subjects, to investigate the independent effect of IR and inflammatory and oxidative stress status on the increasing cIMT, a correlation between these parameters was detected by multiple stepwise linear regression analysis (PGF-2 α , hs-CRP, HOMA-IR, age, gender, BMI-SDS, SBP, and LDL-cholesterol). The statistical significance level was $P < 0.05$.

All calculations were made with the computer program SPSS (Statistical Package for the Social Science Inc, Chicago, United States), version 14.0 software for Windows.

Results

Forty pre-pubertal constitutional lean children and 40 obese children were recruited and compared with 40 healthy subjects similar for age, gender, and

pubertal stage. The mean clinical characteristic and levels of biochemical parameters are summarized in Table 1.

Anthropometric measurements

Significant differences were documented among the three groups according to BMI, BMI-SDS, skin-fold thickness (biceps, triceps, subscapular, and abdominal), fat mass, fat mass %, WC, HC, and WHR (Table 1). In detail, compared with controls and obese subjects, lean children had significantly lower BMI ($P < 0.001$ and $P < 0.001$ respectively), BMI-SDS values ($P = 0.005$ and $P = 0.001$ respectively), skin-fold thickness, and fat mass % (versus controls: biceps $P = 0.011$; triceps $P < 0.001$; abdominal $P < 0.001$; subscapular $P < 0.001$; fat mass % $P < 0.001$ respectively; and versus obese biceps

Table 1 Baseline clinical characteristics and levels of biochemical parameters of lean, obese, and normal control pre-pubertal children.

	Lean	Normal controls	Obese	P ^a
Auxology				
Number	40	40	40	
Age (years)	8.7 $\pm$ 1.5	8.1 $\pm$ 1.1	8.5 $\pm$ 1.7	NS
Gender (M/F)	22M/18F	24M/16F	19M/21F	0.1 ^b
Weight (kg)	22.7 $\pm$ 6.1	25.7 $\pm$ 7.4	45.6 $\pm$ 14.4	<0.001* [†] ‡
BMI (kg/m ²)	13.5 $\pm$ 0.9	16.5 $\pm$ 0.8	25.2 $\pm$ 4.7	<0.001* [†] ‡
BMI-SDS	-2.17 $\pm$ 0.08	0.54 $\pm$ 0.9	5.9 $\pm$ 2.0	<0.001* [†] ‡
Fat mass %	13.6 $\pm$ 4.1	20.69 $\pm$ 7.7	41.5 $\pm$ 7.9	<0.001* [†] ‡
Skin-fold thicknesses bicipital	8.0 $\pm$ 2.4	9.2 $\pm$ 2.8	19.68 $\pm$ 6.9	<0.001* [†] ‡
Skin-fold thicknesses scapular	4.38 $\pm$ 1.5	6.67 $\pm$ 1.9	21.75 $\pm$ 9.3	<0.001* [†] ‡
Skin-fold thicknesses triceps	5.24 $\pm$ 1.9	9.83 $\pm$ 2.9	25.34 $\pm$ 6.1	<0.001* [†] ‡
Skin-fold thicknesses abdominal	4.6 $\pm$ 1.1	7.04 $\pm$ 2.9	20.8 $\pm$ 8.3	<0.001* [†] ‡
WC (cm)	50.0 $\pm$ 5.8	54.8 $\pm$ 5.7	77.2 $\pm$ 13.4	<0.001* [†] ‡
HC (cm)	59.8 $\pm$ 5.8	63.4 $\pm$ 8.5	83.1 $\pm$ 10.8	<0.001 [†] ‡
WHR	0.84 $\pm$ 0.04	0.86 $\pm$ 0.04	0.92 $\pm$ 0.05	<0.001 [†] ‡
SBP (mmHg)	102 $\pm$ 11	97 $\pm$ 9	109 $\pm$ 12	<0.001 [†]
DBP (mmHg)	64 $\pm$ 13	62 $\pm$ 7	67 $\pm$ 6	<0.001 [†]
cIMT				
Right cIMT (mm)	0.37 $\pm$ 0.01	0.31 $\pm$ 0.04	0.40 $\pm$ 0.06	<0.001* [†]
Left cIMT (mm)	0.38 $\pm$ 0.01	0.31 $\pm$ 0.06	0.40 $\pm$ 0.06	<0.001* [†]
Mean cIMT (mm)	0.37 $\pm$ 0.01	0.31 $\pm$ 0.06	0.40 $\pm$ 0.06	<0.001* [†]
Lipid profile				
Total cholesterol (mg/dl)	169 $\pm$ 25	159 $\pm$ 23	169 $\pm$ 19	NS
LDL-cholesterol (mg/dl)	98 $\pm$ 20	96 $\pm$ 10	102 $\pm$ 11	NS
TG (mg/dl)	83 $\pm$ 40	70 $\pm$ 27	101 $\pm$ 54	NS
IR				
Fasting insulin (μ U/ml)	8.9 $\pm$ 5.3	5.3 $\pm$ 2.0	10.9 $\pm$ 6.6	0.001* [†]
Fasting glycemia (mg/dl)	80.6 $\pm$ 7.9	78.3 $\pm$ 8.3	86.5 $\pm$ 8.1	0.002 [†] ‡
C-peptide (mg/dl)	0.86 $\pm$ 0.36	0.86 $\pm$ 0.68	1.42 $\pm$ 0.72	<0.001* [†]
HOMA-IR	1.82 $\pm$ 1.1	1.05 $\pm$ 0.4	2.36 $\pm$ 1.4	<0.001* [†]
G/I	13.3 $\pm$ 8.3	17.04 $\pm$ 7.9	11.8 $\pm$ 7.8	0.003* [†]
Oxidant status				
hs-CRP (mg/l)	0.24 $\pm$ 0.3	0.42 $\pm$ 0.2	2.6 $\pm$ 1.3	$P < 0.001$ [†] ‡
PGF-2 α (ng/ml)	7.0 $\pm$ 2.6	2.5 $\pm$ 2.0	6.9 $\pm$ 3.2	$P < 0.001$ * [†] ‡
Lag phase (min)	33.4 $\pm$ 7.5	47.9 $\pm$ 6.9	27.5 $\pm$ 16.6	$P < 0.001$ * [†] ‡
MDA (nmol/mg)	0.69 $\pm$ 0.15	0.54 $\pm$ 0.18	0.79 $\pm$ 0.22	$P < 0.001$ * [†] ‡
Vit. E (μ mol/l)	36.2 $\pm$ 10.5	36.3 $\pm$ 7.3	35.9 $\pm$ 8.7	NS

*Significant differences by *post hoc* analysis ($P < 0.05$) (lean versus controls). [†]Significant differences by *post hoc* analysis ($P < 0.05$; lean versus obese).

[‡]Significant differences by *post hoc* analysis ($P < 0.05$) (obese versus controls). NS, no significant difference ($P > 0.05$). Values are mean $\pm$ s.d. SDS, s.d. score; BMI, body mass index; WC, waist circumference; HC, hip circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; cIMT, carotid intima-media thickness; TG, triglyceride; HOMA-IR, homeostasis model assessment of insulin resistance; G/I, fasting glucose-insulin ratio; hs-CRP, high sensitivity c-reactive protein; PGF-2 α , urinary isoprostanes; MDA, malondialdehyde; Vit E, plasma vitamin E.

^aOne-way ANOVA.

^b χ^2 test.

$P < 0.011$; triceps $P < 0.001$; abdominal $P < 0.001$; subscapular $P < 0.001$; fat mass % $P < 0.001$ respectively), while these parameters were significantly higher in obese than controls ($P = 0.003$ and $P = 0.005$, biceps, triceps, abdominal, subscapular, and fat mass % = $P < 0.001$ respectively). In addition, WC, HC, and WHR were significantly lower in lean children compared with obese ($P < 0.001$, $P < 0.001$, and $P < 0.001$ respectively) and higher in obese compared with controls ($P < 0.001$, $P < 0.001$, and $P < 0.001$ respectively). Compared with controls, WC was significantly lower in lean subjects ($P = 0.001$), while HC and WHR were similar ($P = 0.13$ and $P = 0.07$ respectively).

SBP and DBP results were statistically different among the three groups (Table 1). In detail, no significant differences were documented for SBP and DBP in lean children compared with controls ($P = 0.03$ and $P = 0.47$ respectively) and obese ($P = 0.08$ and $P = 0.37$ respectively), while obese children showed higher SBP and DBP compared with controls ($P < 0.001$ and $P < 0.001$).

Lipid profile

No difference was found in terms of total cholesterol, LDL-cholesterol, and TG, among the three groups (Table 1).

Oxidant-antioxidant status

Significant differences were documented among the three groups according to hs-CRP, PGF-2 α , lag phase, and MDA (Table 1), while vitamin E levels were similar. In detail, hs-CRP was higher in obese children compared with lean and control children ($P < 0.001$ and $P < 0.001$ respectively), while no differences were found between lean children compared with controls ($P = 0.45$). Furthermore, PGF-2 α and MDA were higher while lag phase was shorter in lean and obese subjects compared with controls (lean $P < 0.001$; $P < 0.001$; $P < 0.001$ and obese $P < 0.001$; $P < 0.001$; $P < 0.001$ respectively), whereas no differences were documented between lean and obese subjects ($P = 0.78$, $P = 0.019$, and $P = 0.53$ respectively).

Insulin-resistance indices

Fasting insulin, fasting glycemia, HOMA-IR, and G/I results were significantly different among the three groups (Table 1). Lean and obese subjects had higher fasting insulin, fasting glycemia, HOMA-IR (lean $P = 0.002$; $P = 0.013$; $P = 0.011$ and obese $P < 0.001$; $P < 0.001$, $P < 0.001$ respectively), and lower G/I values compared with controls ($P = 0.011$, and $P = 0.001$ respectively); while no differences were documented between lean and obese ($P = 0.19$; $P = 0.019$; $P = 0.08$; $P = 0.35$ respectively).

cIMT

Significant differences were documented in left, right, and mean cIMT among the three groups (Table 1). In details compared with controls, the mean value of the left and the right carotid artery was higher in lean and obese children (mean cIMT $P < 0.001$ and $P < 0.001$ respectively), while no significant differences were found between lean and obese (mean cIMT $P = 0.1$). Across the whole population, the mean value of the left and the right carotid artery showed a characteristic J-curve distribution with higher values in the lower and higher part of the BMI (Fig. 1). After adjustment for gender, age, BMI-SDS, SBP, and LDL-cholesterol, the difference in mean cIMT in both lean and obese children remains statistically significant compared with the control group ($P < 0.05$ for trend).

Multiple regression analysis

In order to investigate the independent effect of IR and oxidative stress status on cIMT, a multiple stepwise regression analysis was performed. The mean cIMT was significantly related with PGF-2 α ($\beta = 0.639$, $P < 0.0001$) and HOMA-IR ($\beta = 0.397$; $P < 0.001$), whereas there were no correlations between cIMT and age, gender, BMI-SDS, SBP, LDL-cholesterol, and hs-CRP.

Discussion

This is the first study of a comparable negative effect of both lower and higher adipose tissue storage on carotid arterial wall in constitutional lean and severe obese pre-pubertal children.

Functional and morphological alterations of the vascular wall are universally accepted as an early and

Figure 1 Distribution of the mean value of the left and the right carotid intima-media thickness (mean cIMT) across the lean, control, and obese pre-pubertal children.

pivotal step in the development of atherosclerosis, and the detected increased cIMT could reflect these structural changes (32–34). Previous reports have indicated that cIMT is associated with coronary artery diseases and cardiovascular morbidity and mortality (32–34). Studies in childhood showed significantly increased cIMT in children with hypertension (35), type 1 diabetes (36), familial hypercholesterolemia (37), and severe obese children (9), while no data on increased cIMT in lean children are reported. Furthermore, in line with previous studies on a similar impaired degree of endothelial function in both obese and lean young adults (20), we have demonstrated for the first time a significant increase of cIMT in lean pre-pubertal children compared with controls. Moreover, although both lean and obese children showed significantly higher cIMT compared with controls, no differences were found between the two groups. The documented increase in cIMT in our population is important as it concerns only healthy pre-pubertal children helping to avoid any pubertal-related effects that could influence the precocious impairments of the arterial wall (38). Furthermore, we have excluded other factors such as hypertension, hypercholesterolemia, and smoking, which are well known to induce early alterations of the arterial wall. However, a systematic longitudinal follow-up of these pre-pubertal children is necessary in order to confirm unequivocally the role of the revealed differences of cIMT on cardiovascular disease in adulthood.

Adipose tissue accumulation in patients with generalized or regional obesity and markedly adipose tissue deficiency in patients with genetic or acquired lipodystrophy are both associated with IR and its complications (e.g. type 2 diabetes, hypertriglyceridemia, low levels of high-density lipoprotein cholesterol, and hepatic steatosis) (39–41). In this study, we have demonstrated an impaired insulin sensitivity in both obese and lean pre-pubertal children compared with controls, while no difference was detected between lean and obese subjects, underlying a similar effect of increased and decreased adipose tissue reserve on changes in IR. Although relevant, the underlying mechanisms by which the lack of adipose tissue causes IR remain not completely elucidated and several hypotheses have been proposed (16–19, 39–41). First, patients with lipodystrophies are lacking sufficient adipocytes, and, therefore, the aberrant lipid flux results in a variant storage of fat in non-lipodystrophic tissues, such as the liver and skeletal muscles interfering with insulin responsiveness (at the liver and skeletal muscle) or with altered glucose-stimulated insulin secretion (in pancreatic β -cell) (17). Another hypothesis relates to a possible reduced glucose uptake by skeletal muscles and increased hepatic glucose output due to increased FFA flux in patients with partial lipodystrophies (18, 19). A third hypothesis pertains to the role of adipocytokines, the secretory products of adipose tissue, in causing IR

(18, 19). For example, patients with generalized lipodystrophies have deficiencies of leptin and adiponectin, which may be related to severe IR (19). However, as in this study no information on adipokines or ultrasound determination of aberrant storage TGs in other organs was evaluated, further longitudinal studies are needed in order to offer more information on the underlying relevant mechanisms.

Several studies have clearly demonstrated a tight association between adiposity-related increased IR, oxidative stress, and inflammation in adults as well as in children (9, 11, 32). Impaired insulin sensitivity has continuously been considered to be an important promoting factor of atherosclerosis (11, 42) through chronic inflammation and impaired oxidative stress, two well-known mechanisms directly involved on the development of endothelial dysfunction in both obese adults and children (9, 14, 29, 43). Especially, PGF-2 α can induce vasoconstriction in many vascular beds, promote platelet aggregation, and support proliferation of vascular smooth muscle cells, and they have been considered an important proatherogenic factor on the arterial wall (44–47). We have demonstrated for the first time that lean pre-pubertal children present an impaired oxidant/antioxidant status compared with controls. In fact, lean children showed a significant increase in oxidative markers compared with age, gender, and pubertal stage-matched controls. However, although both lean and obese children showed significantly impaired oxidative status compared with controls, no differences were found between the two groups. In fact, in lean pre-pubertal children, oxidative markers (urinary PGF-2 α , lag phase, MDA, and vitamin E) were similar to those detected in obese children demonstrating similar effects of extremely low or high adipose tissue storage. Impaired insulin sensitivity as well as oxidative stress/inflammatory status has been demonstrated to be directly related to early arterial changes in obese children and adults (9, 11, 32, 42). Similar to those observed in obese subjects, by inducing IR and impaired oxidative status, adipose tissue deficiency in lean children appears to influence changes in the vascular wall. In fact, by multiple stepwise linear regression analysis, we documented an independent effect of IR and oxidative stress status on the increasing cIMT within lean and obese subjects. In fact, cIMT was tightly associated with HOMA-IR, PGF-2 α , and lag phase. According to these results, our data suggest that impaired insulin sensitivity and altered oxidant–antioxidant status, related to adipose tissue depletion, represent key elements for the development of early abnormalities in the arterial wall not only in obese subjects but also in lean pre-pubertal children. These findings represent the first report on the relationship between low adipose tissue reserve and cIMT. Our data appear to be supported by previous studies that reported a J-curve phenomenon between prevalence of disease, including cardiovascular complications, and BMI

suggesting a direct role of adiposity on several diseases including cardiovascular events (1–4). Therefore, obesity as well as leanness appears to similarly induce impaired IR, oxidative stress, and chronic inflammation, which in turn induce endothelial dysfunction leading to cIMT, an early atherosclerotic change in the arterial wall that is universally accepted as a cardiovascular risk factor. However, as this study is a cross-sectional study, further data from a longitudinal study or intervention study are needed in order to confirm this observation.

In conclusion, pre-pubertal lean and obese children present increased oxidative stress and impaired inflammation and insulin sensitivity, which in turn seem to result in a similar impaired endothelial dysfunction and early sign of atherosclerosis. Therefore, impaired adipocyte stores determine an unbalanced endothelial regulation resulting in an increased risk of cardiovascular complications. A complete clarification of the adipose tissue-related endocrine effects represents an important action on the developing of new targets for therapeutic approach in order to prevent and restore the adiposity-related impaired status, early in childhood.

Declaration of interest

All authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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