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Cardiovascular dysfunction and metabolic impairment in pediatric obesity.

Markers of damage and effects of a behavioral intervention

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SUMMARY

Background. Childhood obesity represents a major health concern worldwide due to its well established detrimental effect on cardiovascular (CV) and its negative impact on premature morbidity and mortality.

Aim. To study the effects of pediatric obesity on CV system and to find clinical markers related to CV dysfunction and metabolic impairment, in order to precociously identify children at higher risk of an “unhealthy” metabolic profile and early complications. To investigate the reversibility of CV damage through a healthy-lifestyle program.

Methods. Complete anthropometrical, biochemical and CV assessment has been performed in large cohorts of obese children and adolescents before and after a 12-months behavioral program based on an isocaloric Mediterranen balanced diet plus an exercise traing regimen.

Results. We demonstrated that obesity is associated with an abnormal CV response during childhood and that these alterations are related to the presence of insulin resistance, metabolic syndrome and hyperuricemia. The second study reported a worsened metabolic profile in obese children with an estimated glomerular filtration rate (eGFR) >1 SD or with high serum uric acid levels. Finally, we showed that a healthy-lifestyle program could reverse CV disfunction in children and this effect is related with weight loss and the improvement of metabolic risk and sistemic blood pressure.

Conclusions. CV damage begins precociously, during pediatric age. Our studies provides proof that some clinical markers, like insulin resistance, eGFR and serum uric acid are helpful during risk stratification and should be implemented in clinical practice to identify children at higher risk of obesity-related complications. Behavioral interventions could be an effective strategy to prevent childhood obesity and to reverse its detrimental effects.

RIASSUNTO

Introduzione. A livello globale, l'obesità pediatrica rappresenta uno dei maggiori problemi di salute pubblica a causa dei suoi ben noti effetti sul sistema cardiovascolare (CV) e del suo impatto negativo su morbilità e mortalità precoci.

Scopo. Scopo di questo lavoro è stato valutare gli effetti dell'obesità pediatrica sul sistema CV e identificare marcatori clinici correlati al danno CV e all'alterazione metabolica, al fine di individuare precocemente i bambini a maggior rischio di un profilo metabolico sfavorevole e di sviluppare complicanze precoci. È stata inoltre indagata la reversibilità della disfunzione CV attraverso un programma di cambiamento dello stile di vita.

Metodi. Una valutazione atropometrica, biochimica e CV completa è stata eseguita in ampie coorti di bambini e adolescenti obesi prima e dopo 12 mesi di un trattamento comportamentale basato su una dieta mediterranea isocalorica e su un programma di esercizio fisico.

Risultati. Abbiamo dimostrato che l'obesità è associata ad un'anomala risposta CV, anche in età pediatrica e che tali alterazioni sono correlate alla presenza di insulino-resistenza, sindrome metabolica e iperuricemia. Il secondo studio riporta la presenza di un profilo metabolico peggiore nei bambini obesi con un tasso presunto di filtrazione glomerulare (eGFR) >1 DS e con elevati livelli sierici di acido urico. Infine, abbiamo dimostrato che il cambiamento dello stile di vita, attraverso un programma comportamentale, può migliorare la disfunzione CV in età pediatrica e che tale effetto è correlato con il calo ponderale e con il miglioramento del quadro metabolico e della pressione arteriosa sistemica.

Conclusioni. Il danno CV inizia precocemente, durante l'età infantile. Questi studi forniscono un'evidenza che alcuni marcatori clinici come l'insulino-resistenza, l'eGFR e l'acido urico sierico, sono utili per stratificare il rischio e la loro valutazione dovrebbe essere implementata nella pratica clinica al fine di identificare più precocemente possibile

quei bambini ad alto rischio di sviluppare complicanze obesità-correlate. La terapia comportamentale e il cambiamento dello stile di vita potrebbero essere strategie efficaci per prevenire l'obesità pediatrica e trattare i suoi effetti avversi.

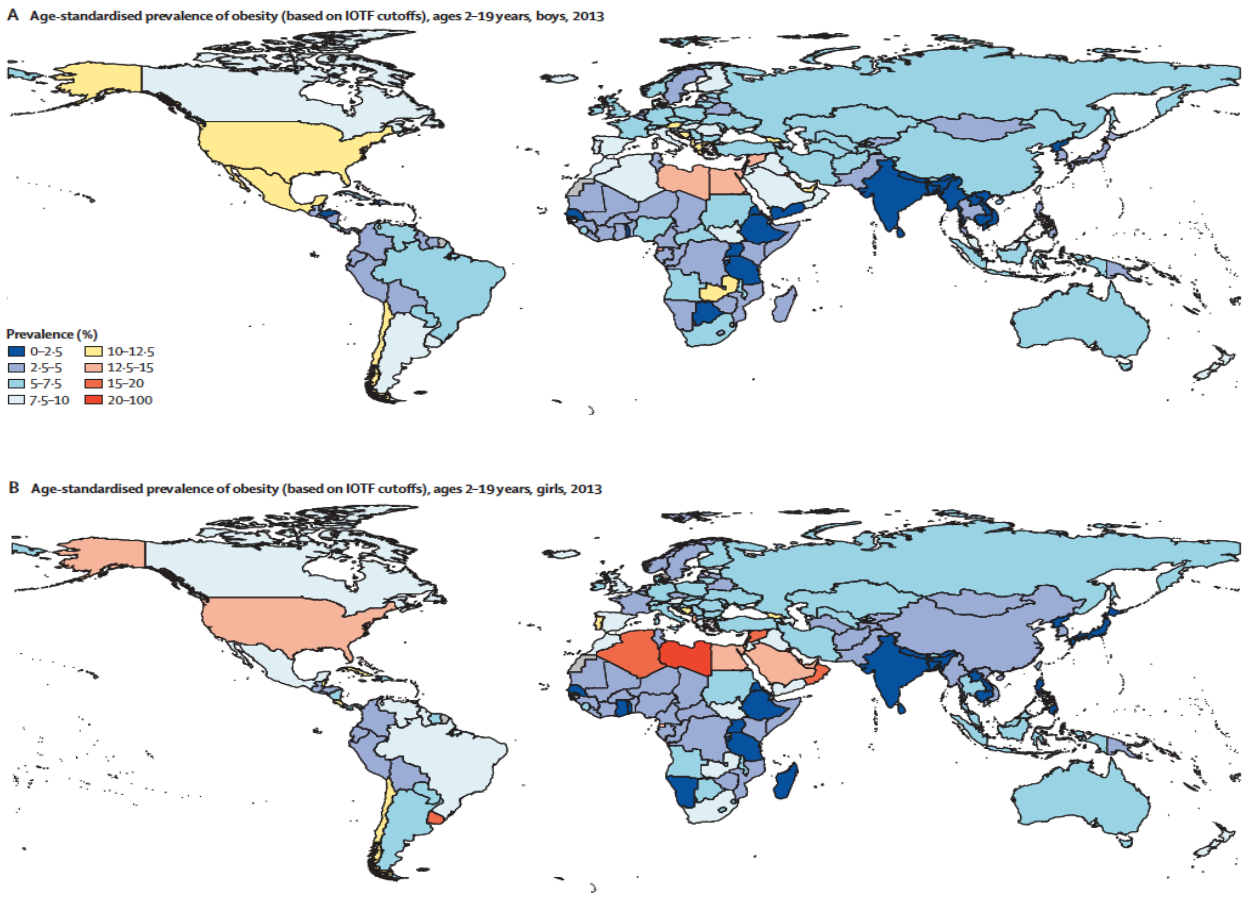
CONTENTS

Chapter 1

General introduction and rationale

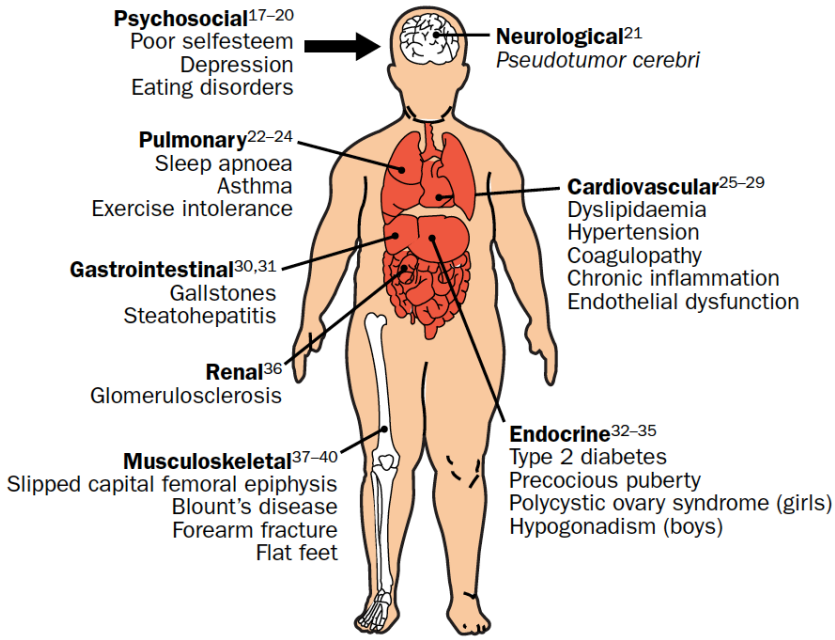
During the past three decades, the prevalence of pediatric obesity has risen worldwide. The definitions of overweight and obesity in children differ between epidemiological studies, making comparisons of cross-sectional prevalence data difficult. Nevertheless, several studies have examined change in prevalence within populations over time, and the results of these analyses are astounding. In western countries, the combined prevalence of childhood overweight and obesity is 47.1% and rates have increased from 16.5% in 1980 to 23.2% in 2013 [1]. In Europe, the prevalence of overweight and obesity between school age children is 12.8% and 7%, respectively [2] and the International Obesity Task Force (IOTF) estimates that by 2020 the prevalence of these conditions will be greater than 35.0% [3].

Figure 1. Global prevalence of obesity in children and adolescents [1].



Childhood obesity is a multisystem disease and causes a wide range of serious complications, increasing the risk of premature morbidity and mortality and raising public-health concerns [4]. In addition, obese children are more prone to become obese adults, with an enhanced risk of obesity-related complications [5].

Figure 2. Complications of pediatric obesity [4].



A cluster of cardiovascular disease risk factors as hypertension, dyslipidaemia, chronic inflammation, increased blood clotting tendency, endothelial dysfunction, and hyperinsulinaemia has been identified in children as young as 5 years of age [6]. Among adolescents and young adults, the presence of cardiovascular disease risk factors correlates with asymptomatic coronary atherosclerosis, and lesions were more advanced in obese subjects [7-9]. Furthermore, in an Israeli cohort including 2.3 million adolescents, the obese group showed a hazard ratio of 3.5 for death from total cardiovascular causes [10]. Moreover, recent studies reported that pediatric obesity is often associated with current cardiovascular abnormalities and there is evolving evidence that clinical indicators

of atherosclerosis such as carotid artery intima-media thickness (CIMT), arterial stiffness and endothelial dysfunction are impaired in children with obesity [11-16]. Furthermore, childhood obesity besides increasing traditional cardiometabolic risk factors, can seriously hamper kidney function and renal dysfunction may start early during childhood, long before a diagnosis of hypertension and type 2 diabetes can be made, and it might be related to pediatric obesity [17].

However, mechanisms by which these effects are mediated have not been fully understood, suggesting that this issue requires immediate attention to prevent progressive cardiovascular and kidney damage. Therefore, the identification of precocious biomarkers and the detection of a “high risk” profile associated with obesity during childhood would allow the early diagnosis of complications and the institution of preventive and therapeutic measures that might reduce morbidity and mortality at long term follow up.

Aim of the thesis

The purpose of this thesis is to analyze the development of obesity-related complications in obese children and adolescents, to detect precocious biomarkers that could help the clinician identifying “high risk” subjects and to assess the potential effect of a healthy-lifestyle intervention reverting cardiovascular dysfunction during pediatric age.

To this purpose, the studies reported in the following chapters were conducted.

The study presented in **Chapter 2** demonstrates that obesity and metabolic syndrome are associated with abnormal cardiovascular response during childhood. This study shows that hyperuricemia can be an early marker of cardiovascular dysfunction suggesting that the routine determination of circulating levels of serum uric acid should be implemented during risk stratification among pediatric age.

In **Chapter 3**, the association of biomarkers of obesity-related renal disease and cardiometabolic risk factors is discussed. This study shows a worsened metabolic profile in

obese children with normal estimated glomerular filtration rate (eGFR) higher than 1 standard deviation or with abnormally high serum uric acid levels suggesting that eGFR and serum uric acid may be helpful in clinical practice to identify an unhealthy metabolic profile in pediatric obesity.

The **Chapter 4** reports the results of a perspective behavioral intervention on cardiovascular response in obese children and adolescents. This study demonstrates that pediatric cardiovascular dysfunction could be partially reversed by a healthy-lifestyle program, the cornerstone of childhood obesity treatment, via weight loss and the improvement of metabolic risk and sistemic blood pressure.

Studies presented in Chapters 2 and 3 have been published in international journals.

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Chapter 2

This study demonstrates that obesity and metabolic syndrome are associated with abnormal cardiovascular response during childhood. Furthermore, hyperuricemia can be an early marker of cardiovascular dysfunction suggesting that the routine determination of circulating levels of serum uric acid should be implemented during risk stratification among pediatric age.

Insulin resistance, serum uric acid and metabolic syndrome are linked to cardiovascular dysfunction in pediatric obesity

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ABSTRACT

Introduction. Childhood obesity is associated with cardiovascular abnormalities but little is known on the potential correlation between early cardiovascular and metabolic alterations.

Aim. Aims of this study were 1) to evaluate early cardiovascular abnormalities in a large population of obese children and adolescents compared with a normal weight counterpart, 2) to investigate their potential association with insulin resistance (IR), serum uric acid (sUA) and metabolic syndrome (MetS).

Methods. This was a single-center case-control study. Eighty obese (OB) subjects (6–16 years) and 20 normal weight (NW) matched controlswere consecutively recruited. In the whole population we performed an anthropometric and a cardiovascular assessment. OB patients also underwent an OGTT and biochemical evaluations.

Results. OB children showed greater left atrial (LA) and ventricular (LV) dimensions and mass and higher carotid artery intima-media thickness (CIMT), compared with NW controls. TheBMI z-score,waist circumference, IR and sUA were positively related with LA and LV dimensions and mass. OB subjects with MetS (46.3%) showed greater LA diameter ($p = 0.001$) and LV area ($p = 0.01$) and volume ($p = 0.04$) compared with OB children without MetS. LA diameter and LV dimensions and mass were significantly dependent on the number of criteria for MetS. Mets, sUA and IR were significant predictors of left heart dimensions and mass in obese children.

Conclusions. Obesity and MetS are associated with abnormal cardiovascular response during childhood. Hyperuricemia can be an earlymarker of cardiovascular dysfunction and the routine determination of circulating levels of sUA should be implemented during risk stratification among pediatric age.

INTRODUCTION

Childhood obesity causes a wide range of severe complications, increasing the risk of premature morbidity and mortality [1]. In addition, obese children are more prone to become obese adults, with higher risk of cardiovascular diseases (CVD) [2]. A cluster of CVD risk factors has been identified in children as young as 5 years of age [3] and, among adolescents, their presence correlates with asymptomatic coronary atherosclerosis [4]. Pediatric obesity has been related to an impaired cardiac structure and function as left atrial (LA) and left ventricular (LV) dimensions and mass significantly greater than lean controls [5–8] and impaired diastolic and systolic function [6,9].

Atherogenesis and arterial wall damage begin during childhood and, there is evolving evidence that clinical indicators of atherosclerosis such as carotid artery intima-media thickness (CIMT), arterial stiffness, and endothelial function are altered in obese children [10,11].

Hyperuricemia has been recognized as a risk factor for CVD in adults being predictive of insulin resistance (IR), metabolic syndrome (MetS) and all its components [12]. However, little is known on the potential association between early cardiovascular alterations and hyperuricemia and MetS in obese children.

The detection of a high cardiovascular risk profile associated with pediatric obesity and the identification of possible early markers of cardiovascular damage during childhood would let an early diagnosis of cardiovascular complications allowing prompt preventive therapeutic measures that might reduce cardiovascular morbidity and mortality at long term follow up.

Therefore, aims of this study were 1) to evaluate early cardiovascular abnormalities in obese children and adolescents compared with a normal weight counterpart, 2) to investigate their potential association with IR, serum uric acid (sUA) and MetS.

SUBJECTS AND METHODS

Study design and population

This was a single-center longitudinal case-control study conducted at Division of Pediatrics in Piedmont Region, Italy. We consecutively enrolled 80 Caucasian obese (OB) children and adolescents, aged 6–16 years, and 20 normal weight, age and sex matched controls (NW). OB patients were eligible if they were generally healthy, obese according to the International Obesity Task Force (IOTF) criteria [13], and not on a weight-loss diet. Exclusion criteria were specific causes of endocrine or genetic obesity, type 1 or type 2 diabetes, previous heart, respiratory, liver and kidney diseases, current or past use of hormonal or interfering therapies (lipid-lowering, hypoglycemic, or antihypertensive treatments). Control subjects were eligible if they were normal weight [13], with no history of organic or psychiatric diseases. The protocol was conducted in accordance with the declaration of Helsinki and was approved by the Local Ethic Committee (CE 95/12). Informed written consent was obtained from all subjects' parents.

Anthropometric and biochemical variables

Height, weight, waist circumference (WC), hip circumference (HC), systolic (SBP) and diastolic (DBP) blood pressure were measured as previously described [14] and bodymass index (BMI) was calculated. Pubertal stages were evaluated according to Tanner criteria.

In the OB group, after a 12-h overnight fast, blood samples were taken for measurement of: glucose (mg/dL), insulin (μUI/mL), total cholesterol (mg/dL), high density lipoproteincholesterol (HDL-c, mg/dL), triglycerides (mg/dL), sUA (mg/dL), using standardized methods in the Hospital's Laboratory [14]. Low density lipoprotein-cholesterol (LDL-c) was calculated by the Friedwald formula. sUA (mg/dL) was measured by Fossati method reaction using uricase with a Trinder-like endpoint. Obese subjects also underwent

an OGTT (1.75 g of glucose solution per kg, maximum 75 g) and samples were drawn for the determination of glucose and insulin every 30 min. Insulin-resistance was calculated using the formula of homeostasis model assessment (HOMA)-IR. Insulin sensitivity at fasting and during OGTT was calculated as the formula of the Quantitative Insulin-Sensitivity Check Index (QUICKI) and Matsuda index (ISI) [28]. Glucose was expressed in mg/dL (1 mg/dL = 0.05551 mmol/L) and insulin in μUI/mL (1 μUI/mL = 7.175 pmol/L) in each formula [15]. Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) were defined according to American Diabetes Association [16] and MetS by using the modified National Cholesterol Education Program/Adult Treatment Panel III (NCEP-ATP III) criteria of Cruz and Goran [17].

Echocardiographic assessment

Transthoracic echocardiogram using a Vivid 7 Pro ultrasound scanner (General Electric Healthcare, USA) was performed by a sonographer and the images were reviewed by an expert pediatric cardiologist, blinded to patients' clinical data. Measurements of left ventricle (LV end-diastolic diameter, LVEDD; LV end-systolic diameter, LVESD; interventricular septum at end diastole, IVSD; LV posterior wall at end diastole, LVPWD), relative wall thickness (RWT), left atrium diameter (LAD), the maximum LA volume, and LV ejection fraction were obtained according to established standards [18]. LV mass (LVM) was derived from the Devereux formula and indexed to body surface area (left ventricular mass index [LVMI]) [27]. Using pulsed wave Doppler, mitral inflow velocities, peak early diastolic velocity (E), peak late diastolic velocity (A), E/A ratio, were measured. Pulsed wave tissue Doppler of the lateralmitral annulus was used for themeasurement of early peak diastolic mitral annular velocity (E'). The E/E' ratio was calculated.

Vascular assessment

Vascular measurements were performed with a high-resolution ultrasonography (Esaote MyLab25™Gold, Esaote, Italy) using a 8 mHz linear transducer and a 5 mHz convex transducer for the abdominal aorta, by an expert sonographer and images were then reviewed offline by an expert vascular surgeon blinded to patients' clinical status. CIMT, abdominal aortic diameter at maximum systolic expansion (Ds) and minimum diastolic expansion (Dd), brachial artery diameters, brachial artery peak systolic velocity (PSV) and end diastolic velocity (EDV) were measured as previously described and aortic strain (S), pressure strain elasticmodulus (Ep), pressure strain normalized by diastolic pressure (Ep*) and brachial artery flow-mediated dilation (FMD) were calculated. While S is the mean strain of the aortic wall, Ep and Ep* are the mean stiffness [19]. The brachial artery maximum diameter recorded following reactive hyperemia was reported as a percentage change of resting diameter (FMD = peak diameter – baseline diameter/baseline diameter) [20].

Statistical analysis

All data are expressed as mean ± standard deviation (SD), absolute values or percentages. A sample of 15 individuals has been estimated to be sufficient to demonstrate a difference of 10% in LV diameter with a SD of 0.44 cm with 90% power and a significance level of 95% in the Student t-test between obese and controls according to published data [6]. A cohort of 75 obese subjects has been estimated to be sufficient to demonstrate differences among numbers of MetS criteria (0–5 criteria according to NCEP-ATPIII classification) [17]. Distributions of continuous variables were examined for skewness and were logarithmically transformed as appropriate. ANCOVAwas used to determine the differences between obese and control subjects. Covariates were sex, age and pubertal stage. Correlation of cardiovascular parameters with continuous values of

BMI zscore, WC, HOMA-IR, ISI, and sUA were examined using Pearson correlation coefficients. Partial correlation was used to correct for covariates. The stepwise regression model with two-tailed probability values and 95% confidence intervals was used to measure the strength of the association between cardiovascular variables and BMI z-score and each specific MetS criteria (abdominal obesity, hypertension, hypertrygliceridemia, low HDL-c, glucose intolerance: model 1) and other metabolic impairments (Model 2: model 1 + sUA; Model 3: model 2 + HOMA-IR). Statistical significance was assumed at $p<0.05$. The statistical analysis was performed with SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Anthropometric and cardiovascular characteristics

Table 1 shows the auxological and cardiovascular data of the 80 OB (age 11.2 ± 2.7 yrs., 47.5% females, 36.3% prepubertal, BMI zscore 2.32 ± 0.51) and 20 age-, sex- and pubertal status-matched NW subjects (age 10.9 ± 2.5 yrs., 60.0% females, 45.0% prepubertal, BMI z-score−0.76±0.98). After adjusting for age, sex and puberty, OB children showed greater LV dimensions (LVEDD $p<0.02$, LVESD $p<0.02$, IVSD $p<0.0001$, LVPWD $p<0.001$), area ($p<0.0001$), volume ($p<0.002$) and LVM ($p<0.0001$), LAD ($p<0.005$) and lower E/A ratio ($p<0.005$) compared with NW subjects. No differences were found in the E/E' ratio, LV systolic function, LVMI and RWT. OB patients had larger abdominal aortic ($p<0.03$) and brachial artery ($p<0.006$) diameters compared with NW controls. CIMT was significantly higher in the OB group ($p<0.0001$) while no difference between the two groups was shown in aortic strain (S) and stiffness (Ep, Ep*) and in FMD of the brachial artery.

Table 1. Clinical and cardiovascular data of 80 obese (OB) and 20 normal weight (NW) subjects.

	OB	NW	p Value
Subjects	80	20	
Age (years)	11.2 ± 2.7	10.9 ± 2.5	ns
Female	38 (47.5%)	12 (60.0%)	ns
Prepubertal	29 (36.3%)	9 (45.0%)	ns
Height (cm)	149.6 ± 15.1	145.3 ± 15.3	**0.0001**
Height (SDS)	1.11 ± 1.21	0.65 ± 1.11	**0.03**
Weight (kg)	69.3 ± 22.6	36.5 ± 10.3	**0.0001**
BMI (kg/m^2)	30.0 ± 5.2	17.0 ± 2.4	**0.0001**
BMI z-score	2.32 ± 0.51	−0.76 ± 0.98	**0.0001**
Waist (cm)	90.2 ± 14.3	60.9 ± 6.6	**0.0001**
Hip (cm)	96.4 ± 15.0	70.4 ± 9.3	**0.0001**
N° WC ≥ 90° p	79 (98.7%)	4 (20.0%)	**0.001**
SBP (mm Hg)	123.7 ± 16.6	105.8 ± 16.1	**0.0001**
DBP (mm Hg)	78.2 ± 10.8	67.1 ± 8.8	**0.0001**
N° SBP or DBP ≥ 90° p	68 (78.7%)	6 (30%)	**0.001**
HR (b/min)	84.2 ± 12.1	78.1 ± 11.4	ns
EF (%)	69.5 ± 8.4	70.8 ± 6.1	ns
FS (%)	40.3 ± 6.7	40.2 ± 5.5	ns
LVEDD (mm)	44.5 ± 0.6	41.2 ± 0.5	**0.02**
LVESD (mm)	27.4 ± 0.5	24.6 ± 1.2	**0.02**
IVSD (mm)	7.6 ± 0.2	5.9 ± 0.4	**0.0001**
LVPWD (mm)	7.8 ± 0.2	6.1 ± 0.5	**0.001**
LAD (mm)	32.0 ± 0.6	27.8 ± 1.3	**0.005**
LV area (cm^2)	25.8 ± 0.6	19.9 ± 1.4	**0.0001**
LV volume (mL)	78.6 ± 3.0	56.3 ± 6.3	**0.002**
LA area (cm^2)	13.1 ± 3.7	12.1 ± 3.6	ns
LA volume (mL)	31.2 ± 12.4	29.4 ± 10.9	ns
LV mass (g)	113.3 ± 6.0	74.3 ± 13.4	**0.0001**
LV mass index (g/m^2)	66.9 ± 22.9	62.5 ± 24.2	ns
RWT	0.35 ± 0.08	0.31 ± 0.06	ns
Mitral E peak (cm/s)	102.0 ± 20.1	99.2 ± 10.3	ns
Mitral A peak (cm/s)	58.2 ± 14.1	50.1 ± 11.2	**0.03**
Mitral E/A ratio	1.8 ± 0.1	2.1 ± 0.1	**0.005**
Mitral lateral E' velocity (cm/s)	19.6 ± 3.2	19.2 ± 2.1	ns
Mitral lateral E/E' ratio	5.2 ± 0.9	5.1 ± 0.7	ns
CIMT (mm)	0.63 ± 0.02	0.46 ± 0.04	**0.0001**
AoDs (mm)	12.8 ± 0.2	11.4 ± 0.4	**0.006**
AoDd (mm)	10.3 ± 0.2	9.3 ± 0.4	**0.03**
S	0.23 ± 0.12	0.25 ± 0.14	ns
Ep (mm Hg)	459 ± 1170	302 ± 681	ns
Ep*	8.3 ± 24.1	4.0 ± 9.2	ns
BAD basal (mm)	3.5 ± 0.1	3.0 ± 0.1	**0.006**
BAD after (mm)	3.5 ± 0.1	3.2 ± 0.2	**0.04**
FMD (%)	3.5 ± 15.6	7.8 ± 13.6	ns
PSV basal (cm/s)	67.2 ± 26.0	71.3 ± 18.9	ns
EDV basal (cm/s)	11.3 ± 6.7	9.3 ± 7.6	ns
PSV immediately after (cm/s)	81.7 ± 27.6	89.2 ± 19.3	ns
EDV immediately after (cm/s)	25.1 ± 10.1	26.5 ± 8.7	ns
PSV after (cm/s)	72.4 ± 27.1	74.6 ± 17.1	ns
EDV afer (cm/s)	13.5 ± 8.0	11.3 ± 6.9	ns

Values are number (%) or means ± SD.

Legend: A, peak velocity of late diastolic transmitral wave; after, 2 min after the release of the pneumatic cuff; AoDd, abdominal aortic diastolic diameter; AoDs, abdominal aortic systolic diameter; BAD brachial artery diameter; BMI, body mass index; CIMT, carotid intima media thickness; DBP, diastolic blood pressure; E, peak velocity of early diastolic transmitral wave; E', peak early diastolic velocity on mitral annulus; EDV, brachial artery end-diastolic velocity; EF, LV ejection fraction; Ep, pressure strain elastic modulus; Ep*, pressure strain normalized for DBP; FMD, brachial artery flow-mediated dilation; FS, LV fractional shortening; HR, heart rate; immediately after, the release of the pneumatic cuff; IVSD, interventricular septum diastolic dimension; LA, left atrium; LAD, LA end-systolic diameter; LV, left ventricle; LVEDD, LV end-diastolic dimension, LVESD, LV end-systolic dimension; LVPW, LV posterior wall diastolic dimension; N°, number of subjects; ns, not significant; p, percentile; PSV, brachial artery peak systolic velocity; RWT, relative wall thickness; S, aortic strain; SBP, systolic blood pressure; SDS, standard deviation score; WC, waist circumference.

Associations between cardiovascular measurements and metabolic parameters

In the whole population, after adjusting for age, sex and pubertal status, both BMI z-score and WC were positively associated with LV dimensions (LVEDD, LVESD, IVSD, LVPWD), area and volume, LVM, LVM index, RWT, LAD, CIMT, abdominal aortic and brachial artery diameters and negatively relatedwith the E/A ratio. A negative relationship was found between BMI z-score and aortic stiffness.

After adjusting for age, sex and puberty, insulin-resistance at fasting was positively correlated with LV dimensions (LVEDD, IVSD, LVPWD), area and volume, LVM and LVMI, RWT, LAD, LA area and volume, abdominal aortic diameter and negatively with the E/A ratio while insulin sensitivity during OGTT was negatively related with IVSD, LVPWD, LVM, RWT, LAD and abdominal aortic diameter.

sUA was directly associated with LVEDD, LVESD, LVM, LV area and volume, brachial artery diameters and negatively with the E/A ratio, even after adjustment for confounding factors (Table S1).

Associations between cardiovascular measurements and SBP and DBP are shown in Table S2 in the supplementary appendix. As regards lipid profile, after adjustment for confounding factors, total cholesterol was positively correlated with LVPWD and RWT; HDL-c was negatively related with LAD; LDL-c was directly associated with CIMT and triglycerides were positively correlated with LAD. Total cholesterol, LDL-c and triglycerides were negatively related with the E/A ratio.

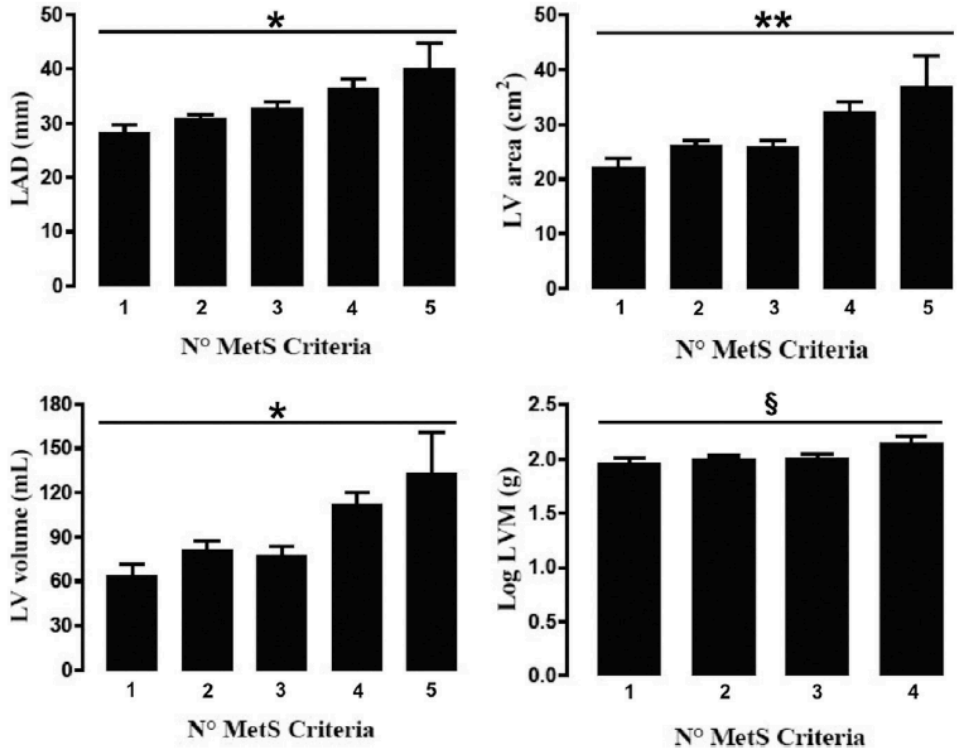
Impact of MetS and sUA

Out of OB children, 37 (46.3%) matched the NCEP ATPIII criteria for MetS. Patients with MetS had greater LAD (p=0.001) and LV area (p=0.01) and volume (p=0.04) compared with OB subjects without MetS.

LAD and LV area, volume and LVM were significantly dependent on the number of criteria of MetS. Increasing the number of matched criteria for MetS, LAD, LV area, volume and LVM significantly increased (Fig. 1). This correlation persisted after adjustment for HOMA-IR for LAD, LV area and volume while was lost for LVM.

Figure 1. MetS criteria-dependent left atrium diameter (LAD) (mm), left ventricle (LV) area (cm^2), LV volume (mL) and LV mass (LVM) (g) in 80 obese children and adolescents. Log transformation was used for skewed variables (LVM).

*p<0.001; ** p<0.0001; §p<0.04. Significance was maintained when the model included homoeostasis model assessment of insulin resistance (HOMA-IR) as covariates for LAD, LV area and volume while was lost for LVM.



Stepwise multiple regression analysis showed that BMI z-score, HDL-c ≤10th percentile and triglycerides ≥90th percentile were independent predictors of LAD (model 1 and 2). In model 3, only BMI z-score and HDL-c in addition to HOMA-IR were confirmed as significant predictors of increased LAD. LV area was predicted by BP ≥90th percentile and IGT (model 1). In model 2, only sUA and BP were significant predictors. In model 3, HOMA-IR and BP were found as significant predictors. LV volume was predicted by IGT and HDL-c ≤10th percentile (model 1). In model 2 and 3, sUA and HOMA-IR were the only significant predictors. Finally, LVM was predicted by BMI z-score and triglycerides ≥90th percentile. In model 2 and 3, BMI z-score, sUA, HOMA-IR and triglycerides were the only significant predictors (Table 2).

Table 2. Stepwise multiple regression analysis of BMI z-score, metabolic syndrome (MetS) components, serum uric acid (sUA) and insulin resistance (HOMA-IR) (as independent variables) on left atrium diameter (LAD), left ventricular (LV) area and volume and Log LV mass (dependent variables).

Dependent variable	Significant effects	B (95% CI)	β	p-Value
Model 1				
LAD (mm)	BMI z-score	2.286 (1.070;3.501)	0.375	<0.0001
	HDL-c ≤ 10th p	0.255 (0.036;0.473)	0.229	<0.02
	Triglycerides ≥ 90th p	0.278 (0.023;0.534)	0.218	<0.03
R:0.568				
Model 2 (sUA)				
LAD (mm)	BMI z-score	2.286 (1.070;3.501)	0.375	<0.0001
	HDL-c ≤ 10th p	0.255 (0.036;0.473)	0.229	<0.02
	Triglycerides ≥ 90th p	0.278 (0.023;0.534)	0.218	<0.03
R:0.568				
Model 3 (HOMA-IR)				
LAD (mm)	BMI z-score	2.081 (0.828;3.333)	0.341	<0.001
	HDL-c ≤ 10th p	0.275 (0.059;0.490)	0.247	<0.01
	HOMA-IR	0.042 (0.006;0.077)	0.240	<0.02
R:0.574				
Model 1				
LV area (cm^2)	BP ≥ 90th p	4.395 (1.137;7.653)	0.290	<0.009
	IGT	7.016 (1.548;12.484)	0.275	<0.01
R:0.428				
Model 2 (sUA)				
LV area (cm^2)	sUA	1.900 (0.928;2.873)	0.402	<0.0001
	BP ≥ 90th p	3.761 (0.636;6.886)	0.248	<0.02
R:0.513				
Model 3 (HOMA-IR)				
LV area (cm^2)	HOMA-IR	0.633 (0.207;1.060)	0.316	<0.004
	BP ≥ 90th p	4.150 (0.920;7.379)	0.273	<0.01
R:0.453				
Model 1				
LV volume (mL)	IGT	32.633 (5.271;59.995)	0.266	<0.02
	HDL-c ≤ 10th p	14.123 (0.140;28.105)	0.225	<0.05
R:0.365				
Model 2 (sUA)				
LV volume (mL)	sUA	10.284 (5.443;15.125)	0.449	<0.0001
R:0.449				
Model 3 (HOMA-IR)				
LV volume (mL)	sUA	8.177 (3.073;13.281)	0.357	<0.002
	HOMA-IR	2.370 (0.189;4.551)	0.242	<0.03
R:0.502				
Model 1				
Log LV mass (g)	BMI z-score	1.003 (0.615;1.390)	0.495	<0.0001
	Triglycerides ≥ 90th p	0.105 (0.023;0.187)	0.245	<0.01
R:0.598				
Model 2 (sUA)				
Log LV mass (g)	BMI z-score	0.765 (0.356;1.174)	0.378	<0.0001
	sUA	0.034 (0.009;0.058)	0.276	<0.007
	Triglycerides ≥ 90th p	0.100 (0.021;0.178)	0.233	<0.01
R:0.647				
Model 3 (HOMA-IR)				
Log LV mass (g)	BMI z-score	0.687 (0.279;1.095)	0.339	<0.001
	sUA	0.029 (0.004;0.053)	0.235	<0.02
	HOMA-IR	0.012 (0.001;0.023)	0.198	<0.04
	Triglycerides ≥ 90th p	0.080 (0.001;0.159)	0.186	<0.05
R:0.671				

Legend: BP, blood pressure; CI, confidence interval; HDL-c, HDL-cholesterol; IGT, impaired glucose tolerance; p, percentile.

Model 1: independent variables were BMI z-score and MetS components, (waist circumference ≥90th percentile; BP ≥90th percentile; triglycerides ≥90th percentile; HDL-c ≤10th percentile; impaired fasting glucose; impaired glucose tolerance).

Model 2: independent variables were those of Model 1 plus sUA.

Model 3: independent variables were those of Model 2 plus HOMA-IR.

DISCUSSION

In this study, we found that pediatric obesity negatively affected cardiac remodelling and impaired vascular structure. Furthermore, IR and sUA and the presence of MetS might play an additional adverse role during heart remodelling among children and adolescents.

Cardiovascular alterations in pediatric obesity

Our study showed marked variations in the LA and LV dimensions in OB children compared to NW. These findings are in line with some previous studies reporting that LA and LV dimensions are significantly greater in OB children compared to NW [6,8,10,21]. Several investigations have also reported greater LVM in OB children [5,6,8,10,22], and this has been detected as early as 2 years of age [22]. Interestingly, we found no differences when normalizing LVM to body surface area between the two groups [9,21] possibly related to the overcorrection of measurements (normalized for parameters including weight) required for the obese group. Moreover, hypertrophy and dilatation of the LV might represent a physiological response to the enhanced preload and afterload dependent to the increased BMI.

Regarding heart function, we found a significant reduction in the E/A ratio in the OB group (mainly related to the increased inflow velocities during atrial contraction/A-velocities) and

no significant changes in E'-velocities or in the E/E'-ratio with a preserved global LV systolic function. Despite the well-known association between diastolic dysfunction and subsequent impaired systolic functional changes in the adulthood, this correlation is still conflicting when applied in pediatric subjects.

The majority of studies, showed no difference between obese and normal weight children in the peak early mitral inflow velocity (E) and in the early diastolic mitral velocity assessed with PWTDI (E') as parameters for diastolic function [7,9,21]. According with our data, the lack of significant changes in early filling characteristics suggests an increased dependency of atrial filling related to a decreased LV early relaxation [9,21].

In line with this, we also found increased systolic and diastolic abdominal aortic and brachial artery diameters in the OB group, which might represent an early marker of vascular remodelling.

In fact, the assessment of CIMT is a sensitive clinical marker of atherosclerosis, predictive of CV morbidity and mortality in the adulthood and high-risk population. Interestingly, we found greater CIMT in obese patients, even in younger children, not related to the pubertal status and mainly due to the exposure to cardiovascular risk factors.

Potential mechanisms linking obesity to cardiovascular dysfunction

The excess of adipose tissue, enhanced metabolic activity and the subsequent increased preload, predispose to LA and LV dilatation, LV remodelling and hypertrophy as a compensatory mechanism for systolic and diastolicwall stress [23]. Obesity is associated with changes in the vascular system related to the development of early atherosclerosis, arterial hypertension and increase afterload. In this study the BMI z-score, waist circumference and systemic blood pressure are positively correlated with increasing CIMT. Surprisingly, we found an inverse relationship between the BMI z-score and arterial stiffness that might reflect an early compensatory mechanism depicted by the arteries that

try to contrast the afterload via structural changes (increased diameter and thickness) before the rise in stiffness.

According to previous data, in our obese population, the HOMA-IR correlates with increasing heart and aortic dimensions and decreasing diastolic function while no association was found between IR and CIMT and arterial stiffness [24]. IR can potentially induce to a decreased myocardial glucose uptake and increased fatty acid oxidation resulting in the accumulation of toxic intermediates of fatty acid metabolism that finally lead to myocardial dysfunction and arteries enlargement. Furthermore, compensatory hyperinsulinemia induces LV hypertrophy through binding of insulin to cardiac insulin-like growth factor 1 receptors [25].

In our OB group, MetS was associated with greater heart dimension and mass with straightforward linear raises when increasing the number of matched criteria for MetS. Subjects who met the MetS criteria, presented worst metabolic parameters (dyslipidemia, dysglycemia) with higher prevalence of hypertension and altered BMI with a significant impact on cardiac structural alterations. Our data highlight the influence ofMetS also in the pediatric subset, suggesting the usefulness of an early MetS investigation implemented with prompt cardiovascular imaging examination and aggressive therapeutic strategy in the obese children in order to prevent future cardiovascular dysfunction.

Another interesting finding of the current study is the association between sUA and cardiovascular adverse remodelling. The association between sUA and levels of individual MetS components and their clustering has been previously reported [25–27]. The Bogalusa Heart Study showed that elevated sUA plays a crucial role in the pathogenesis of MetS with an influence that begins during adolescence, suggesting that sUA may aid in the early identification and treatment of high risk individuals for MetS [27]. Indeed, in a group of 299 overweight/obese children aged 8–18 years from the STYJOBS/EDECTA cohort, sUA was the best predictor of unhealthy obesity [28]. Furthermore, recent

investigations, has suggested that sUA level is an independent predictor of hypertension in adulthood [12]. In addition, Viazzi et al. showed that sUA was directly related to SBP and DBP independently of puberty, gender, BMI z-score, HOMA and renal function in a cohort of children and adolescents [29]. These data were confirmed by Feig et al. that showed a correlation between the reduction of sUA and normalization of blood pressure in 66% of hyperuricemic adolescents with hypertension, as compared to 3% in controls [30]. Finally, a reduction in sUA improved body weight and related CVD risk factors in young patients with hyperuricemia [31]. Moreover, sUA is an independent risk factor for heath failure and is associated with increased cardiovascular mortality in adults and children [12]. Out of our knowledge, this is the first study that demonstrates an association between sUA and LV volume and mass that is independent of BMI z-score, MetS and insulin resistance in a large cohort of obese pediatric patients. Studies conducted in adult populations suggest that hyperuricemia is associated with cardiac remodelling and LV hypertrophy and that sUA could be a marker of subclinical myocardial dysfunction [32], but this data were not fully confirmed during childhood. Reschke et al. showed, in a population of 49 hypertensive children of which 21 overweight/obese and not evaluated for the presence of MetS, that 1 mg/dL increase in sUA over the baseline value was associated with an increase in LVM of 20.2 g. However, this association was no longer significant after adjustment for confounding factors [33]. Increased sUA levels may contribute to the echocardiographic abnormalities associated with obesity through effects on endothelial dysfunction and inflammation. Although uric acid seems to have antioxidant activity in the extracellular environment, once entered the cells, including vascular and heart muscle cells and adipocytes, might promote several detrimental effects. Injurious impacts of sUA include intracellular reactive oxygen species production, an inhibitory effect on nitric oxide formation, induction of platelet aggregation, and pro-inflammatory activity [12], also in children [34]. Furthermore, sUA activates the renin-angiotensin system causing

hypertension. The renin-angiotensin system, in turn, has been proposed to cause LV hypertrophy and cardiac fibrosis though mechanisms including BP increase, direct action of angiotensin II on cardiac myocytes, and effects of aldosterone [32]. As suggested by Borghi and Cicero, sUA requires more attention in the evaluation of the metabolic risk profile of obese children and adolescents [35] and, based on our findings, it could be an early marker of cardiovascular dysfunction in this population allowing a prompt and effective preventive measures.

Study limitations

Our study has several potential limitations. The major limit is the relatively small size of the population. Moreover, our study was not a randomized controlled trial. We perform a prospective data collection, which is certainly susceptible to selection bias. Finally a more extensive use of vascular imaging modalities including speckle tracking echocardiography and cardiac MRI would have certainly improved the results of the current study.

CONCLUSION

In conclusion, this study shows that obesity andMetS are associated with abnormal cardiovascular response during childhood. Moreover, hyperuricemia can be an early marker of cardiovascular dysfunction and the routine determination of circulating levels of sUA should be implemented during risk stratification among children and adolescents. Further larger studies and randomized trials are warranted to confirm our findings.

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SUPPLEMENTARY TABLES

Table S1. Partial correlation for body mass index (BMI) z-score, waist circumeference (WC, cm), homeostasis model assessment (HOMA-IR), Matsuda index (ISI) and serum uric acid (sUA, mg/dL) with cardiovascular measurements.

	BMI z-score		**WC** (cm)		**HOMA-IR**		**ISI**		**sUA** (mg/dL)	
	r	***P value***	**r**	***P value***	**r**	***P value***	**r**	***P value***	**r**	***P value***
HR (b/min)	0.085	ns	0.114	ns	0.174	ns	-0.137	ns	-0.060	ns
EF (%)	-0.115	ns	-0.105	ns	0.060	ns	0.016	ns	-0.045	ns
FS (%)	-0.104	ns	0.015	ns	0.058	ns	-0.102	ns	-0.070	ns
LVEDD (mm)	**0.218**	**0.05**	**0.355**	**0.001**	**0.266**	**0.02**	0.059	ns	**0.264**	**0.03**
LVESD (mm)	**0.247**	**0.02**	**0.322**	**0.002**	0.065	ns	0.052	ns	**0.270**	**0.02**
IVSD (mm)	**0.390**	**0.0001**	**0.420**	**0.0001**	**0.364**	**0.001**	**-0.291**	**0.02**	0.001	ns
LVPWD (mm)	**0.441**	**0.0001**	**0.556**	**0.0001**	**0.386**	**0.001**	**-0.275**	**0.02**	0.132	ns
LAD (mm)	**0.431**	**0.0001**	**0.451**	**0.0001**	**0.385**	**0.001**	**-0.385**	**0.001**	0.131	ns
LV area (cm^2)	**0.294**	**0.01**	**0.515**	**0.0001**	**0.366**	**0.001**	-0.023	ns	**0.269**	**0.02**
LV volume (mL)	**0.278**	**0.01**	**0.470**	**0.0001**	**0.379**	**0.001**	0.047	ns	**0.290**	**0.02**
LA area (cm^2)	0.175	ns	0.199	ns	**0.290**	**0.01**	-0.061	ns	-0.050	ns
LA volume (mL)	0.142	ns	0.167	ns	**0.317**	**0.007**	-0.026	ns	-0.005	ns

LV mass (g)	**0.438**	**0.0001**	**0.546**	**0.0001**	**0.445**	**0.0001**	**-0.269**	**0.03**	**0.225**	**0.05**
LV mass index (g/m^2)	**0.266**	**0.02**	**0.245**	**0.02**	**0.287**	**0.01**	-0.028	ns	0.134	ns
RWT	**0.351**	**0.002**	**0.332**	**0.002**	**0.279**	**0.02**	**-0.286**	**0.02**	0.030	ns
Mitral E/A ratio	**-0.244**	**0.03**	**-0.214**	**0.05**	**-0.253**	**0.04**	0.291	ns	**-0.229**	**0.05**
Mitral E/E' ratio	-0.198	ns	-0.050	ns	-0.148	ns	0.155	ns	-0.080	ns
CIMT (mm)	**0.281**	**0.01**	**0.376**	**0.0001**	0.065	ns	0.056	ns	0.056	ns
AoDs (mm)	**0.267**	**0.01**	**0.356**	**0.001**	0.084	ns	**-0.268**	**0.03**	0.118	ns
AoDd (mm)	0.197	ns	**0.244**	**0.02**	**0.259**	**0.02**	-0.073	ns	0.025	ns
S	0.114	ns	0.147	ns	-0.123	ns	-0.029	ns	0.125	ns
Ep (mmHg)	**-0.228**	**0.04**	-0.037	ns	0.116	ns	-0.005	ns	-0.060	ns
Ep*	**-0.313**	**0.005**	-0.092	ns	0.063	ns	0.045	ns	-0.072	ns
BAD basal (mm)	**0.302**	**0.008**	**0.392**	**0.0001**	-0.058	ns	0.089	ns	**0.321**	**0.007**
BAD after (mm)	**0.293**	**0.01**	**0.386**	**0.0001**	-0.145	ns	0.189	ns	**0.347**	**0.003**
FMD (%)	-0.187	ns	-0.154	ns	-0.114	ns	0.172	ns	-0.192	ns
PSV basal (cm/sec)	-0.090	ns	-0.080	ns	0.165	ns	-0.127	ns	-0.012	ns
EDV basal (cm/sec)	0.034	ns	0.133	ns	0.193	ns	-0.130	ns	0.011	ns
PSV immediately after (cm/sec)	-0.121	ns	-0.162	ns	**0.271**	**0.03**	**-0.277**	**0.05**	0.004	ns

EDV immediately after (cm/sec)	-0.013	ns	-0.016	ns	0.198	ns	-0.082	ns	0.002	ns
PSV after (cm/sec)	0.021	ns	-0.076	ns	0.191	ns	-0.165	ns	0.072	ns
EDV after (cm/sec)	0.028	ns	0.090	ns	0.211	ns	-0.142	ns	0.042	ns

Values in bold represent significant results. Partial correlation was adjusted for gender, age and Tanner stage. Log transformation was used for skewed variables.

For abbreviations see Table 1.

Table S2. Partial correlation for systolic (SBP, mmHg) and diastolic (DBP, mmHg) blood pressure with cardiovascular measurements.

	SBP		DBP	
	r	***P value***	**r**	***P value***
HR (b/min)	0.174	ns	0.115	ns
EF (%)	0.125	ns	0.085	ns
FS (%)	**0.202**	**0.05**	0.187	ns
LVEDD (mm)	**0.501**	**0.0001**	**0.327**	**0.002**
LVESD (mm)	**0.359**	**0.001**	**0.279**	**0.008**
IVSD (mm)	**0.289**	**0.006**	**0.240**	**0.02**
LVPWD (mm)	**0.257**	**0.02**	**0.249**	**0.02**
LAD (mm)	**0.357**	**0.001**	**0.373**	**0.0001**
LV area (cm^2)	**0.402**	**0.0001**	**0.205**	**0.05**
LV volume (mL)	**0.335**	**0.002**	0.192	ns
LA area (cm^2)	**0.219**	**0.04**	0.187	ns
LA volume (mL)	**0.240**	**0.03**	0.176	ns
LV mass (g)	**0.483**	**0.0001**	**0.409**	**0.0001**
LV mass index (g/m^2)	**0.354**	**0.0001**	**0.303**	**0.005**
RWT	0.185	ns	0.176	ns
Mitral E/A ratio	0.163	ns	0.115	ns
Mitral E/E' ratio	-0.009	ns	-0.184	ns
CIMT (mm)	**0.240**	**0.02**	**0.263**	**0.01**
AoDs (mm)	0.176	ns	**0.289**	**0.006**
AoDd (mm)	0.193	ns	**0.283**	**0.007**
Aortic Strain, S	0.095	ns	0.114	ns

Ep (mmHg)	**0.286**	**0.006**	0.173	ns
Ep*	**0.220**	**0.04**	0.184	ns
BAD basal (mm)	0.135	ns	0.163	ns
BAD after (mm)	0.114	ns	0.162	ns
FMD (%)	0.167	ns	0.185	ns
PSV basal (cm/sec)	0.142	ns	0.167	ns
EDV basal (cm/sec)	0.076	ns	0.152	ns
PSV immediately after (cm/sec)	0.087	ns	0.093	ns
EDV immediately after (cm/sec)	0.136	ns	0.147	ns
PSV after (cm/sec)	**0.226**	**0.03**	0.187	ns
EDV after (cm/sec)	0.056	ns	0.115	ns

Values in bold represent significant results. Partial correlation was adjusted for gender, age and Tanner stage. Log transformation was used for skewed variables.

For abbreviations see Table 1.

Chapter 3

This study shows a worsened metabolic profile in obese children with normal estimated glomerular filtration rate (eGFR) higher than 1 standard deviation or with abnormally high serum uric acid levels suggesting that eGFR and serum uric acid may be helpful in clinical practice to identify an unhealthy metabolic profile in pediatric obesity.

High-normal estimated glomerular filtration rate and hyperuricemia positively correlate with metabolic impairment in pediatric obese patients

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ABSTRACT

Background. Childhood obesity represents a major health concern worldwide due to its well established detrimental effect on cardiovascular and its potential negative effect on kidney functions. However, biomarkers that can help diagnose early stages of kidney damage in obese children represent an unmet clinical need.

Objectives. In this study, we asked whether the prevalence of microalbuminuria, estimated glomerular filtration rate (eGFR) or hyperuricemia recorded in a wide cohort of obese children and adolescents would positively correlate with cardiometabolic dysfunction in these subjects.

Methods. We carried out a cross-sectional study on 360 obese children and adolescents between the ages of 3–18 years, enrolled in a tertiary care center. Clinical and biochemical evaluations including oral glucose tolerance tests (OGTTs) were performed on all patients. Microalbuminuria was defined as urinary albumin-to-creatinine ratio (u-ACR) of 30–300 mg/g. All data are expressed as mean ± standard deviation (SD), absolute values or percentages. Sex age-specific and eGFR SDs were used for statistical analyses. Serum uric acid ≥5.5 mg/dL was considered abnormal.

Results. The prevalence of microalbuminuria was 6.4%. Except for a lower insulinogenic-index, no correlations between microalbuminuria and cardiometabolic risk factors were detected. eGFR was <-1 SD and >1 SD in 1.4% and 60.8% of subjects, respectively. Subjects with an eGFR >1 SD had higher systolic blood pressure, liver enzymes, insulin resistance, glucose and insulin during OGTT, lower insulin sensitivity and a more prevalent microalbuminuria. Hyperuricemia (27.5%) increased the odds of hypertension, HDL ≤10th percentile and glucose ≥155.0 mg/dL after 60 minutes of OGTT.

Conclusions. A worse cardiometabolic profile was observed in subjects with an eGFR >1 SD compared to other subgroups. Therefore, pediatric obese patients with eGFR >1 SD or

hyperuricemia should be closely monitored for microalbuminuria and post-challenge glucose and insulin secretion, all potential indicators of renal dysfunction in these young patients.

INTRODUCTION

The rising prevalence of pediatric obesity is one of the most challenging public health issues worldwide. Most of the major concerns derive from the fact that childhood obesity, besides increasing traditional cardiometabolic risk factors, can seriously hamper kidney function [1]. In this regard, the recent global obesity epidemic has coincided with a dramatic rise in the prevalence of end-stage renal disease (ESRD) [1]. To make matters worse, emerging evidence suggests that renal dysfunction may start early during childhood, long before a diagnosis of hypertension with type 2 diabetes can be made, and it might be related to pediatric obesity [1].

Since onset of the obesity-associated renal disease is generally subtle and asymptomatic, there is clearly an urgent need of biomarkers that could allow early detection of kidney dysfunction in obese children. In this regard, mounting evidence indicates that in adults the prevalence of microalbuminuria, which is often associated with nephropathy and atherosclerosis, positively correlates with the degree of obesity [2,3]. Furthermore, a relationship between microalbuminuria and obesity has also been reported in children and adolescents [1], although long-term studies in these patients have yet to be conducted. Interestingly, the association between estimated glomerular filtration rate (eGFR) and some cardiometabolic risk factors appears non-linear as either low- or high-normal eGFR has been associated with increased risk of metabolic diseases and mortality [2]. However, whether eGFR represents a *bona fide* cardiometabolic risk indicator, especially in pediatric patients, still remains to be determined [2,4].

In addition to microalbuminuria, hyperuricemia is another well-established risk factor for chronic kidney disease (CDK) in adults [5]. This is probably due to the detrimental effects exerted by uric acid once it permeates a cell, which obviously counteracts its antioxidant activity in the extracellular environment [6]. Therefore, hyperuricemia has a negative impact on both metabolism and longevity independent of traditional cardiometabolic risk factors [5]. However, data concerning hyperuricemia in obese children and adolescents are still lacking.

Here, we have conducted a cross-sectional study on a wide cohort of obese pediatric patients to determine 1) the prevalence of microalbuminuria; 2) the distribution of age- and sex-specific eGFRs; 3) the prevalence of hyperuricemia; and 4) any correlations between microalbuminuria, eGFR and uric acid and other known cardiometabolic markers.

MATERIALS AND METHODS

Study design

This was a cross-sectional study. Study quality was assessed. We consecutively recruited Caucasian children and adolescents, aged 3–18 years, referred to our Pediatric Endocrine Service from January 2011 to June 2014 for simple obesity. Subjects were eligible if generally healthy, overweight or obese, according to the IOTF criteria [7], and naïve to a weight-loss diet. Among children that had been previously subject to biochemical investigations due to any medical condition, only healthy children were included in the study. Subjects who refused to perform an oral glucose tolerance test (OGTT) were included only if they underwent fasting biochemical evaluations. Exclusion criteria were diagnosed or suspected endocrine or genetic obesity, type 1 diabetes and previous kidney diseases. Subjects referred to our Service for known comorbidities of obesity (e.g. glucose alterations, arterial hypertension, dyslipidemia, liver steatosis, hyperuricemia etc.) were

also excluded to avoid interferences due to previous lifestyle or pharmacological interventions.

The protocol was conducted in accordance with the declaration of Helsinki and was approved by the Local Ethic Committee of AOU Maggiore della Carità of Novara (CE 95/12). Informed consents was administered to all patients and parents of minors prior to the evaluations, and the study was carefully explained by the research team to all parents and children. Only those patients who provided a written informed consent were included in the study.

Anthropometric and biochemical measurements

Height was measured to the nearest 0.1 cm using a Harpenden stadiometer. Body weight was measured with light clothing to the nearest 0.1 kg using a mechanical column weighing scale (Wunder, weighing capacity 200 Kg). Body mass index (BMI) was calculated as body weight divided by squared height (kg/m2). The BMI standard deviation score (BMI-SDS) was calculated by the least median squares (LMS) method as previously described [8]. Waist circumference (WC) was measured at the high point of the iliac crest around the abdomen and was recorded to the nearest 0.1 cm. A non-elastic flexible tape was used, with the subjects being kept in a standing position with minimal respiration and no clothing covering the waist area or compressions on the skin. The waist-to-height ratio was calculated as well. Pubertal stages were determined by physical examination, using the criteria of Marshall and Tanner. Systolic BP (SBP) and diastolic BP (DBP) were measured three times at 2-minute intervals using a mercury sphygmomanometer with an appropriate cuff size after participants had been sitting quietly for at least 15 minutes, with their right arm being supported at the level of the heart, and feet resting flat on the floor, prior to other physical evaluations and at least 30 minutes after blood sampling. Mean values were used for all these analyses. Hypertension was determined only if BP values

recorded at enrollment and testing day were both found elevated. After a 12-hour overnight fast, blood samples were taken for measurement of: glucose (mg/dL), insulin (μUI/mL), total cholesterol (mg/dL), high density lipoprotein (HDL)-cholesterol (mg/dL), triglycerides (mg/dL), aspartate aminotransferase (AST, IU/L), alanine aminotransferase (ALT, IU/L), uric acid (mg/dL), creatinine (mg/dL), IGF1 (ng/mL), 25-hydroxy (OH) vitamin D (ng/mL), using standardized methods in the Hospital's Laboratory [9]. Low-density lipoprotein (LDL)-cholesterol was calculated by the Friedwald formula. AST-to-ALT ratio was calculated. Uric acid (mg/dL) was measured by the Fossati method using uricase with a Trinder-like endpoint. Serum creatinine concentration (mg/dL) was measured by the enzymatic method. Glucose was determined by the hexokinase method (Slein Method, Advia 1200/1800/2400 Autoanalyzer; Bayer Diagnostics, Leverkusen, Germany) with an intra-assay coefficient of variation of 0.7–2.3% (range 0.0 mg/dL-700.0 mg/dL). Insulin was determined by an immunoassay method (Advia Centaur1; Bayer Diagnostics, Leverkusen, Germany) with an intra- and inter-assay coefficient of variation of 3.2–4.6% and 2.6–5.9%, respectively (range 0.5 mU/L-300.0 mU/L). Urine albumin (mg/L) was determined by an advanced immunoturbidimetric assay, and urine creatinine (mg/dL) was measured using the enzymatic method.

Subjects also underwent OGTT (1.75 g of glucose solution per kg, maximum 75 g), and samples were drawn for the determination of glucose and insulin every 30 minutes. The area under the curve (AUC) was calculated according to the trapezoidal rule. Insulin resistance was calculated using the formula for homeostasis model assessment (HOMA)-IR. Insulin sensitivity at fasting and during OGTT was calculated with the formula of the Quantitative Insulin-Sensitivity Check Index (QUICKI) and Matsuda index (ISI). Insulinogenic (InsI) and disposition (DI) indexes were also calculated as previously reported [10]. The stimulus for insulin secretion in the increment in plasma glucose as the insulinogenic index was calculated as the ratio of the changes in insulin and glucose

concentration from 0 to 30 minutes (InsI). Beta-cell compensatory capacity was evaluated by the disposition index defined as the product of the ISI and InsI (DI) [11]. Glucose was expressed in mg/dL (1 mg/dL = 0.05551 mmol/L) and insulin in µUI/mL (1 µUI/mL = 7.175 pmol/L) in each formula.

Definitions

Subjects were classified as overweight or obese according to age- and sex-specific IOTF cut-offs [7]. WC percentiles were stratified according to sex and age, identifying abdominal obesity as the presence of WC ≥90th percentile or a waist-to-height ratio of 0.5 [10]. SBP and DBP values were evaluated according to percentiles for age, sex and height, and arterial hypertension was defined as SBP or DBP >95th percentile. Triglycerides, LDL- and HDL-cholesterol percentiles for age and sex were classified according to the Lipid Research Clinic Pediatric Prevalence Study. Dyslipidemia was defined as the presence of triglycerides ≥90th percentile, HDL- cholesterol ≤10th percentile or LDL ≥90th percentile. Impaired fasting glucose and impaired glucose tolerance were defined as fasting plasma glucose ≥100–125 mg/dL (5.6 to 6.9 mmol/ L) and 2-hour post-OGTT, glucose ≥140–199 mg/dL (7.8 to 11.0 mmol/L), respectively. Uric acid ≥5.5 mg/dL was considered abnormal [12]. According to the NKF-K/DOQI Guidelines for chronic kidney disease (CKD) in children and adolescents [13], the eGFR was calculated using the modified Schwartz's formula [14]: eGFR (mL/min/1.73 m2) = [0.413 x patient's height (cm)] / serum creatinine (mg/dL). The normal renal function of patients [mean eGFR ± stan- dard deviation (SD) in mL/min/1.73 m2] was calculated based on age and gender according to NKF-K/DOQI Guidelines [13]. 2–12 year-old males and females: 133±27 mL/min/1.73 m2; 13– 21 year-old males: 140±30 mL/min/1.73m2; and 13–21 year-old females: 126±22 mL/min/1.73 m2. Because only 6 subjects had eGFR lower or higher than ±2SD, the population was divided into four categories according to age and gender ±1SD (range: <-1 SD; -1-0 SD; 0–1 SD;

>1 SD). All subjects collected first-morning urine samples at rest. Urine albumin-to creatinine- ratio (u-ACR; mg/g) was calculated as follows: [urine albumin (mg/dL)/urine creatinine (g/dL)]. Microalbuminuria was defined as u-ACR ranging from 30 to 300 mg/g [15]. We collected two more samples from the subjects found positive for microalbuminuria to confirm the measurement. Microalbuminuria was diagnosed if all the three samples were found positive.

Statistical analysis

All data are expressed as mean ± SD, absolute values or percentages. In the case of microalbuminuria, the u-ACR mean values of the three first-morning samples were used as continuous variables. With an expected prevalence of 14% of microalbuminuria [6], a confidence level of 99.0% and a margin of error of 5.0%, a population size of 320 individuals was estimated sufficient to reflect our target population. Skewed variables were logarithmically transformed. ANOVA was used to determine the differences among sex, the presence of microalbuminuria, hyperuricemia, and the eGFR subgroups with a Bonferroni post-hoc test for multiple comparisons in the latter. Analysis of covariance (ANCOVA) was also used for hyperuricemia and eGFR and covariates were age, sex, puberty and BMI (Model 1) or WC (Model 2), according to the significant relationship with dependent variables. Logistic regression was used to determine the association of microalbuminuria, eGFR and uric acid with the odds ratio (OR, 95% CI) of each cardiometabolic risk factor. Covariates of model 1 and 2 were also used in logistic regression for hyperuricemia and eGFR. Correlations as well as partial correlations were performed. Significance was assumed at p <0.05. The analysis was carried out with SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA). The database of the study is available upon request for validation or collaboration purposes as it includes other data (e.g. family history and other biochemical variables) not yet analyzed.

RESULTS

Anthropometric and metabolic characteristics of patients

Nineteen out of 379 subjects selected were excluded because they did not satisfy inclusion criteria (fifteen subjects did not have adequate blood sampling, and 4 were without at least 3 urine collections). The final dataset included 360 participants (180 males and 180 females), aged 3 to 18 years, with a mean age of 10.9±3.0 years. Of the 360 participants, 18 subjects did not undergo OGTT (fifteen refused, 3 had analyses missing for technical problems), but had a complete fasting biochemical evaluation. Among patients 88% of them were obese, and 12% overweight. Almost all subjects had a WC ≥90th percentile (97.8%) with an overall mean of the waist-to-height ratio of 0.63±0.11, without differences between sexes. The clinical and biochemical characteristics of subjects are reported in Table 1. Hypertension was diagnosed in 216 (60.0%) subjects. Eighty-eight subjects (24.4%) had triglycerides ≥90th percentile; 148 (41.1%) had HDL-cholesterol ≤10th percentile; and 29 (8.0%) had LDL-cholesterol ≥90th percentile. Twenty subjects (5.5%) had impaired fasting glucose, 19 (5.2%) impaired glucose tolerance and 4 (1.1%) both metabolic dysfunctions. One patient had type 2 diabetes.

Table 1. Clinical and biochemical features of the study population according to sex.

		Overall	M	F
Subjects		360	180	180
Age (years)		10.9±3.0	10.7±2.8	11.1±3.3
Puberty	PP	155 (43.1%)	98 (54.4%)	57 (31.7%) †
	P	205 (56.9%)	82 (45.6%)	123 (68.3%) †
BMI (kg/m^2)		28.12±4.52	27.83±3.85	28.40±5.09
Obesity IOTF		317 (88.0%)	159 (88.3%)	158 (87.8%)
BMI SDS (kg/m^2)		2.09±0.46	2.06±0.41	2.13±0.50
Waist circumference (cm)		90.8±13.4	90.6±12.0	91.1±14.6
Waist/Height ratio		0.63±0.11	0.62±0.10	0.64±0.11
SBP (mmHg)		126±16.1	126±16.8	126±15.3
SBP percentile		90±14.9	89±15.3	90±14.5
DBP (mmHg)		79±10.8	79±10.8	79±10.7
DBP percentile		87±15.3	87±15.1	87±15.3
Total cholesterol (mg/dL)		145.6±27.7	144.6±26.9	146.8±28.5
HDL-c (mg/dL)		42.8±8.7	43.1±8.6	42.5±8.7
LDL-c (mg/dL)		87.3±23.5	86.9±23.4	87.7±23.7
Triglycerides (mg/dL)		77.8±43.5	72.4±35.8	83.1±9.6 *
AST (IU/L)		23.8±7.0	25.2±7.0	22.5±6.8 †
ALT (IU/L)		24.3±13.0	26.4±15.8	22.2±8.9 *
AST/ALT ratio		1.10±0.39	1.11±0.38	1.10±0.39
Uric acid (mg/dL)		4.87±1.20	4.97±1.30	4.76±1.07
IGF-1 (ng/mL)		286.0±129.7	259.2±129.7	313.1±124.2 †
eGFR (mL/min/1.73m^2)		119.78±19.70	120.6±19.4	118.91±19.95
GlcT0' (mg/dL)		87.9±7.3	88.3±7.1	87.5±7.5
GlcT30' (mg/dL)		134.8±22.4	136.9±23.0	132.7±22.5 *
GlcT60' (mg/dL)		115.1±25.9	116.1±26.5	114.0±25.3
GlcT90' (mg/dL)		108.9±20.0	108.7±19.5	107.5±21.3
GlcT120' (mg/dL)		108.0±21.6	109.9±18.3	106.0±18.3 *
AUC Glc (mg/dL*h/dL)		13816.4±2866.7	14035.7±3513.6	13587.8±1965.3
Mean Glc (mg/dL)		111.7±20.5	113.4±24.6	109.9±14.9
InsT0' (mUI/L)		16.4±11.4	14.7±10.0	18.1±12.4 *
Mean Ins (mUI/L)		75.6±62.3	72.9±67.5	78.3±56.6
HOMA-IR		3.66±2.80	3.37±2.72	3.96±2.85 *
ISI		4.71±4.70	4.77±3.78	4.66±5.50 *
QUICKI		0.33±0.05	0.33±0.04	0.33±0.05 *
InsI		2.14±4.04	2.05±4.88	2.22±2.96
DI		6.79±16.84	6.38±11.96	7.22±20.73
u-ACR (mg/g)		11.30±26.99	9.11±20.64	13.48±32.01 *

Data are expressed as mean±SD. p value <0.01*; < 0.0001†. OGTT data are available for 342 or 345 subjects.

Legend: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; BMI: body mass index; DI: disposition index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; F: female; GlcT0': fasting glucose; GlcT30',T60',T90',T120': post-challenge glucose; HDL-c: high

density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; InsI: insulinogenic index; InsT0': fasting insulin; IOTF: International Obesity Task Force; ISI: insulin sensitivity index; LDL-c: low density lipoprotein cholesterol; M: male; P: pubertal; PP: prepubertal; QUICKI: quantitative insulin-sensitivity check index; SBP: systolic blood pressure; u-ACR: urinary albumin-to-creatinine ratio.

Microalbuminuria and associations between eGFR, uric acid, and other cardiometabolic variables

Microalbuminuria was detected in 6.4% (23/360) of subjects. In patients with or without microalbuminuria, uric acid was ≥5.5 mg/dL in 34.7% (8/23) and 27.0% (91/337) of them, respectively (p=0.278). All patients with microalbuminuria had eGFR >0 SD. In particular, 69.6% (16/23) of them had eGFR >1 SD. In the entire cohort without microalbuminuria, 91.7% (309/337) had eGFR >0 SD, and in 60.2% (203/337) of them the eGFR was >1SD. Subjects with microalbuminuria had lower insulin levels at 30-minute post-OGTT (81.8± 72.8 mUI/L *vs* 122.3±163.5; p<0.02) and lower insulinogenic index (InsI) (0.74±5.08 *vs* 2.24± 4.04; p<0.05) than those without it. No correlations between u-ACR as a continuous variable and cardiometabolic alterations were found.

eGFR evaluation and its association with microalbuminuria, uric acid, and other cardiometabolic variables

In 1.4% (5/360) of patients, eGFR was <-1 SD, while in 4 of them it was <-2 SD. Furthermore, eGFR was >1SD in 60.8% (219/360) of subjects, with 2 of them displaying an eGFR >2SD. Anthropometric and metabolic characteristics of subjects according to eGFR categories are reported in Table 2.

Table 2. Anthropometric and metabolic characteristics of the study population according to eGFR stratified for percentiles, age and sex dependent, based on NKF-K/DOQI Guidelines.

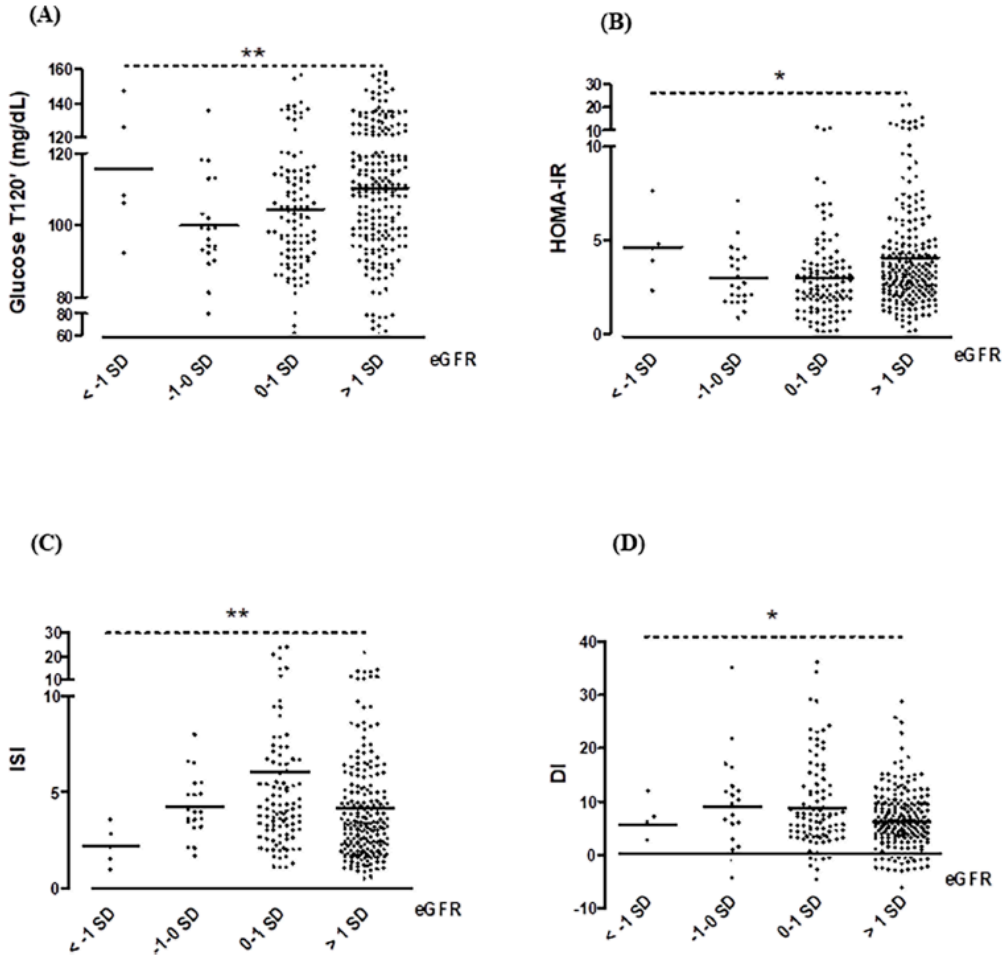
		Categories of eGFR				*P* value
		< - 1 SD	- 1–0 SD	0–1 SD	> 1 SD	
Subjects		5 (1.4%)	23 (6.4%)	113 (31.4%)	219 (60.8%)	
Age (years)		11.1±4.0	11.7±3.0	10.7±3.1	10.9±2.9	*ns*
Sex	M	3 (60%)	5 (21.7%) **	44 (38.9%) ‡‡	128 (58.5%)	*< 0.0001*
	F	2 (40%)	18 (78.3%) **	69 (61.1%) ‡‡	91 (41.5%)	
Puberty	PP	2 (40%)	5 (21.7%)	57 (50.4%)	91 (41.5%)	*ns*
	P	3 (60%)	18 (78.3%)	56 (49.6%)	128 (58.5%)	
BMI (kg/m^2)		30.13±6.74	29.50±5.43	27.50±4.98	28.25±4.06	*ns*
BMI SDS (kg/m^2)		2.35±0.63	2.21±0.55	2.03±0.48	2.11±0.43	*ns*
Waist circumference (cm)		97.1±20.3	93.8±14.1 §	87.5±13.0 ‡‡	92.1±13.0	*< 0.009*
Waist/Height ratio		0.64±0.08	0.63±0.09	0.61±0.08	0.641±0.11	*ns*
SBP (mmHg)		135±10	128±15.5	123±16.0 ‡‡	128±16.2	*< 0.02*
SBP percentile		98±1.3	92±9.23	86±17.5 ‡‡	91±13.7	*< 0.01*
DBP (mmHg)		86±4.1	83±10.9	79±11.1	79±10.6	*ns*
DBP percentile		98±1.9	92±8.3	87±14.5	86±16.1	*ns*
Total cholesterol (mg/dL)		141.8±21.3	138.9±27.5	141.6±25.6	148.5±28.6	*ns*
HDL-c (mg/dL)		38.8±8.9	42.3±9.2	43.5±9.4	42.6±8.2	*ns*
LDL-c (mg/dL)		86.4±12.5	83.9±26.9	83.4±22.7	89.7±23.6	*ns*
Triglycerides (mg/dL)		83.6±54.3	70.5±22.6	74.6±39.6	80.0±46.8	*ns*
AST (IU/L)		23.6±10.7	19.5±6.0 §,**	22.9±5.9 ††	24.8±7.4	*< 0.002*
ALT (IU/L)		31.8±25.3	23.7±10.2	20.8±8.3 ‡‡	25.9±14.5	*< 0.001*
AST/ALT ratio		0.86±0.29 ‡	0.89±0.31 ¶	1.20±0.37 ‡‡	1.08±0.39	*< 0.001*
Uric acid (mg/dL)		4.70±0.43	5.30±0.89	4.72±1.16	4.90±1.24	*ns*
IGF-1 (ng/mL)		255.5±102.6	324.4±113.7	280.8±133.1	286.6±130.3	*ns*
GlcT0' (mg/dL)		86.6±4.9	87.04±5.75	86.9±7.2	88.5±7.5	*ns*
GlcT30' (mg/dL)		152.5±28.0 †	123.5±18.5 $^{\S,\#}$	134.2±23.7	135.8±22.3	*< 0.0001*
GlcT60' (mg/dL)		122.2±36.4	106.7±19.5	111.0±27.2	117.6±25.3	*ns*
GlcT90' (mg/dL)		121.0±24.7 †	96.0±16.4 **	104.1±19.17 ‡‡	110.8±20.6	*< 0.001*
GlcT120' (mg/dL)		115.6±21.1	100.0±13.6 **	104.4±17.4 ‡‡	110.3±20.6	*< 0.009*
AUC Glc (mg/dL*h/dL)		22287.0±17283. 9 †	12614.2±1278.7 **	13393.9.±2008.8 ††	13930.7.±1868.0	*< 0.004*
Mean Glc (mg/dL)		168.8±117.3 *	102.9±9.5 **	108.5±15.0 ††	112.6±14.3	*< 0.005*
InsT0' (mUI/L)		21.7±8.8	13.7±7.1	13.7±9.7 ‡‡	17.9±12.3	*< 0.006*
Mean Ins (mUI/L)		106.0±39.8	60.2±29.7	65.8±47.5	80.9±69.9	*ns*
HOMA-IR		4.62±1.92	2.97±1.50	2.95±2.13 ‡‡	4.08±3.12	*< 0.02*
ISI		2.18±1.01	4.24±1.16	6.05±6.85 ‡‡	4.18±3.40	*< 0.005*
QUICKI		0.31±0.01	0.33±0.02	0.35±0.05 §§	0.32±0.04	*< 0.001*
InsI		2.14±1.27	2.81±3.75	2.20±1.91	2.04±4.79	*ns*
DI		5.56±4.48	9.09±9.04	10.30±13.70 ‡‡	4.92±18.61	*< 0.05*
u-ACR (mg/g)		7.35±3.81	4.83±5.18	9.91±18.83	12.78±31.72	*ns*

Data are expressed as mean±SD. p value -1SD vs -1-0 SD: <0.05*; <0.01†. -1SD vs 0-1SD: <0.05‡. -1-0 SD vs 0±1 SD: <0. 05§; <0.0001¶; -1-0 SD vs >1SD: <0.05$^{\#}$; <0.01**. 0±1 SD vs >1SD: < 0.05††; < 0.01‡‡; < 0.0001§§. ns: not significant. ANOVA analysis with a Bonferroni post-hoc test was used. OGTT data are available for 342 or 345 subjects. Legend: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; BMI: body mass index; DI: disposition index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; F: female; GlcT0': fasting glucose; GlcT30',T60',T90',T120': post-challenge glucose; HDL-c: high density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; InsI: insulinogenic index; InsT0': fasting insulin; IOTF: International Obesity Task Force;

ISI: insulin sensitivity index; LDL-c: low density lipoprotein cholesterol; M: male; P: pubertal; PP: prepubertal; QUICKI: quantitative insulin-sensitivity check index; SBP: systolic blood pressure; u-ACR: urinary albumin-to-creatinine ratio.

Compared with subjects with eGFR <-1SD, patients with eGFR >1SD showed higher SBP, AST, ALT, glucose and insulin during OGTT, insulin resistance (Fig 1 , Panel A and B); they also had lower sensitivity indexes after both crude analysis and ANCOVA (Fig 1 , Panel C and D).

Figure 1. Relationship between glucometabolic parameters and stratified estimated glomerular filtration rate (eGFR) levels. (Panel A) Plasma glucose levels (mg/dL) after 2-hour post-glucose tolerance test (OGTT) (T120'); (Panel B) homeostatic model assessment of insulin resistance (HOMA-IR); (Panel C) insulin sensitivity index (ISI); (Panel D) disposition index (DI); p value <0.05*; <0.01** after ANOVA corrected for confounding factors (i.e. sex, puberty, BMI).



Subjects with an eGFR within 0 and 1 SD had higher odds to have triglycerides <90th percentile in both crude and controlled models (model 2; OR 1.750; CI 1.002±3.056; p < 0.04).

eGFR was correlated with many variables in the crude model. After adjusting for age, sex, puberty, and BMI, eGFR was positively associated with WC, fasting insulin, glucose levels at 90 and 120 minutes, AUC and mean glucose, insulin at 120 minutes, mean insulin and u-ACR, and negatively associated with DPB, uric acid, ISI, and QUICKI. After adjusting for age, sex, puberty, and WC, eGFR was positively associated with fasting insulin, glucose levels at 90 and 120 minutes, AUC and mean glucose, HOMA-IR and u-ACR, and negatively associated with DPB, uric acid, ISI, and QUICKI (Table 3).

Table 3. Partial correlations between eGFR and microalbuminuria, uric acid and other cardiometabolic variables.

eGFR	Model 1		Model 2	
	r	*P* value	r	*P* value
Age (years)				
BMI (kg/m^2)			-0.062	*ns*
BMI SDS (kg/m^2)				
Waist circumference (cm)	**0.150**	*< 0.005*		
Waist/Height ratio				
SBP (mmHg)	0.034	*Ns*	0.016	*ns*
DBP (mmHg)	**- 0.122**	*< 0.002*	**-0.131**	*< 0.01*
Total cholesterol (mg/dL)	0.092	*Ns*	0.084	*ns*
HDL-c (mg/dL)	0.065	*Ns*	0.071	*ns*
LDL-c (mg/dL)	0.067	*Ns*	0.066	*ns*
Triglycerides (mg/dL)	0.026	*Ns*	-0.002	*ns*
AST (IU/L)	0.094	*Ns*	0.085	*ns*
ALT (IU/L)	0.037	*Ns*	0.012	*ns*
AST/ALT ratio	-0.11	*Ns*	-0.004	*ns*
Uric acid (mg/dL)	**-0.172**	*< 0.0001*	**-0.217**	*< 0.0001*
IGF-1 (ng/mL)	0.021	*Ns*	0.042	*ns*
GlcT0' (mg/dL)	0.078	*Ns*	0.090	*ns*
GlcT30' (mg/dL)	0.032	*Ns*	0.039	*ns*
GlcT60' (mg/dL)	0.077	*Ns*	0.083	*ns*
GlcT90' (mg/dL)	**0.174**	*< 0.001*	**0.176**	*< 0.001*
GlcT120' (mg/dL)	**0.164**	*< 0.002*	**0.168**	*< 0.002*
AUC Glc (mg/dL*h/dL)	**0.132**	*< 0.01*	**0.128**	*< 0.001*
Mean Glc (mg/dL)	**0.131**	*< 0.01*	**0.140**	*< 0.01*
Mean Ins (mUI/L)	**0.109**	*< 0.04*	0.080	*ns*
HOMA-IR	**0.164**	*< 0.02*	**0.141**	*< 0.009*
ISI	**-0.131**	*< 0.01*	**-0.108**	*< 0.05*
QUICKI	**-0.176**	*< 0.01*	**-0.140**	*< 0.009*
InsI	0.009	*Ns*	0.002	*ns*
DI	-0.047	*Ns*	-0.041	*ns*
u-ACR (mg/g)	**0.124**	*< 0.02*	**0.128**	*< 0.01*

ns: not significant. OGTT data are available for 342 or 345 subjects.

Legend: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; BMI: body mass index; DI: disposition index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; GlcT0': fasting glucose; GlcT30',T60',T90',T120': post-challenge glucose; HDL-c: high density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; InsI: insulinogenic index; InsT0': fasting insulin; ISI: insulin sensitivity index; LDL-c: low density lipoprotein cholesterol; QUICKI: quantitative insulin-sensitivity check index; SBP: systolic blood pressure; u-ACR: urinary albumin-tocreatinine ratio.

Model 1: controlled for sex, age, puberty and BMI.

Model 2: controlled for sex, age, puberty and waist circumference.

Hyperuricemia evaluation and its association with microalbuminuria, eGFR and other cardiometabolic variables

Hyperuricemia was present in 27.5% (99/360) of subjects, of whom 8.1% (8/99) had microalbuminuria, whereas microalbuminuria was found in 5.7% (15/261) of subjects without hyperuricemia.

Interestingly, 64.6% (64/99) of subjects with hyperuricemia had eGFR >1SD, and 26.3% (26/99) were between 0 and 1SD. In contrast, no subject with eGFR <-1 SD had hyperuricemia.

Conversely, 59.4% (155/261) of subjects without hyperuricemia had eGFR >1 SD, and 33.3% (87/261) were between 0 and 1 SD.

Subjects with hyperuricemia were older, had higher BMI, BMI SDS, waist circumference, SBP, DBP, triglycerides, ALT and IGF-1 levels and lower HDL-cholesterol, AST, AST to ALT ratio, eGFR and u-ACR compared with those with normal acid uric levels. Moreover, subjects with hyperuricemia showed higher glucose and insulin, either at fasting or as responses to OGTT, associated with higher insulin resistance and lower insulin sensitivity than those without hyperuricemia. After controlling for confounding factors, subjects with hyperuricemia maintained higher levels of IGF-1 and lower eGFR compared to those with normal acid uric levels (Table 4).

Table 4. Anthropometric and metabolic characteristics of the study population according to uric acid.

	URIC ACID *P* value			
	Normal	High	Model 1	Model 2
Age (years)	10.2±3.0	12.7±2.5		
BMI (kg/m^2)	27.1±4.5	30.8±4.7		*ns*
BMI SDS (kg/m^2)	2.03±0.46	2.27±0.54		*ns*
Waist circumference (cm)	87.5±13.4	100.3±12.4	*< 0.0001*	
Waist/Height ratio	0.62±0.10	0.64±0.12	*ns*	
SBP (mmHg)	123.2±16.1	134.5±17.7	*< 0.01*	*ns*
SBP percentile	88.7±15.0	92.1±13.7	*< 0.01*	*ns*
DBP (mmHg)	77.8±10.7	84.2±10.8	*< 0.05*	*ns*
DBP percentile	85.6±16.2	90.2±12.4	*< 0.05*	*ns*
Total cholesterol (mg/dL)	146.9±28.1	142.1±26.4	*ns*	*ns*
HDL-c (mg/dL)	43.8±8.7	40.1±7.9	*ns*	*ns*
LDL-c (mg/dL)	88.2±23.8	84.8±22.8	*ns*	*ns*
Triglycerides (mg/dL)	74.6±43.5	86.1±49.7	*ns*	*ns*
AST (IU/L)	24.2±7.0	22.9±6.6	*ns*	*ns*
ALT (IU/L)	23.1±13.1	27.5±16.6	*ns*	*ns*
AST/ALT ratio	1.16±0.39	0.95±0.30	*ns*	*ns*
Uric acid (mg/dL)	4.3±0.8	6.3±0.7	*ns*	*ns*
IGF-1 (ng/mL)	258.6±115.5	353.8±132.4	*< 0.04*	*< 0.03*
eGFR (mL/min/1.73m^2)	121.8±19.4	113.6±18.5	*ns*	*< 0.001*
25-OH VitD (ng/mL)	20.3±9.2	20.3±9.6	*ns*	*ns*
GlcT0' (mg/dL)	87.3±8.9	90.4±12.3	*ns*	*ns*
GlcT30' (mg/dL)	135.3±23.5	133.2±20.6	*ns*	*ns*
GlcT60' (mg/dL)	111.7±25.8	123.2±26.4	*ns*	*ns*
GlcT90' (mg/dL)	106.4±20.7	112.6±22.0	*ns*	*ns*
GlcT120' (mg/dL)	106.6±18.7	111.8±19.7	*ns*	*ns*
AUC Glc (mg/dL*h/dL)	1370.2±2870.7	14109.1±1943.1	*ns*	*ns*
Mean Glc (mg/dL)	110.7±20.6	114.3±14.9	*ns*	*ns*
InsT0' (mUI/L)	146.1±13.1	22.3±18.9	*< 0.01*	*ns*
InsT30' (mUI/L)	123.6±186.2	108.4±80.8	*ns*	*ns*
InsT60' (mUI/L)	80.0±86.7	109.7±104.8	*ns*	*ns*
InsT90' (mUI/L)	68.9±65.2	86.6±75.2	*ns*	*ns*
InsT120' (mUI/L)	71.2±92.7	100.5±126.3	*ns*	*ns*
AUC Ins (mUI*h/dL)	9508.7±8260.3	10988.4±7915.6	*ns*	*ns*
Mean Ins (mUI/L)	72.9±62.6	85.6±64.2	*ns*	*ns*
HOMA-IR	3.27±4.25	5.33±6.84	*< 0.05*	*ns*
ISI	5.13±4.79	3.66±2.91	*ns*	*ns*
QUICKI	0.34±0.04	0.32±0.05	*ns*	*ns*
InsI	2.17±4.50	2.06 ±2.50	*ns*	*ns*
DI	7.25±6.84	5.62±6.18	*ns*	*ns*
u-ACR (mg/g)	11.59±26.93	10.53±24.50	*ns*	*ns*

Data are expressed as mean±SD. ns: not significant. OGTT data are available for 342 or 345 subjects.

Legend: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; BMI: body mass index; DI: disposition index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; F: female; GlcT0': fasting glucose; GlcT30',T60',T90',T120': post-challenge glucose; HDL-c: high density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; InsI: insulinogenic index; InsT0': fasting insulin; InsT30',T60',T90',T120': post-challenge glucose; ISI: insulin sensitivity index; LDL-c: low density lipoprotein cholesterol; M: male; QUICKI: quantitative insulin-sensitivity

check index; SBP: systolic blood pressure; u-ACR: urinary albumin-to-creatinine ratio; uric acid: high (≥5.5 mg/dL); 25OH VitD: 25-0H vitamin D.

Model 1: controlled for sex, age, puberty and BMI.

Model 2: controlled for sex, age, puberty and waist circumference.

Uric acid levels were positively associated with ALT, IGF-1, HOMA-IR, fasting insulin, glucose levels at 60, 90 and 120 minutes, AUC and mean glucose, insulin at 60 minutes, and negatively associated with HDL-cholesterol, AST to ALT ratio, eGFR, and QUICKI, also when corrected for covariates (Table 5).

Table 5. Partial correlations between uric acid and microalbuminuria, eGFR and other cardiometabolic variables.

URIC ACID	Model 1		Model 2	
	r	*P* value	r	*P* value
Age (years)				
BMI (kg/m^2)			0.015	*ns*
BMI SDS (kg/m^2)				
Waist circumference (cm)	**0.169**	*< 0.002*		
Waist/Height ratio				
SBP (mmHg)	**0.105**	*< 0.05*	0.078	*ns*
DBP (mmHg)	0.076	*ns*	0.088	*ns*
Total cholesterol (mg/dL)	0.052	*ns*	0.044	*ns*
HDL-c (mg/dL)	**-0.153**	*< 0.004*	**-0.150**	*< 0.005*
LDL-c (mg/dL)	0.081	*ns*	0.079	*ns*
Triglycerides (mg/dL)	**0.105**	*< 0.04*	0.087	*ns*
AST (IU/L)	0.018	*ns*	0.009	*ns*
ALT (IU/L)	**0.115**	*< 0.03*	**0.104**	*< 0.05*
AST/ALT ratio	-0.118	*< 0.02*	**-0.122**	*< 0.02*
IGF-1 (ng/mL)	**0.187**	*< 0.0001*	**0.217**	*< 0.0001*
eGFR (mL/min/1.73m^2)	**-0.172**	*< 0.001*	**-0.217**	*< 0.0001*
GlcT0' (mg/dL)	0.070	*ns*	0.074	*ns*
GlcT30' (mg/dL)	0.096	*ns*	0.089	*ns*
GlcT60' (mg/dL)	**0.209**	*< 0.0001*	**0.206**	*< 0.0001*
GlcT90' (mg/dL)	**0.118**	*< 0.03*	**0.111**	*< 0.04*
GlcT120' (mg/dL)	**0.137**	*< 0.01*	**0.136**	*< 0.01*
AUC Glc (mg/dL*h/dL)	**0.176**	*< 0.001*	**0.168**	*< 0.002*
Mean Glc (mg/dL)	**0.179**	*< 0.001*	**0.178**	*< 0.001*
InsT0' (mUI/L)	**0.181**	*< 0.001*	**0.014**	*< 0.009*
Mean Ins (mUI/L)	**0.114**	*< 0.003*	0.072	*ns*
HOMA-IR	**0.140**	*< 0.008*	**0.117**	*< 0.03*
ISI	**-0.120**	*< 0.02*	-0.096	*ns*
QUICKI	**-0.150**	*< 0.0001*	**-0.113**	*< 0.03*
InsI	-0.037	*ns*	-0.049	*ns*
DI	-0.090	*ns*	-0.091	*ns*
u-ACR (mg/g)	-0.085	*ns*	-0.080	*ns*

ns: not significant.

Legend: ALT: alanine aminotransferase; AST: aspartate aminotransferase; AUC: area under the curve; BMI: body mass index; DI: disposition index; DBP: diastolic blood pressure; eGFR: estimated glomerular filtration rate; GlcT0': fasting glucose; GlcT30',T60',T90',T120': post-challenge glucose; HDL-c: high density lipoprotein cholesterol; HOMA-IR: homeostatic model assessment of insulin resistance; InsI: insulinogenic index; InsT0': fasting insulin; ISI: insulin sensitivity index; LDL-c: high density lipoprotein cholesterol; QUICKI: quantitative insulin-sensitivity check index; SBP: systolic blood pressure; u-ACR: urinary albumin-to-creatinine ratio.

Model 1: controlled for sex, age, puberty and BMI.

Model 2: controlled for sex, age, puberty and waist circumference.

Lastly, hyperuricemia was associated with hypertension (OR: 2.086, CI 1.257±3.460, p <0.003), HDL-cholesterol ≤10th percentile (OR: 2.001, CI 1.252±3.198; p <0.003) and glucose ≥155.0 mg/dL at 60 minutes after OGTT (OR: 2.350, CI 1.045±5.282; p <0.03) in both crude and controlled models.

DISCUSSION

This study shows a worsened metabolic profile in obese children with normal eGFR higher than 1SD or with abnormally high serum uric acid levels. In particular, post-OGTT glucose levels were found to be higher, albeit within standard cutoffs, in those individuals with eGFR >1 SD. Furthermore, subjects with microalbuminuria did not display a major impairment in their cardiometabolic alterations, although all of them had eGFR >0 SD.

In recent years, a positive correlation between microalbuminuria and obesity in adult patients has been clearly established [3]. However, in pediatric patients such correlation appears to be less obvious and only partially understood probably due to the lack of consistent data on large-sized cohorts. In this regard, here we show a prevalence of 6.4% of microalbuminuria in a large cohort of obese children and adolescents, which is in good

agreement with previous data reporting prevalence of microalbuminuria ranging between 0.3% and 10.1% in similar patient groups [16-21]. These studies, including ours, however appears to underestimate the prevalence of microalbuminuria when compared to other reports showing a much higher prevalence of microalbuminuria in obese children, which could go as high as 37.0% [22,23]. This discrepancy could be explained by different modalities of urine sample collection [22] or by the measurement of urinary albumin excretion rate per minute time rather than u-ACR [23]. Moreover, other variables such as postural changes and exercise before the testing session, as well as ethnicity, might account for data variability [24], even though no evidence indicating that this is indeed the case in obese children has been found in previous studies [18,19].

Associations between microalbuminuria and other cardiometabolic markers in obese children and adolescents have been reported in some [16,18,20-22,25] but not all studies [17,19,26], including ours. Although this lack of correlation could be explained by the young mean age of our patients or the low prevalence of microalbuminuria registered in these latter, our findings do not support a routine assessment of microalbuminuria in all obese children and adolescents. Interestingly, we find a lower insulinogenic index in subjects with microalbuminuria, which is in good agreement with a recent study demonstrating a positive correlation between microalbuminuria and HbA1c in obese Korean adolescents [21]. In this regard, it has been hypothesized that the prevalence of microalbuminuria progressively increased as plasma glucose values climbed through the `normal range' into the impaired range of glucose tolerance, suggesting that the effect of glucose may be continuous. Furthermore, frequent daily postprandial states of relatively higher glucose levels could increase oxidative stress on the vessels leading to increased urinary albumin excretion secondary to endothelial dysfunction [16].

Moreover, because HbA1c and insulinogenic-index are both related to insulin-resistance, also the latter could be a responsible for this alteration. This is in agreement with the evidence that insulin-resistance contributes to micro and macrovascular disease [1,16].

Since our results appear to indicate that subjects with eGFR >0SD have microalbuminuria and a suboptimal metabolic phenotype, further longitudinal studies on larger populations are clearly needed to fully establish whether eGFR could be used as a useful marker to stratify high-risk obese youths.

According to NKF-K/DOQI guidelines [13], we also report eGFR <-1SD in 1.4% of our population, who was free of known CKDs. Based on a normal population distribution, a much greater percentage would be expected to be below -1SD and again below normal -2SD. The fact that the majority of our subjects were stratified above 0 and, to a greater extent, 1SD indicates a quite alarming skewed distribution of eGFR in obesity, in agreement with some [27], but not all authors [20,28]. In fact, differently by us, the distribution observed in the National Health and Nutrition Examination Survey (NHANES) resulted toward lower eGFR beyond what is expected. However, the NHANES cohort included only adolescents aged 12±19 years of general U.S. population [28], while our population was much younger. These contrasting findings could be due also to different obesity trajectories or different formulas used to determine eGFR from serum creatinine concentration (i.e Jaffè technique vs Schwartz's formula) [14]. Therefore, previous reports have clearly shown that adulthood obesity is associated with glomerular hyper-perfusion and hyper-filtration as an early sign of physiologic maladaptation leading, in part, to afferent arteriolar vasodilatation [1]. Of note, we show that microalbuminuria was present only in subjects with eGFR >0SD in good agreement with a previous report [29]. Thus, our findings, together with the observation that childhood obesity positively correlates with a fast decline of eGFR over time, with a 2- to -3 fold higher risk of

developing ESRD [30], should prompt physicians to evaluate the possibility of renal dysfunction in obese children.

We also show that subjects with eGFR >1SD presented with an increased burden of cardiometabolic alterations as recent studies in adults seem to suggest [2]. In particular, in our young patients with eGFR >1SD, systolic blood pressure, glucose, and insulin levels in response to OGTT and insulin resistance were higher, whereas insulin sensitivity was lower compared to other subgroups, suggesting a glucose dysregulation mainly after OGTT. The same condition was also present in patients with eGFR <-1SD, suggesting a U-shaped relationship, even though the low number of subjects in the left part of the curve does not allow us to draw any firm conclusions about its generalizability. A similar U-shaped effect between eGFR, blood pressure and microalbuminuria has been recently reported by Di Bonito P. et al. [29], although these authors could not find a significant relationship between eGFR and the glyco-insulinemic profile at fasting. This discrepancy might be due to the different eGFR cutoff in adults used in this study.

Overall, our findings raise the possibility that eGFR >1SD may be an early predictor of dysglycemia and pre-diabetes, a possibility that could be further explored by investigating the relationships among post-challenge glucose, insulin levels and kidney function in youths. Our data are also consistent with findings by Matsushita et al., who have recently shown that the inclusion of eGFR and u-ACR among traditional risk predictors greatly improved the discrimination of cardiometabolic outcomes in adults [4]. However, additional longitudinal studies are clearly needed to establish the evolution and distribution of eGFR in obese children and explain its pathophysiological significance over time.

In our study, we show an inverse correlation between eGFR and uric acid, which is in line with previous reports on several adult populations [6] and adolescents with type 1 diabetes [31].

While in the past hyperuricemia was thought to result from a decreased uric acid clearance due to kidney damage, it now seems that uric acid per se might play a role in the natural history of GFR decline [6]. In this regard, here we show that hyperuricemic patients are at increased risk of having a 1-hour post-OGTT glycemia ≥155.0 mg/dL. This cut-off seems to be associated with an increased metabolic risk in subjects with a post-challenge normal glucose tolerance as well as with the development of an overt type 2 diabetes rather than fasting glucose [32]. Moreover, serum uric acid levels are closely related to both early-phase insulin secretion and 2-hour post-challenge glucose levels in adults with apparently normal glucose regulation [33].

To the best of our knowledge, this is the first study in a pediatric obese population where a positive association between uric acid and glucose response after OGTT has been found. In line with previous evidence [5], we confirm a worse cardiometabolic profile in subjects with hyperuricemia, which increased the odds of hypertension and HDL-cholesterol ≤10th percentile.

In agreement with recent studies in adults [34], we also observed an association between uric acid and ALT, which suggests that uric acid may be an independent risk factor for liver diseases.

Although serum uric acid seems a good predictor of renal and cardiometabolic diseases, its normal values in children and adolescents are still undefined. In this regard, we report an age-dependent effect on serum uric acid levels. Thus, the fixed cut-off is probably improper in the pediatric population, while a distribution according to age and sex may be more appropriate.

All in all, our results are limited to a Caucasian population. We included only Caucasian children and adolescents because ethnic influences on microalbuminuria and serum uric acid have been reported [18,19,24,35]. Therefore, further studies on more heterogeneous populations are needed.

Our study has some limitations. First of all, the cross-sectional design does not allow us to conclude that there is a causal relationship between variables; longitudinal studies might clarify this aspect. Moreover, a normal-weight control group is lacking, because the study was performed in a tertiary referral center. Another limit is that microalbuminuria was measured on spot morning urine samples; however, spot u-ACR correlates very well with the urine collection at the 24-hour time point [19]. Furthermore, we failed to observe a normal distribution of eGFR in obesity, and a very low percentage of subjects could be stratified in the extreme tails (±2SD). Thus, studies on larger populations are needed to confirm our data and investigate the metabolic phenotype of those with an eGFR above or below ±2SD.

On the other hand, our study includes a large sample of subjects as well as the availability of OGTT for the majority of patients. Moreover, microalbuminuria was confirmed on three samples, and eGFR was stratified according to the pediatric cut-off, unlike most publications on pediatric obesity.

In conclusion, our study suggests that eGFR may be helpful in clinical practice to identify an unhealthy metabolic profile in pediatric obesity. Thus, more attention should be paid to this relatively inexpensive parameter. Therefore, in subjects with an eGFR >1SD or hyperuricemia, we encourage to investigate the early-phase insulin secretion and 2-hour post-challenge glucose levels. Serum uric acid seems to be another useful tool to diagnose subjects at high risk of metabolic impairment. However, studies based on larger population are needed to establish normal references values according to age and sex.

Finally, based on our data, we strongly recommend the inclusion of microalbuminuria only in routine screenings of pediatric obese patients with eGFR greater than 1 SD. Further studies on large-sized pediatric cohorts are needed to confirm our finding also in obese children with an eGFR less than -2SD or greater than 2SD.

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Chapter 4

This chapter reports the results of a perspective behavioral intervention on cardiovascular response in obese children and adolescents. This study demonstrates that pediatric cardiovascular dysfunction could be partially reversed by a healthy-lifestyle program, the cornerstone of childhood obesity treatment, via weight loss and the improvement of metabolic risk and sistemic blood pressure.

Effects of a healthy-lifestyle intervention on cardiovascular dysfunction in pediatric obesity

Manuscript in preparation

ABSTRACT

Introduction. Healthy-lifesyle interventions are the cornerstone treatment of childhood obesity. Pediatric obesity is associated with cardiovascular abnormalities but the reversibility of these alterations via behavioral programs are still debated.

Aim of this study was to determine the effects of a 12 months healthy-lifestyle intervention based on diet plus physical activity on cardiovascular structure and function in obese children and adolescents.

Methods. In this single-center perspective study we assessed changes in anthropometric, biochemical and a cardiovascular variables in 55 obese subjects (6–16 years) before and after a 12-months behavioral program based on an isocaloric Mediterranen balanced diet plus an exercise traing regimen (45-60 minutes daily sessions of aerobic training). Compliance was defined as a negative change in BMI z-score ≥10% from baseline.

Results. At 12 months (T12), we observed a significant improvement of metabolic parameters (blood glucose during the OGTT, insulin resistance, HDL-cholesterol, and insulin sentivity) in compliant subjects (C). Treatment was effective in C subjects in increasing the mitral peak early diastolic velocity E ($p<0.04$) and the E/A ratio ($p<0.02$). Furtermore, in patients with a reduction in the number of NCEP-ATPIII metabolic syndrome criteria, behavioral intervention reduces LV area ($p<0.01$) and volume ($p<0.05$). Finally, intervention reduces carotid intima-media thickness ($p<0.05$) in subjects showing a decrease of the sistemic blood pressure.

Conclusions. In obese children, cardiovascular impairement could be reversed by a healthy-lifestile intervention. To adopt prompt behavioral programs in childhood obesity is crutial both for prevention and for treatment of precocius complications and could have an exponential impact on long-term morbidity and mortality and global public health.

INTRODUCTION

The rising prevalence of childhood obesity worldwide is one of the most important public health issues. Pediatric obesity is associated with a broad range of severe complications, increasing the risk of premature morbidity and mortality [1]. As a consequence of this pediatric obesity epidemic, an increase in the incidence of coronary heart disease and in the number of cardiovascular deaths is expected to occur in young adulthood. By 2035, it is estimated that the prevalence of coronary heart disease will increase from 5 to 16%, with more than 100.000 excess cases attributable to the increased obesity [2]. In obese adolescents, the hazard ratio for death in early adulthood from coronary heart disease is 4.9, 2.6 from stroke, 2.1 for sudden death, and 3.5 for death from total cardiovascular causes [3]. Furthermore, we and others demonstrated that even during childhood, obesity impaired cardiac and vascular structure and function [4-10] and that some metabolic factors such as insulin resistance, serum uric acid and the presence of metabolic syndrome (MetS) might play an adverse role during heart remodelling among children and adolescents [4]. Seen this alarming data, a promt and aggressive therapeutic strategy in obese children is mandatory in order to reverse modifiable obesity-related risk factors, to prevent cardiovascular dysfunction and ultimately to reduce cardiovascular deaths. The treatment of pediatric obesity is usually based on exercise, dietary, and behavioral interventions. However, little is known about the reversibility of cardiac abnormalities in obese children and adolescents undergoing a lifestyle intervention program.

Therefore, the primary purpose of this study was to determine the effects of a 12 months healthy-lifestyle intervention based on diet plus physical activity on cardiovascular structure and function in obese children and adolescents.

SUBJECTS AND METHODS

Study design and population

This was a perspective single-center study performed at the Pediatric Endocrinology Unit, Department of Health Sciences, University of Piemonte Orientale, Novara, Italy. We enrolled from December 2015 to February 2017 subjects aged 6-16 years if they were generally healthy, obese according to the International Obesity Task Force (IOTF) criteria [11], and not on a weight-loss diet. Exclusion criteria were endocrine or genetic obesity, type 1 or type 2 diabetes, previous heart, respiratory, liver and kidney diseases, current or past use of hormonal or interfering therapies (lipid-lowering, hypoglycemic, or antihypertensive treatments). The protocol was conducted in accordance with the declaration of Helsinki and the Local Ethic Committee approved the study (CE 95/12). Informed written consent was obtained from all subjects' parents.

Intervention

Patients were evaluated at baseline (T0) and after 12 months (T12) of a behavioral intervention program. A trained pediatric endocrinologist and a nutritionist assessed the habitual diet and administered an isocaloric Mediterranen balanced diet. To evaluate food consumption, foods were classified according to the Italian Institute of Research on Food and Nutrition [12]. Food frequencies questionnaires, validated for a wide range of ages [13], were completed by parents. Moreover, subjects underwent a physical activity regimen including 45-60 minutes daily sessions of aerobic training (fast walking, running, ball games, or swimming). Compliance to diet and training was assessed at baseline and every 3 months for one year with specific questionnaires. The nutritional counselling and the reinforcement of healthy lifestyle habits were performed at the same time intervals.

Procedures

At baseline and after the 12 months-behavioral intervention, we evaluated anthropometric and biochemical variables including an OGTT and we performed an echocardiographic and a vascular assessment (for details see chapter 2) [4]. For the purpose of this study and based on previous adults' studies [14,15], compliance was defined as a negative change in BMI z-score ≥10% from baseline.

Statistical analysis

All data are expressed as mean ± standard deviation (SD), absolute values or percentages. Skewed variables were log transformed. The Wilcoxon signed-rank test was used to assess changes in the anthropometric, biochemical and cardiovascular variables.

A two-way repeated measure ANOVA was performed to evaluate the time effect, the treatment effect and the interaction effects of: the negative change in BMI z-score ≥10%, the reduction of HOMA-IR, the number of MetS criteria according to NCEP-ATPIII classification [16], of SBP and of DBP on the dependent variables (cardiovascular parameters). Sum of squares type III was used. The following covariates were also subsequently introduced: sex and pubertal status.

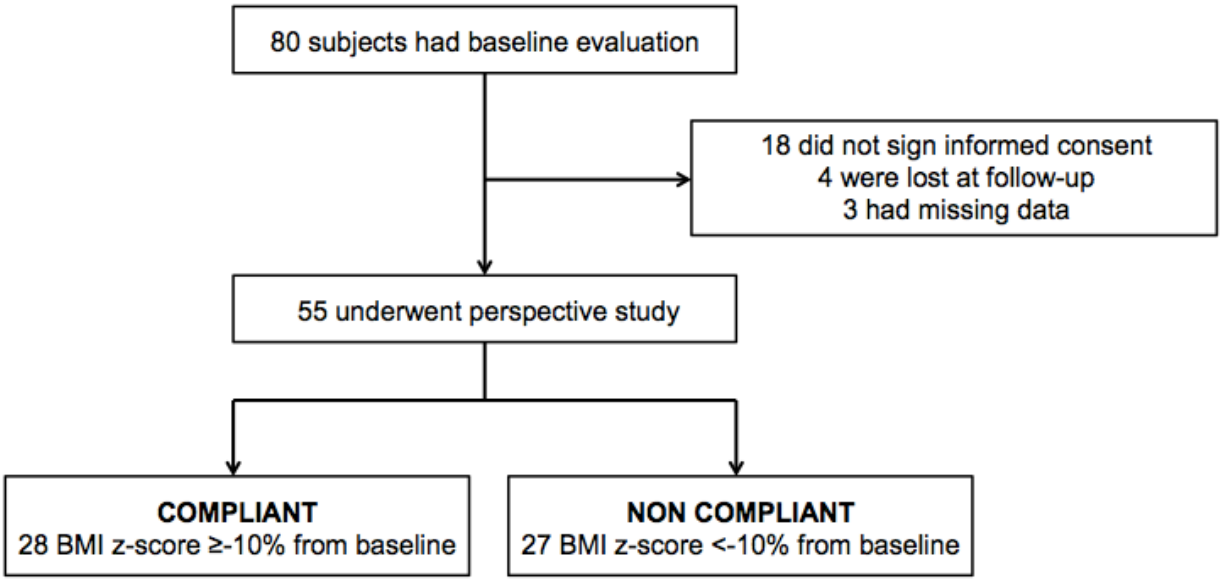
Statistical significance was determined at a p value of <0.05. All the statistical analyses were performed using R Statistical Software and SPSS for Windows version 17.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Of the 80 subjects who underwent baseline evaluation (see chapter 2), 62 accepted the longitudinal study while 18 refused to sign the consent for the subsequent follow-up visits and were excluded. During the study protocol, 4 patients were lost at follow-up and in 3

subjects some data (2 subjects did not perfom biochemical evaluation for the impossibility to find an ev access, 1 subject did not have ecochardiografic and vascular assessment) were not recorded and they were excluded from the final study population. Out of 55 patient, 28 (51%) were considered compliant to behavioral program on the basis of self-reported questionnaires and a negative change in BMI z-score ≥10% from baseline (Figure 1).

Figure 1. Study flow diagram.



The baseline characteristics were similar in the compliant and non-compliant group and are shown in table 1.

Table 1. Baseline characteristics of 28 compliant (C) and 27 non-compliant (NC) obese subjects.

	C	NC	*P value*
Subjects	28	27	
Age (years)	11.2±2.7	11.4±2.8	ns
Female	13 (44.8%)	13 (48.1%)	ns
Prepubertal	11 (40.7%)	9 (31.0%)	ns
Height (cm)	149.5±13.7	151.6±17.0	ns
Height (SDS)	1.0±1.1	1.1±1.2	ns
Weight (kg)	67.3±21.0	75.2±25.4	ns
BMI (kg/m^2)	29.3±5.2	31.8±5.7	ns
BMI z-score	2.23±0.51	2.50±0.57	ns
Waist (cm)	88.9±14.2	95.1±13.7	ns
SBP (mmHg)	123.3±18.2	126.0±15.9	ns
DBP (mmHg)	78.0±11.8	77.7±10.0	ns

Values are number (%) or means±SD.

Legend: BMI, body mass index; DBP, diastolic blood pressure; ns, not significant; SBP, systolic blood pressure; SDS, standard deviation score.

Anthropometric and biochemical variables

After the 12 months behavioral intervention, compliant subjects showed a significant reduction of BMI ($p<0.0001$), BMI z-score ($p<0.0001$), blood glucose at baseline ($p<0.006$) and after 120 minutes during the OGTT ($p<0.007$) and insulin resistance ($p<0.002$) and a significant increase of HDL-cholesterol ($p<0.0001$) and insulin sentivity ($p<0.01$). In contrast, non-compliant subjects had higher waist circumference ($p<0.03$), DBP ($p<0.01$) and a worse insulin sensitivity ($p<0.01$) at T12 compared to baseline (Table 2).

Table 2. Changes in anthropometric and biochemical variables during the 12-months behavioral intervention in compliant (C) and non-compliant (NC) obese children.

	C			NC		
N°	**28**			**27**		
	T0	**T12**	***P value***	**T0**	**T12**	***P value***
BMI (kg/m^2)	29.3±5.2	26.1±4.0	0.0001	31.8±5.7	31.3±4.8	ns
BMI z-score	2.20±0.51	1.60±0.64	0.0001	2.50±0.57	2.42±0.53	0.009
Waist (cm)	88.9±14.2	86.7±11.4	ns	95.1±13.7	97.8±11.3	0.03
SBP (mmHg)	123.3±18.2	124.5±12.3	ns	126.0±15.9	126.4±15.7	ns
DBP (mmHg)	78.0±11.8	77.2±10.0	ns	77.7±10.0	82.8±9.0	0.01
Glucose T0 (mg/dl)	87.7±5.7	84.3±6.5	0.006	88.5±7.9	87.6±6.6	ns
Glucose T120 (mg/dl)	111.4±15.4	99.4±13.6	0.007	112.9±21.5	111.0±21.3	ns
Total-c (mg/dl)	139.9±33.4	139.5±24.9	ns	145.8±25.0	146.1±24.4	ns
HDL-c (mg/dl)	43.9±10.0	48.8±9.0	0.0001	39.9±8.6	41.7±9.9	ns
LDL-c (mg/dl)	81.3±27.6	78.2±22.7	ns	87.0±23.9	84.0±26.4	ns
TG (mg/dl)	69.4±32.7	61.9±27.6	ns	93.3±58.3	101.9±97.2	ns
Uric acid (mg/dl)	5.1±1.8	5.1±1.3	ns	4.9±1.1	4.9±1.3	ns
HOMA-IR	4.3±2.8	3.0±1.4	0.002	5.7±3.6	5.9±3.6	ns
ISI	3.4±1.6	4.4±1.9	0.01	3.4±3.4	2.5±1.4	0.01
QUICKI	0.32±0.03	0.33±0.02	0.02	0.31±0.03	0.30±0.02	**ns**

Values are means±SD.

Legend: BMI, body mass index; DBP, diastolic blood pressure; HOMA, homeostasis model assessment; ISI, Matsuda index; N°, number of subjects; ns, not significant; QUICKI, Quantitative Insulin-Sensitivity Check Index; SBP, systolic blood pressure; TG, triglycerides; Total-c, total cholesterol.

Echocardiographic and vascular assessment

In compliant subjects we found a significant decrease of the heart rate ($p<0.01$) and of carotid artery intima-media thickness (CIMT, $p<0.01$) and a significant increase of left atrial area ($p<0.04$), mitral peak early diastolic velocity (E, $p<0.001$), and abdominal aortic diameter at minimum diastolic expansion ($p<0.03$) at T12 compared to baseline values. Non-compliant patients had higher left ventricular end-diastolic diameter (LVEDD, $p<0.04$)

at T12 compared to T0. Even in this group an increase of the E ($p<0.007$) and a decrease of the CIMT ($p<0.01$) was shown after 12 months (Table 3).

Table 3. Changes in cardiovascular variables during the 12-months behavioral intervention in compliant (C) and non-compliant (NC) obese children.

	C			**NC**		
N°	**28**			**27**		
	T0	**T12**	***P value***	**T0**	**T12**	***P value***
HR (b/min)	81.1±12.8	73.8±10.3	0.01	85.8±10.7	84.7±12.7	ns
EF (%)	69.8±7.9	71.1±7.9	ns	70.0±9.9	69.4±6.8	ns
FS (%)	39.7±6.3	40.5±4.6	ns	42.9±7.4	40.1±5.7	ns
LVEDD (mm)	45.4±6.0	47.1±6.6	ns	46.1±7.7	47.0±5.1	0.04
LVEDD z-score	-0.65±0.91	-0.24±0.91	ns	-1.35±1.28	-0.94±1.19	ns
LVESD (mm)	27.5±4.8	28.0±5.1	ns	28.2±7.1	28.4±3.4	ns
LVESD z-score	-0.74±1.07	-0.52±1.13	ns	-1.16±1.13	-1.03±1.24	ns
IVSD (mm)	7.6±1.6	7.6±1.7	ns	7.7±2.0	8.2±1.9	ns
IVSD z-score	-0.28±0.94	-0.49±0.80	ns	-0.09±1.05	0.30±1.06	ns
LVPWD (mm)	7.5±1.8	7.5±1.2	ns	8.5±2.8	7.7±2.1	ns
LVPWD z-score	0.20±0.20	0.31±1.00	ns	0.46±0.95	0.35±1.08	ns
LAD (mm)	32.0±5.2	33.0±4.9	ns	33.7±6.1	33.4±5.4	ns
LAD z-score	1.12±1.25	0.83±1.22	ns	1.26±1.00	1.54±0.85	ns
Ao (mm)	24.0±3.2	25.0±3.8	ns	25.0±5.3	26.5±4.1	ns
LA/Ao ratio	1.40±0.21	1.34±0.22	ns	1.35±0.22	1.30±0.19	ns
LV mass (g)	113.1±47.2	123.2±40.9	ns	131.7±85.1	121.0±53.2	ns
LV MI (g/m^2)	66.8±18.3	73.9±17.8	ns	72.0±30.9	64.8±17.5	ns
LV mass z-score	-0.001±1.48	0.11±1.17	ns	0.26±1.49	-0.15±1.07	ns
RWT	0.33±0.07	0.33±0.07	ns	0.37±0.09	0.36±0.08	ns
LV area (cm^2)	26.5±6.0	26.9±5.7	ns	26.9±7.2	26.5±5.0	ns
LV volume (mL)	83.0±27.3	84.8±26.9	ns	86.3±36.9	83.5±23.4	ns
LA area (cm^2)	12.9±3.5	14.4±2.8	0.04	13.9±4.6	13.9±3.1	ns
LA volume (mL)	32.5±13.9	37.2±12.5	ns	31.6±12.8	34.9±12.2	ns
Mitral E (cm/sec)	1.02±0.20	1.12±0.18	0.001	1.04±0.24	1.21±0.59	0.007
Mitral A (cm/sec)	0.55±0.14	0.56±0.11	ns	0.60±0.13	0.65±0.23	ns
Mitral E/A ratio	1.91±0.43	2.02±0.44	ns	1.70±0.44	1.80±0.47	ns
CIMT (mm)	0.62±0.17	0.52±0.09	0.01	0.61±0.11	0.55±0.05	0.01
AoDd (mm)	10.0±1.6	10.8±1.7	0.03	10.8±2.4	11.2±1.6	ns

AoDs (mm)	12.5±1.9	13.1±1.5	ns	13.2±2.3	13.5±1.8	ns
S	0.25±0.10	0.23±0.12	ns	0.24±0.15	0.21±0.11	ns
Ep (mmHg)	201.8±128.3	249.1±161.5	ns	478.7±1140.1	253.4±149.2	ns
Ep*	2.7±1.8	3.4±2.2	ns	6.3±15.5	3.0±1.8	ns
BAD basal (mm)	3.40±0.74	3.49±0.54	ns	3.53±0.73	3.44±0.57	ns
BAD after (mm)	3.48±0.68	3.38±0.88	ns	3.70±0.75	3.48±0.94	ns
FMD (%)	3.4±14.6	0.9±10.5	ns	6.5±18.4	1.8±24.6	ns

Values are means±SD.

Legend: A, peak velocity of late diastolic transmitral wave; AoDd, abdominal aortic diastolic diameter; AoDs, abdominal aortic systolic diameter; BAD brachial artery diameter; CIMT, carotid intima-media thickness; E, peak velocity of early diastolic transmitral wave; EF, LV ejection fraction; Ep, pressure strain elastic modulus; Ep*, pressure strain normalized for diastolic blood pressure; FMD, brachial artery flow-mediated dilation; FS, LV fractional shortening; HR, heart rate; IVSD, interventricular septum diastolic dimension; LA, left atrium; LAD, LA end-systolic diameter; LV, left ventricle; LVEDD, LV end-diastolic dimension, LVESD, LV end-systolic dimension; LVPW, LV posterior wall diastolic dimension; N°, number of subjects; ns, not significant; RWT, relative wall thickness; S, aortic strain.

Effects of the 12-month behavioral intervention

A two-way repeated measure ANOVA was performed to evaluate the time effect, the treatment effect and the interaction effects on all the cardiovascular dependent variables according to the negative change in BMI z-score ≥10% from baseline (Table 4). We found a significant effect of the interaction (time*treatment) for the mitral peak early diastolic velocity E (F:4.562, $p<0.04$) and E/A ratio (F:5.614, $p<0.02$), even when adjusted for sex and pubertal stage. We did not find any significant effect of the interaction analysing subjects who did and did not show a reduction of insulin resistance (HOMA-IR). Out of 55 patients, 20 (36.4%) showed a reduction in the number of metabolic syndrome criteria based on NCEP-ATPIII classification. Reducing the numer of MetS criteria, LV area and volume decreased with a significant effect of the interaction (LV area, F:5.918, $p<0.01$; LV volume, F:3.863, $p<0.05$), even when adjusted for sex and pubertal stage (Figure 2). Furthermore, 24 (43.6%) subjects showed a decrease of the SBP and 20 (36.4%) of the

DBP. In both children who presented a reduction of SBP or DBP, a significant effect of the interaction was shown for CIMT (SBP, F:3.940, p<0.05; DBP, F:7.988, p<0.007) even weighted for confounding factors (Figure 3).

Table 4. Cardiovascular variations obtained with multivariable analysis of repeated measure according to the negative change in BMI z-score ≥10% from baseline.

	Time	Treatment	Interaction
HR (b/min)	F:6.443	F:8.459	F:2.243
	p<0.01	p<0.005	
EF (%)	F:0.182	F:0.112	F:0.292
FS (%)	F:0.456	F:1.952	F:1.969
LVEDD (mm)	F:5.714	F:0.026	F:0.063
	p<0.02		
LVEDD z-score	F:5.423	F:6.266	F:0.237
	p<0.02	p<0.01	
LVESD (mm)	F:1.482	F:0.001	F:0.167
LVESD z-score	F:1.279	F:2.931	F:0.154
IVSD (mm)	F:0.686	F:0.466	F:0.686
IVSD z-score	F:0.274	F:6.642	F:2.241
		p<0.01	
LVPWD (mm)	F:2.971	F:1.890	F:2.228
LVPWD z-score	F:1.493	F:0.295	F:2.821
LAD (mm)	F:0.824	F:0.459	F:0.285
LAD z-score	F:0.067	F:4.303	F:1.954
		p<0.04	
Ao (mm)	F:3.532	F:2.540	F:0.422
LA/Ao ratio	F:1.793	F:1.083	F:0.079
LV mass (g)	F:0.977	F:0.026	F:0.832
LV mass index (g/m^2)	F:0.001	F:0.154	F:2.288
LV mass z-score	F:0.544	F:0.001	F:1.662
RWT	F:4.515	F:0.536	F:1.146
	p<0.03		
LV area (cm^2)	F:0.001	F:0.001	F:0.251
LV volume (mL)	F:0.029	F:0.026	F:0.302
LA area (cm^2)	F:1.296	F:0.067	F:1.856
LA volume (mL)	F:4.865	F:0.734	F:0.628

	p<0.03		
Mitral E (cm/sec)	F:9.976	F:0.782	F:4.562
	p<0.0001		p<0.04*
Mitral A (cm/sec)	F:1.231	F:8.224	F:0.983
		p<0.006	
Mitral E/A ratio	F:0.876	F:3.213	F:5.614
			p<0.02§
CIMT (mm)	F:8.809	F:2.608	F:1.518
	p<0.004		
AoDd (mm)	F:4.431	F:2.158	F:0.371
	p<0.04		
AoDs (mm)	F:2.248	F:1.682	F:0.176
Aortic Strain, S	F:1.587	F:0.325	F:0.017
Ep (mmHg)	F:0.642	F:1.660	F:1.437
Ep*	F:0.793	F:1.168	F:1.669
BAD basal (mm)	F:0.006	F:0.048	F:0.757
BAD after (mm)	F:1.636	F:0.734	F:0.150
FMD (%)	F:1.234	F:0.282	F:0.065

A two-way repeated measure ANOVA was performed to evaluate the time effect, the treatment effect and the interaction effects on the dependent variables.

After adjusting for sex and pubertal status: *p<0.05, §p<0.02.

Legend: see Table 3 legend.

Figure 2. Variations of LV area and volume in 12 months in subjects with (blu bars) or without (red bars) a reduction of the number of metabolic syndrome criteria according to NCEP-ATPIII classification [16].

Data are expressed as marginal mean ± SEM. Data are significant in interaction (*p<0.01; **p<0.05). T0: baseline. T12: after 12 months of behavioral intervention.

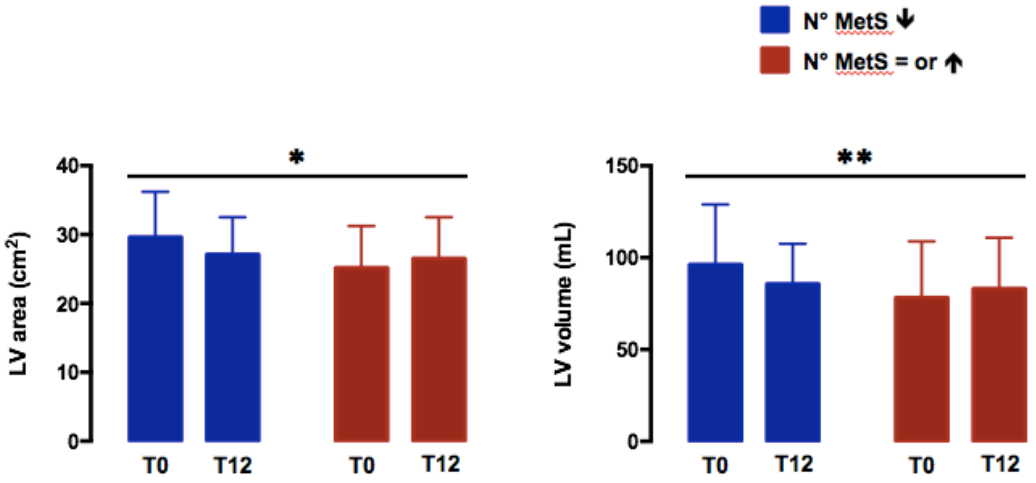
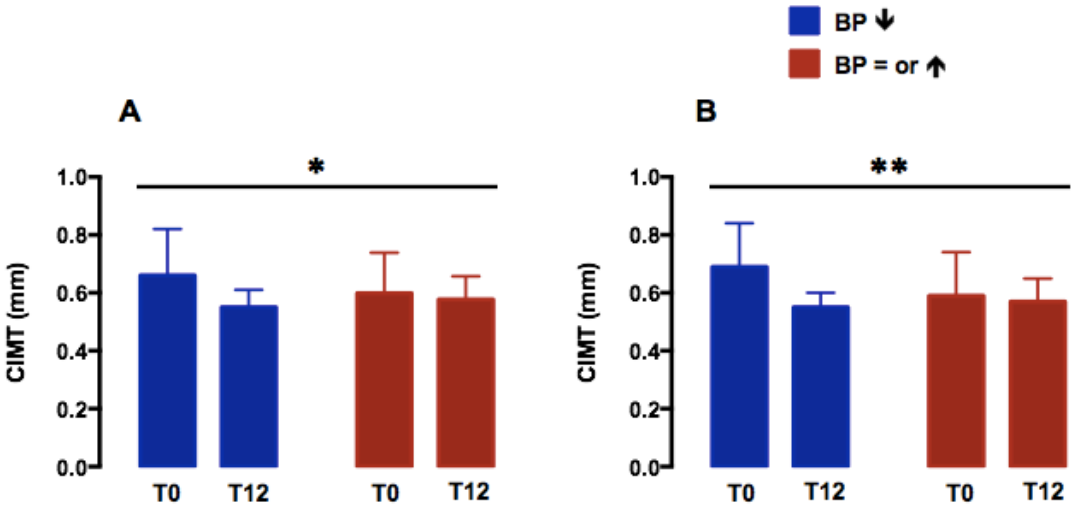


Figure 3. Variations of CIMT in 12 months in subjects with (blu bars) or without (red bars) a reduction of SBP (panel A) and DPB (panel B).

Data are expressed as marginal mean ± SEM. Data are significant in interaction (*p<0.05; **p<0.007). T0: baseline. T12: after 12 months of behavioral intervention.



DISCUSSION

In this study, we found an improvement of cardiovascular dysfunction in obese children after a 12 months intervention based on an isocaloric Mediterranean diet and aerobic exercise training.

Lifestyle interventions of healthy eating, exercise, and reducing sedentary activity are the cornerstones therapies of obesity in childhood and adolescence. In adults, behavioral intervention shown a weight-loss efficacy of between 5 and 10%, often resulting in CV risk factors improvements [17,18]. Furthermore, pediatric lifestyle intervention trials have also reported improvements in body composition and metabolic parameters [19-24]. In line with this, we also found that children who showed a significant change in BMI z-score from baseline, improved glucose metabolism, HDL-cholesterol and insulin sensitivity with a parallel reduction of insulin resistance.

In a previous study conducted on a population of obese children and adolescents that included the subjects analyzed for this perspective study, we found significant differences in cardiovascular structure and function compared with a group of normalweight patients [4]. However, cardiovascular parameters fall all in the normal ranges and highlighting the cardiac remodeling and the impaired vascular function in obese pediatric subjects is difficult with standard imaging techniques and without a control population. As the direct cardiovascular effects of childhood obesity are relatively mild and subtle, the effect of intervention programs on cardiac remodeling and cardiovascular function is difficult to measure. In this study, we found a significant increase of the E/A ratio (mainly related to the increased early diastolic velocity/E-velocities). The effect of weight loss on diastolic function in children has been previously reported. In a study by Ippisch et al. in morbidly obese adolescents aged 13-19 years, a significant reduction of BMI after 10 months from bariatric surgery was accompanied with a decrease of mitral A-velocities, an increase of

the E/A ratio and of mitral E'-velocities [25]. In addition, an exercise-training program of 8 weeks improved diastolic function (mitral inflow A-velocities decreased, mitral Doppler tissue derived E'- and A'-velocities increased, and E/E' ratio decreased) without significant changes of BMI in a group of obese children aged 8-14 years [26]. Ingul et al. recently performed a very small pilot study in 10 obese adolescents and reported that a 13-week aerobic interval-training program normalized systolic and diastolic cardiac function among obese adolescents compared with a normalweight counterparts [27]. Furthermore, Zeybek et al. found that a low carbohydrate diet during 6 months significantly reduced BMI and improved right ventricle diastolic function [28]. Taken together, these data suggest that the diastolic dysfunction is reversed by weight loss. Moreover, previous studies have shown that a reverse heart remodeling occurs after the normalization of functional changes. This outline the importance to continue the long-term follow-up of our patients and to improve strategies aimed to prevent the usually seen high drop-out rate of lifestyle intervention programs.

An interesting finding of the current study is the significant effect of metabolic syndrome on left ventricular area and volume; in children with a reduction of the number of MetS criteria after the 12 months intervention, LV area and volume significantly decrease compared with patients with the same or a greater number of MetS criteria from the baseline. As previously shown, in obese children MetS was associated with greater heart dimension and mass with straightforward linear raises when increasing the number of matched criteria for MetS [4]. Subjects who met the MetS criteria, presented worst metabolic parameters (dyslipidemia, dysglycemia) with higher prevalence of hypertension and altered BMI with a significant impact on cardiac structural alterations. Reducing the number of MetS criteria, as reducing CV risk factors, could change heart remodeling. Our data confirm the influence of MetS also in the pediatric age, suggesting the usefulness of programs aimed at preventing or reversing MetS and the related future cardiovascular

dysfunction.

In the current study, we found that in children in whom intervention reduces blood pressure, CIMT significantly decreased. Systemic hypertension is a common complication of childhood obesity. Elevated BP has been related to endothelial and smooth muscle cell dysfunction, arterial stiffness [29], and increased left ventricular mass [30]. Systemic hypertension in obese children might be due to impaired endothelial function or activation of the sympathetic nervous system or insulin resistance [29]. In adults, a reduction of 5 mmHg in diastolic BP is associated with a 35% decrease of stroke [31]. Farpour-Lambert et al. showed, in a randomized controlled trial enrolling 44 obese prepubertal children that a 3 months exercise training programme significantly reduced systolic and diastolic BP and improved arterial stiffness and CIMT [32]. Other pediatric studies have demonstrated improvements in flow-mediated dilatation and intima-media thickness with diet alone, exercise alone or diet and exercise [14,33,34]. Furthermore, in adults, 1 SD increase in CIMT has been associated with a 2-fold raised risk of ischemic stroke or myocardial infarction [35]; thus changes of CIMT could determine important health gains at a population level.

Our study has several potential limitations. The major limit is the relatively small size of the population. Moreover, our study was not a randomized controlled trial. We perform a prospective data collection, which is certainly susceptible to selection bias. Finally a more extensive use of vascular imaging modalities including speckle tracking echocardiography and cardiac MRI would have certainly improved the results of the current study.

In conclusion, this study shows that a healthy-lifestyle intervention could reverse cardiovascular dysfunction in obese children. This effect is probably mediated by several mechanisms as the reduction of body weight, the improvement of metabolic status and the reduction of systemic blood pressure.

Further larger studies and randomized trials are warranted to confirm our findings and

future research should investigate the molecular basis of these changes.

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Chapter 5

Conclusions and future perspectives

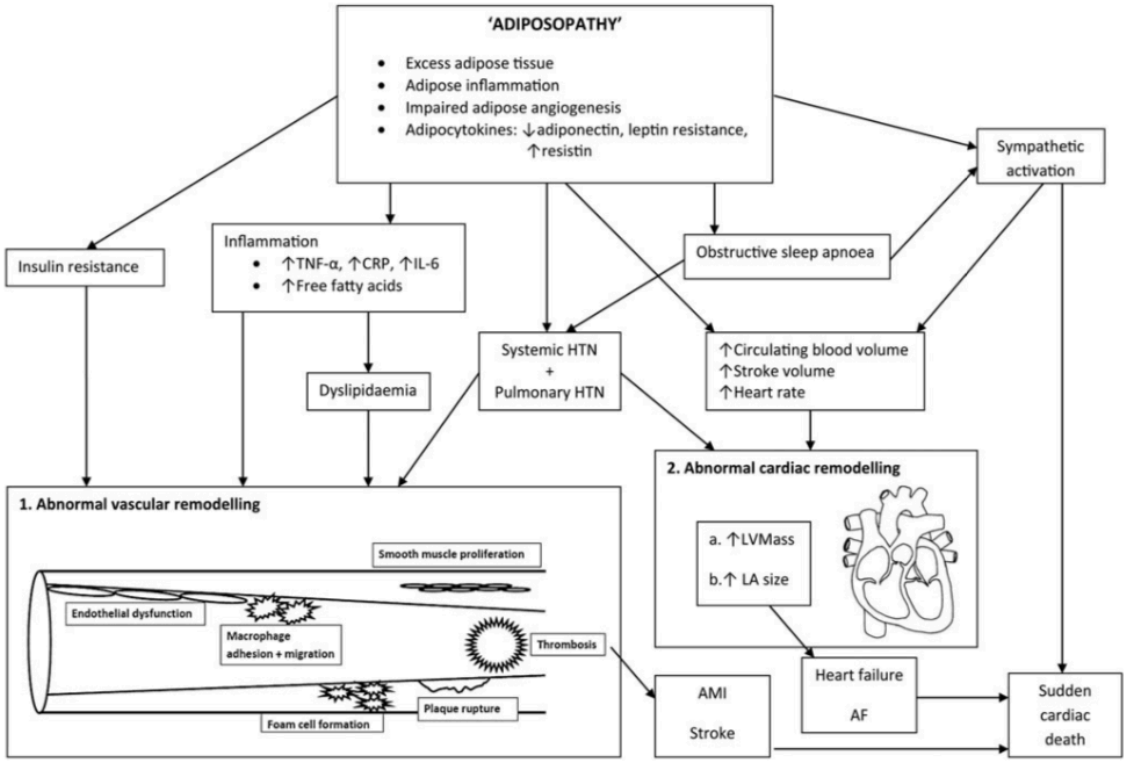
The childhood obesity epidemic is one of the most alarming public health issues worldwide. Pediatric obesity is associated with a wide range of severe complications, exponentially increasing the risk of premature morbidity and mortality and negatively impacting the prevalence and the age of cardiovascular events and death [1,2]. This thesis demonstrates that, beyond traditional cardiovascular risk factors, pediatric obesity is currently associated with cardiovascular impairement. This data requires immediate attention in order to precociously identify children at greater risk of cardiovascular dysfunction. With this purpose, the finding of clinical markers, like serum uric acid, insulin resitance and estimated glomerular filtration rate, helpful to recognize an “unhealthy” metabolic profile and to stratify risk among pediatric population is crucial. Furtermore, it is well known that childhood obesity is highly predictive of adult obesity [3] while recent evidences show that some serious complications and adverse events could be prevented by reversing this trend and gaining a normal weight before puberty [4]. In this thesis, we show that cardiovascular damage could be revesed by a healthy-lifestyle program, the cornestone of obesity treatment during pediatric age and this highlight the importance to establish large behavioral programs with trained personnel, in qualify units, and with a close follow-up to prevent drop-outs and to obtain long-term results. In this perspective, the role of the pediatric community to prevent obesity and its complications, to precocously identify children at higher risk and to promptly establish therapeutic measures is crucial for the whole community and the pediatric age represents a window of opportunities for the future public health.

Future perspectives

The cumulative burden of “traditional” cardiovascular risk factors related to obesity has been related to cardiovascular impairment and atherosclerosis. However, recent studies have focused on novel mediators of cardiovascular dysfunction. It is well known that

obesity is characterized by insulin resistance and a low-grade inflammation. Particularly, dysfunctional visceral adipose tissue could be implicated in inflammation, oxidative stress, and angiogenesis (Figure 1) [5]. Furthermore, it has been established that free fatty acids (FFA) represent an important link between obesity, insulin resistance, and inflammation [6].

Figure 1. Pathophysiology of cardiovascular dysfunction in "adiposopathy" of obesity [5].



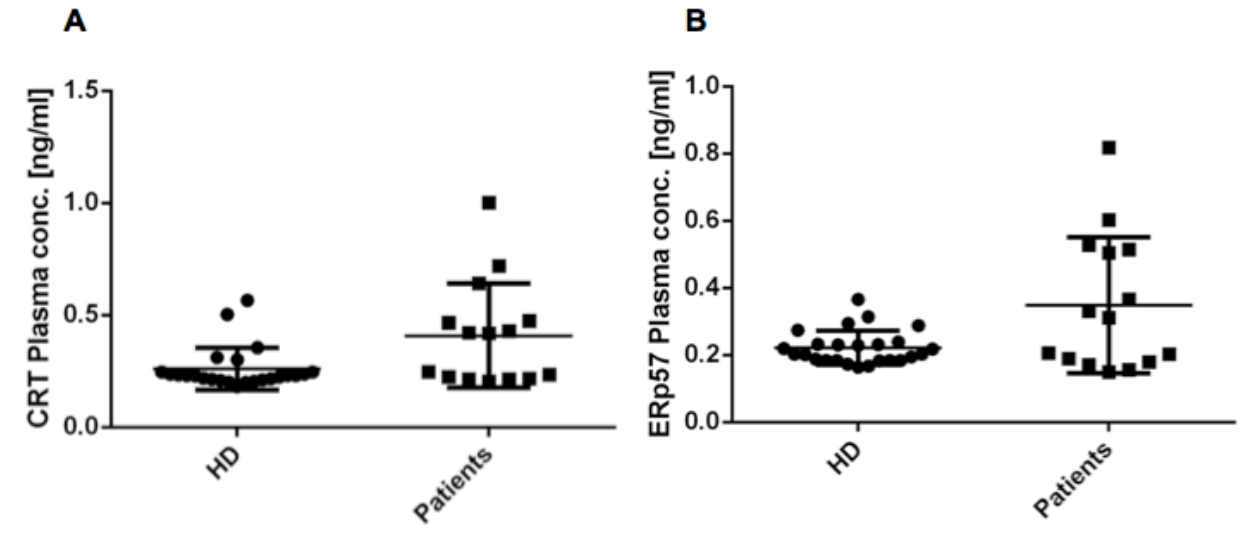
However, seen that not all insulin resistant subjects have elevated plasma FFA levels other mechanisms should be involved. One of these appears to be endoplasmic reticulum (ER) stress [7]. The ER is a main site for protein and for lipid and sterol synthesis. Ribosomes attached to the ER membranes release newly synthesized peptides into the ER lumen, where protein chaperones and foldases assist in the proper posttranslational modification and folding of these peptides. The folded proteins are then released to the Golgi complex for final modification. If the influx of misfolded or unfolded peptides exceeds

the ER folding capacity, ER stress ensues. Three proximal ER stress sensors have been identified. They are inositol-requiring enzyme (IRE)-1, PKR-like ER protein kinase (PERK), and activating transcription factor-6. These sensors trigger activation of pathways, termed the unfolded protein response (UPR), which act to reduce the ER stress.

In vitro and animal models suggest that ER stress could represent a response to an excessive macronutrient intake and that ER stress can lead to the development of insulin resistance and inflammation via the phosphorylation and activation of C-jun N- terminal kinase (JNK), the activation and nuclear translocation of nuclear factor κB (NFκB), and the production of reactive oxygen species (ROS) [8,9]. So far, however, ER stress has only been reported in some rodent models of obesity and few studies exist only in adult obese human subjects [10].

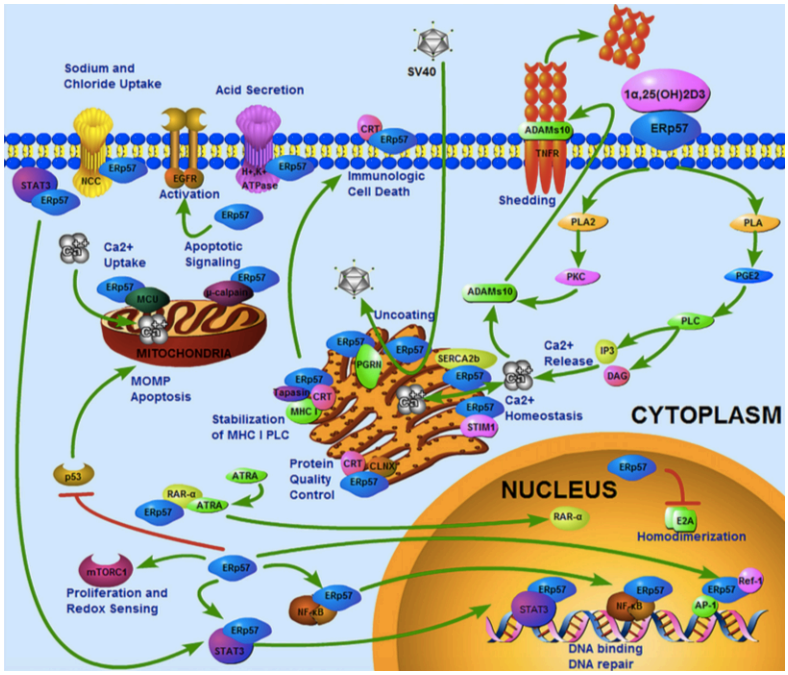
Our preliminary data show that calreticulin (CRT) and ERp57, ER stress-responsive proteins, have higher concentrations in plasmatic samples of 14 obese children compared to a group of normalweight age- sex- and pubertal status matched controls (Figure 2) suggesting activation of UPR and therefore the presence of ER stress.

Figure 2. Preliminary data: calreticulin (CRT) (panel A) and ERp57 (panel B) plasmatic levels (ng/mL) in 14 obese children and lean controls.



The chaperone CRT facilitates protein folding by shielding unfolded protein regions from surrounding proteins, thus preventing aggregation. In addition, CRT and calnexin (CLNX) act in the quality-control machinery of the ER that monitors the glycosylation status of proteins and determines whether a molecule is exported to the Golgi complex or targeted for ER-associated degradation [8]. ERp57 participates in protein folding in association with CRT and CLNX (Figure 3) [11].

Figure 3. Multiple binding partners and functions of ERp57. ERp57 can be localized to the ER, nucleus, cytoplasm, mitochondria and plasma membrane. In the ER, ERp57's b-b′ domains associate with the P-domain of CRT/CLNX and ERp57 a and a′ active domains mediate the catalysis of CRT/CLNX bound substrates [11].



The finding of the presence of ER stress from pediatric age could lead novel insights to the complex mechanisms involved in obesity-related insulin resistance and inflammation and therefore atherosclerosis and cardiovascular damage. Furthermore, we will investigate if a healthy-lifestyle intervention and the reduction of nutrients intake could attenuate the ER stress, improving insulin resistance and inflammation and finally leading to a better metabolic profile.

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