



## Epicardial fat, abdominal adiposity and insulin resistance in obese pre-pubertal and early pubertal children



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### ABSTRACT

**Objective:** To assess the cross-sectional association of epicardial fat with insulin resistance, major abdominal adipose depots, and cardiovascular disease (CVD) risk factors in obese pre-pubertal and early pubertal children.

**Methods:** By using magnetic resonance imaging in 30 pre-pubertal and early pubertal patients [21 males, Tanner Stage I-II, median age 11.2 (2.95) y, BMI z-score  $2.56 \pm 0.11$  SDS], visceral (VAT), subcutaneous (SAT), epicardial adipose tissues (EAT) and hepatic fat fraction (HFF) were estimated. Lipid profile, liver function tests, circulating adipokines and markers of inflammation [leptin, adiponectin, tumor necrosis factors- $\alpha$  (TNF- $\alpha$ ), C-reactive protein (CRP), interleukins 6 and 10 (IL-6, IL-10)] were assayed. Insulin resistance was estimated by the homeostasis model assessment of insulin resistance (HOMA-IR). Body composition was measured by dual-energy X-ray absorptiometry.

**Results:** In 14 insulin resistant children (HOMA-IR  $>2.5$ ), median values of EAT were significantly higher than in insulin sensitive mates [ $54.0$  (35.45)  $\text{cm}^3$  vs.  $27.2$  (17.03)  $\text{cm}^3$ ;  $p = 0.03$ ]. Moreover, EAT performed no differently in identifying insulin resistant patients (AUC 0.737; 95% CI 0.538–0.936;  $p = 0.028$ ) from VAT (AUC 0.772; 95% CI 0.599–0.945;  $p = 0.011$ ); SAT (AUC 0.795; 95% CI 0.628–0.962;  $p = 0.006$ ); and HFF (AUC 0.777; 95% CI 0.607–0.947;  $p = 0.010$ ). Stepwise regression analysis showed that EAT ( $\beta = 0.025$ ; 95% CI 0.012–0.038,  $p = 0.001$ ) and CRP ( $\beta = 0.622$ ; 95% CI 0.069–0.238,  $p = 0.002$ ) predicted HOMA-IR ( $R^2 = 0.71$ ;  $p = 0.001$ ), while VAT, SAT and HFF were excluded from the model.

**Conclusions:** In pre-pubertal and early pubertal obese children, EAT is a significant marker of increased insulin resistance and associated cardiovascular risk.

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## 1. Introduction

The epicardial adipose tissue (EAT) is a part of the visceral fat deposited within the pericardic sac around the heart, between the visceral layers of the pericardium and the outer wall of the myocardium. EAT shares a common embryological origin with the intra-abdominal visceral adipose tissue (VAT) and, as such, it is a metabolically active adipose tissue [1]. Hence, although EAT

represents only 1% of the total body fat mass in physiological conditions, it may be particularly important in the pathophysiology of the cardiovascular disease (CVD) risk associated with obesity [1]. The pericardial fat is usually referred to as the fat which is anterior to the epicardial one and, therefore, located between the visceral and parietal pericardium. However, not all experts adopt the same nomenclature [2].

Owing to its close proximity to the heart and coronary vasculature, the epicardial fat can secrete large amounts of pro-inflammatory adipokines and free fatty acids which locally may promote CVD [1]. In particular, perivascular adipose tissue surrounding the coronary arteries may cause *in situ* vascular dysfunction [3,4] and favor coronary atherosclerosis [5–7]. A significant relationship between epicardial fat volume and presence of coronary atherosclerosis measured by multislice computed tomography has been reported in both subjects with asymptomatic coronary artery disease [5] and with type 2 diabetes [6]. A thickness

**Abbreviations:** DXA, dual-energy X-ray absorptiometry; CRP, C-reactive protein; HFF, hepatic fat fraction; HOMA-IR, homeostasis model assessment of insulin resistance; IL-6, interleukin-6; IL-10, interleukin-10; EAT, epicardial adipose tissue; MRI, magnetic resonance imaging; SAT, subcutaneous adipose tissue; TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; VAT, visceral adipose tissue.

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of 2.4 mm is worldwide recognized as being the optimal cut-off to predict the presence of significant CAD in asymptomatic patients [7]; while a cut-off EAT volume of 75 ml show 72% sensitivity and 70% specificity for detecting the presence of atherosclerosis [5].

Thickness and volumes of either epicardial or pericardial adipose tissues close to the heart have been found quantitatively associated with various CVD risk factors and particularly with waist circumference and visceral fat as estimated also by magnetic resonance imaging (MRI) [8]. Nevertheless, in most of the studies, the association of either epicardial or pericardial fat with CVD risk factors resulted independent of the amount of visceral fat [2–16]. In the Framingham Heart Study, the fat volume within the pericardium and the intra-thoracic fat volume (the whole fat within the thorax minus the formed depot) correlated with coronary and aortic calcium, respectively [2] and with multiple markers of inflammation and oxidative stress [14]. Other studies showed that pericardial fat volume [16–18], area [12,13], and thickness [8,10,19–21] measured by computerized tomography scan [18,19], MRI [12,13,17], echocardiography [8,19–22], and at autopsy [16] correlate with blood pressure [8,12,13], coronary artery calcium [5–7,10], insulin resistance [13,20], early left ventricular dysfunction [20] and left mass [16,23].

The few studies, performed in children, have investigated ultrasonographically thickness of the subepicardial adipose tissue [15,23]. Abaci et al. [15] provided normative values of subepicardial fat thickness in 30 normal weight children and found increased thickness in 46 obese age-matched patients. Albeit no significant correlation was found between epicardial adipose thickness and degree of insulin resistance, a cut-off point of 4.1 mm identified insulin resistant children [defined as pre-pubertal children having a value of Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) >2.5 and as pubertal ones with HOMA-IR >4] with 90% sensitivity and 61% specificity. More recently, Mazur et al. [23] failed to find in 52 enrolled children any relationship between increased EAT thickness and either insulin resistance or metabolic syndrome after correction for waist circumference.

No study has hitherto investigated the association between EAT and VAT, both estimated by an accurate technique, their interplay in relation to insulin resistance, metabolic abnormalities and other major abdominal adipose tissues (i.e., the Subcutaneous Adipose Tissue, SAT; and the Hepatic Fat Fraction, HFF) in obese pre-pubertal and early pubertal children.

Aim of the present study was to evaluate in obese pre-pubertal and early pubertal children, cross-sectional associations of volumes of epicardial adipose tissue as estimated by short-axis slice MRI with insulin resistance estimated by the HOMA-IR, cardiovascular risk factors and fat accumulation within the major abdominal fat reservoirs.

## 2. Materials and methods

Thirty consecutive Caucasian obese pre-pubertal and early pubertal children were recruited at the Obesity and Nutrition Outpatient Clinic of the Pediatric Section of the University Hospital of Verona.

Inclusion criteria were: no previous treatment for obesity, Tanner stage I–II, no systemic, genetic, and endocrine disease, and no use of medication. Puberty development was clinically assessed on the basis of secondary sex characteristics. Tanner stages for pubic hair, breast configuration, and genital status were used as reference [24].

The study protocol included estimation of anthropometrics, body composition by Dual X-ray Energy Absorptiometry (DXA), localized adiposity by MRI (1.5-T Avanto, Siemens, Erlangen, Germany), estimation of fasting glucose, insulin, lipid profile,

alanine aminotransferase (ALT), aspartate aminotransferase (AST),  $\gamma$ -glutamyl-transferase ( $\gamma$ -GT), adipokines (leptin, adiponectin) and circulating markers of inflammation (C-reactive protein, CRP; Tumor Necrosis Factor- $\alpha$ , TNF- $\alpha$ ; Interleukin-6 and 10, IL-6 and IL-10). HOMA-IR was computed as: fasting plasma glucose  $\times$  fasting insulin/22.5, with glucose and insulin expressed in  $mmol\ l^{-1}$  and  $\mu U\ ml^{-1}$  [25].

Oral glucose tolerance test was performed in all children and impaired glucose tolerance or type 2 diabetes was diagnosed according to the criteria of the American Diabetes Association [26].

The protocol was in accordance with the 1975 Declaration of Helsinki, and its revision in 1983, and was approved by the Ethical Committee of the University Hospital of Verona. Informed consent was obtained from the patients' parents or responsible guardians.

### 2.1. Anthropometrics and body composition estimation

Height was measured to the nearest 0.5 cm on a standardized height board. Weight was determined to the nearest 0.1 kg on a standard physician's beam scale, with the subject dressed only in light clothes and without shoes. BMI was calculated as weight (kilograms) divided by height (meters) squared. Body composition was assessed by using a DPX-L densitometer (Lunar Corp., Madison, WI). The subjects were scanned in light clothing while lying flat on their backs. On the day of each test, the DPX-L was calibrated according to the manufacturer's instructions. The percent body fat mass (FM%) was calculated as (total body fat/body weight)  $\times$  100, where body fat and weight are expressed in kilograms.

### 2.2. Case definition

Overweight was defined as a BMI  $\geq$  85th (1.036 SDS) and <95th (1.645 SDS) percentiles; obesity as a BMI  $\geq$  95th percentile (1.645 SDS) for age and sex [27]. Hyper-triglyceridemia was defined as fasting triglycerides  $\geq$  150 mg/dl; low HDL-cholesterol as fasting HDL <40 mg/dl [28].

Systolic (SBP) and diastolic blood pressure (DBP) were measured three times while the subjects were seated, and the measurements were averaged for the analysis. Hypertension was defined as a value of systolic blood pressure  $\geq$  130 mm Hg or diastolic blood pressure  $\geq$  85 mm Hg [28].

Insulin resistance was defined as a HOMA-IR  $\geq$  2.5 [29].

### 2.3. Laboratory assessment

Fasting glucose concentration, lipids, and liver enzymes were measured by standard in-house methods. Plasma insulin levels were measured by chemiluminescent immunometric assays (Euro/DPC Ltd, Llanberis, UK). Plasma adiponectin was measured by enzyme-linked immunosorbent assay (ELISA, B-Bridge International, Mountain View, CA) according to the manufacturer's instructions. LDL-cholesterol level was estimated by the Friedewald formula [30]. Plasma CRP and leptin were both assessed by ELISA (DBC, London, Ontario, Canada). Plasma adiponectin was measured by ELISA (B-Bridge International, Mountain View, CA). IL-6, IL-10, and TNF- $\alpha$  were measured by a multiplex sandwich ELISA (Pierce Biotechnology, Rockford, IL). The reported limits of detection of these assays were 0.01 mg/l, 0.5 ng/ml, 0.23  $\mu$ g/ml, 0.2 pg/ml, 0.2 pg/ml, and 0.8 pg/ml for highly sensitive CRP, leptin, adiponectin, IL-6, IL-10, and TNF- $\alpha$ , respectively.

### 2.4. Acquisition of MRI images

Measurement of abdominal fat distribution and HFF was performed as elsewhere described [31]. A single slice at the L4 level

was used to measure adipose tissue distribution. The VAT compartment is bounded by the internal margin of the abdominal muscle walls and includes intraperitoneal, preperitoneal, and retroperitoneal adipose tissues. The SAT compartment includes the adipose tissues outside the VAT boundary. With the use of a cursor, a free-hand region of interest (ROI) was drawn around deep SAT (DSAT) and superficial SAT (SSAT). The mean signal intensity  $\pm$  standard deviation of the adipose tissue was obtained from these ROI. Hepatic fat accumulation was measured using MRI along with the Dixon method [32]. A hepatic fat fraction cut-off of 5.5% was chosen as the threshold to diagnose steatosis [33].

For the EAT assessment, we used a dark blood prepared T1-weighted multislice turbo spin-echo pulse sequence with a water suppression prepulse to obtain a transversal four-chamber view and short-axis images in the same orientations used for the cine short-axis images. Imaging parameters were as follows: time of repetition = 800 ms, time to echo = 24 ms, slice thickness = 4 mm, interslice gap = 2 mm, and field of view = 30–34 cm. The EAT amount was calculated by using the modified Simpson's rule with integration over the image slices. Epicardial fat was defined as the fat between the myocardial border and the internal visceral layer of the pericardium. The border between epicardial and pericardial fat was localized by visual inspection of the entire cine series in all slices. EAT areas were then traced manually on consecutive end-systolic short-axis images beginning at the mitral valve and ending at the last slice containing cardiac adipose tissue. The areas obtained for each slice were added together and multiplied by slice thickness to yield the volume. EAT mass was obtained by multiplying epicardial fat volumes by fat density ( $0.9196 \text{ g ml}^{-1}$ ).

### 2.5. Statistical analysis

Continuous data are reported as median and Interquartile Range (IQR), unless otherwise indicated, with categorical data as counts and percentages. The one-way analysis of variance and the Mann–Whitney *U* test were used for inter-group comparisons as appropriate. The nonparametric Spearman correlation coefficient was used.

Backward linear regression analyses were performed to identify independent correlates of HOMA-IR. The parameters successively considered were estimates of fat distribution (EAT, VAT, SAT and HFF) and, successively, markers of inflammation. The significant model which explained the largest variability of HOMA-IR was reported as the best model. Receiver operating characteristic curve

analysis was used to evaluate specificity and sensitivity of estimates of abdominal and epicardial adiposity to identify insulin resistant children (HOMA-IR  $>2.5$ ).

The *p* value was set as statistically significant at  $p < 0.05$ . Data analysis was performed by using SPSS statistical software (SPSS V12.0, Inc., Chicago, IL).

### 3. Results

Table 1 summarizes anthropometric and metabolic parameters in the sample population as a whole, as well as stratified by tertiles of epicardial fat.

Table 2 reports data on total body and abdominal adiposity, adipokines and inflammatory molecules in the same groups. Patients in the highest tertile of EAT had higher body weight ( $p = 0.002$ ), absolute amount of body fat mass ( $p = 0.01$ ), BMI ( $p = 0.007$ ), and subcutaneous adipose tissue ( $p = 0.03$ ) than children in the lowest tertiles.

When patients from the first and the second tertiles were combined and compared to the ones in the highest tertile, the latter showed also significantly higher waist circumference ( $p = 0.02$ ), VAT ( $p = 0.05$ ), diastolic blood pressure ( $p = 0.04$ ), and  $\gamma$ -GT ( $p = 0.04$ ).

Sex distribution was not different among EAT tertiles ( $p = 0.2$ ).

In the whole sample, gender differences were observed just in leptin concentration [males vs. females: 26.9 (15.13) vs. 70.51 (68.9) ng/ml;  $p = 0.001$ ].

#### 3.1. Insulin resistance and metabolic syndrome

In the fourteen children (46%) classified as having insulin resistance, EAT median values were significantly higher [ $54.0 (35.45) \text{ cm}^3$ ] than in insulin sensitive mates [ $27.2 (17.03) \text{ cm}^3$ ;  $p = 0.03$ ]. No difference was observed between the ones having [ $N = 15, 50\%$ ; EAT =  $48.2 (37.2) \text{ cm}^3$ ] or the ones with not hepatic steatosis [EAT =  $37.3 (31.2) \text{ cm}^3$ ;  $p = 0.3$ ].

The distribution of metabolic abnormalities clustering into the metabolic syndrome was significantly different between sexes and among tertiles of epicardial fat: 12 boys presented with 1 abnormality and 4 with two; 7 girls presented with simple obesity, 2 with one abnormality, none of them with 2 or more abnormalities ( $p = 0.02$ ). Two boys resulted intolerant at the glucose tolerance test. EAT median values were significantly higher in the

**Table 1**  
Anthropometrics, metabolic profile and insulin resistance in the sample as a whole and divided into tertiles of epicardial fat. Data are reported as median and Interquartile Range (IQR). *P* refers to statistical significance at the ANOVA. Alanine Aminotransferase; ALT, Aspartate Aminotransferase, AST; Homeostasis Model Assessment of Insulin Resistance, HOMA-IR.

	Whole sample (N = 30)	I tertile	II tertile	III tertile	<i>p</i>
Age (years)	11.2 (2.91)	11.7 (4.7)	10.9 (2.4)	11.5 (2.35)	0.4
Body weight (kg)	65.5 (26.4)	57.4 (24.5)	58.1 (30.7)	78.8 (23.3)	<b>0.02</b>
Height (cm)	150 (19)	144 (29.5)	149.5 (27)	151.5 (11.5)	0.4
BMI ( $\text{kg/m}^2$ )	28 (6.9)	27.5 (5.2)	27.4 (5.8)	34 (7.8)	<b>0.007</b>
BMI z-score (SDS)	2.6 (0.83)	2.30 (0.85)	2.62 (0.7)	2.94 (0.7)	0.2
Waist circumference (cm)	85 (15)	81 (13)	82 (17.2)	91 (16)	0.07
SBP (mm/hg)	119 (13.5)	110 (15.5)	118.5 (14)	120 (11.7)	0.2
DBP (mm/hg)	70 (18)	65 (14)	68.5 (16.5)	78.5 (25.5)	0.1
Fasting glucose (mg/dl)	84 (7)	83.5 (9.25)	85.9 (7.50)	84.5 (11.25)	0.7
Fasting insulin ( $\mu\text{UI/ml}$ )	11.6 (9.9)	10.6 (11.7)	11 (10.9)	15.6 (13.2)	0.3
HOMA-IR	2.41 (2.04)	2.18 (2.41)	2.4 (1.98)	3.35 (2.6)	0.2
Cholesterol (mg/dl)	174 (31.7)	156.5 (55)	176 (35.25)	178.5 (22)	0.6
HDL-cholesterol (mg/dl)	44.5 (13)	46.5 (10)	46 (16.25)	42.5 (12.25)	0.9
LDL-cholesterol (mg/dl)	109 (19.4)	94.5 (43.5)	108.3 (16.6)	111.5 (22.05)	0.5
Triglycerides (mg/dl)	99 (41)	80.5 (51.5)	77 (52.5)	105 (44.75)	0.7
ALT ( $\mu\text{UI/ml}$ )	29 (10)	37.5 (4.50)	31.5 (10.5)	30.5 (6.75)	0.6
AST ( $\mu\text{UI/ml}$ )	31 (7)	28 (30.5)	29.5 (27.5)	25.5 (16.75)	0.9
$\gamma$ -GT ( $\mu\text{UI/ml}$ )	16 (10)	14.5 (3.25)	16 (17.25)	26 (5.75)	0.09

Bold represents statistically significant.

**Table 2**

Total body, abdominal, epicardial adiposity and inflammatory profile in the sample as a whole and divided into tertiles of epicardial fat. Data are reported as median and Interquartile Range (IQR). *P* refers to statistical significance at the ANOVA. C-Reactive Protein, CRP; Tumor Necrosis Factor-alpha, TNF-alpha; Interleukin-6 and 10, IL-6, IL-10.

	Whole sample (N = 30)	I tertile	II tertile	III tertile	<i>p</i>
Fat free mass (kg)	38.6 (15.9)	34.6 (12.3)	34.2 (17.7)	46.1 (12.8)	0.06
Fat mass (kg)	25.7 (11.4)	22.4 (10)	23.3 (17.7)	30.7 (11.5)	<b>0.01</b>
Fat mass (%)	40 (7.3)	40 (7.4)	39.3 (10.6)	43.3 (5.8)	0.3
Epicardial fat (cm <sup>3</sup> )	38.8 (37.0)	18.4 (9.0)	38.8 (16.2)	64.6 (26.5)	<b>&lt;0.0001</b>
Visceral fat (cm <sup>2</sup> )	68.2 (41.6)	57.6 (37.3)	59.3 (45.5)	82.8 (31.8)	0.1
Subcutaneous fat (cm <sup>2</sup> )	165.4 (70.5)	151.7 (32.7)	155.1 (53.1)	219.0 (102.7)	<b>0.03</b>
Hepatic fat fraction (%)	4.9 (21.0)	3.4 (10.7)	3.9 (27.6)	9.08 (38.5)	0.4
Leptin (ng/ml)	33.2 (17.4)	30.08 (29.5)	35.5 (30.2)	34.2 (71.5)	0.7
Adiponectin (μg/ml)	7.9 (4.3)	6.5 (3.2)	10.01 (6.9)	8.99 (3.82)	0.4
CRP (mg/l)	2.9 (5.97)	4.53 (7.14)	2.9 (4.2)	2.4 (7.7)	0.7
TNF-alpha (pg/ml)	0.4 (2.7)	0.35 (2.91)	1.8 (6.5)	0.35 (3.2)	0.3
IL-6 (pg/ml)	3.9 (6.0)	3.6 (3.97)	8.7 (14.9)	3.9 (5.10)	0.4
IL-10 (pg/ml)	0.3 (0.9)	0.3 (0.3)	1.2 (3.9)	0.3 (0.7)	0.2

Bold represents statistically significant values.

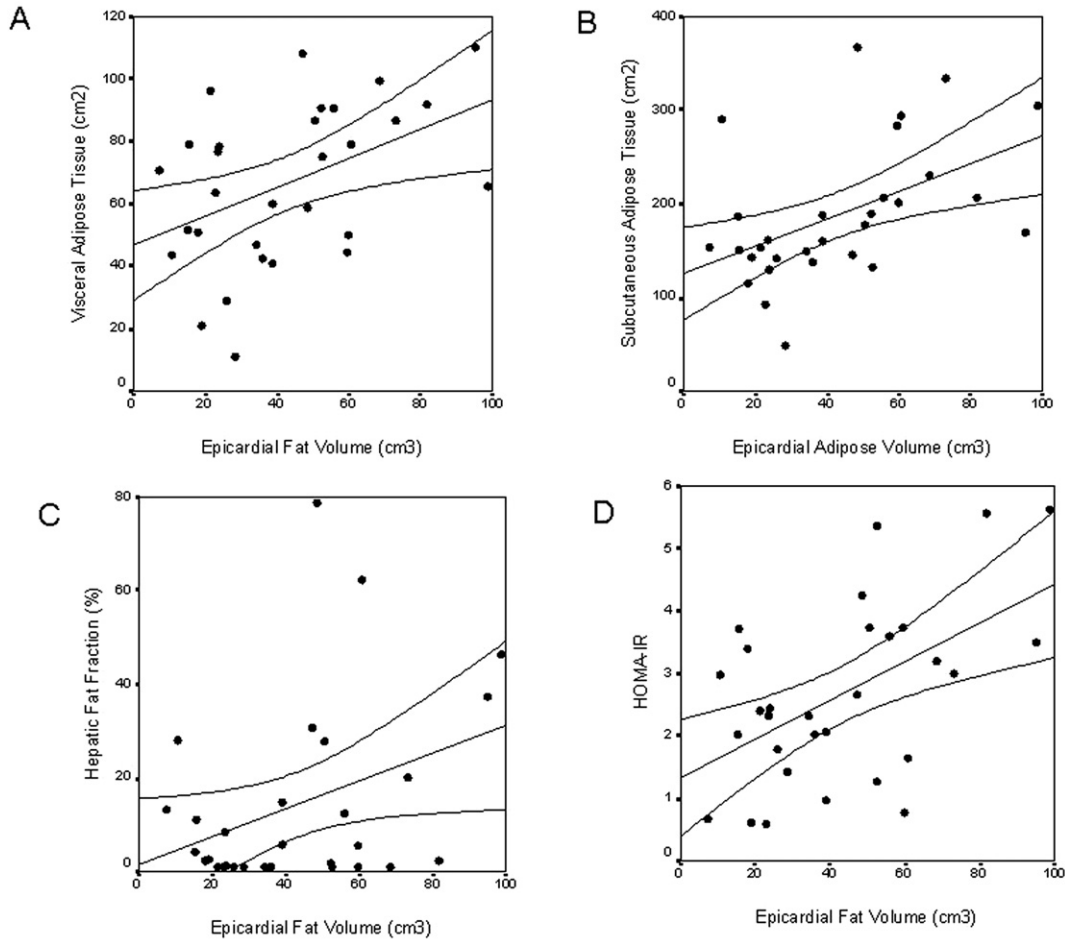
6 patients with two or more abnormalities than in the subjects with simple obesity or complicated by only one abnormality (*p* = 0.014).

3.2. Correlation and linear regression analyses

In Fig. 1, linear regressions between volumes of EAT and visceral (Panel A, *R*<sup>2</sup> = 0.202; *p* = 0.01), subcutaneous (Panel B, *R*<sup>2</sup> = 0.242;

*p* = 0.006), hepatic fat fraction (Panel C, *R*<sup>2</sup> = 0.137; *p* = 0.04) and HOMA-IR (Panel D, *R*<sup>2</sup> = 0.285; *p* = 0.002) were plotted.

At the Spearman correlation analysis, significant relationships were observed between EAT and body weight (*r*<sub>o</sub> = 0.553, *p* = 0.002); kg of fat mass (*r*<sub>o</sub> = 0.563, *p* = 0.001); waist circumference (*r*<sub>o</sub> = 0.473, *p* = 0.008); visceral (*r*<sub>o</sub> = 0.405, *p* = 0.03) and subcutaneous adipose tissue (*r*<sub>o</sub> = 0.545, *p* = 0.002); HOMA-IR (*r*<sub>o</sub> = 0.420, *p* = 0.02); systolic blood pressure (*r*<sub>o</sub> = 0.414, *p* = 0.03) and γ-GT (*r*<sub>o</sub> = 0.776, *p* = 0.003).



**Fig. 1.** Linear regressions between epicardial adipose tissue (EAT) and visceral fat area (VAT; Panel A; *R*<sup>2</sup> = 0.2018; *p* = 0.013; *y* = 0.4656 *x* + 46.727); subcutaneous fat area (SAT, Panel B; *R*<sup>2</sup> = 0.2418; *p* = 0.006; *y* = 1.4692 *x* + 125.36), hepatic fat fraction (HFF; Panel C; *R*<sup>2</sup> = 0.1371; *p* = 0.04; *y* = 0.2976 *x* + 1.5043) and HOMA-IR (Panel D; *R*<sup>2</sup> = 0.2852; *p* = 0.002; *y* = 0.0309 *x* + 1.3338).

VAT was significantly correlated with HOMA-IR ( $r_o = 0.492$ ,  $p = 0.006$ ); LDL-cholesterol ( $r_o = 0.453$ ,  $p = 0.014$ ); and CRP ( $r_o = 0.724$ ,  $p = 0.001$ ).

SAT was correlated with body weight ( $r_o = 0.702$ ,  $p < 0.0001$ ); BMI ( $r_o = 0.840$ ,  $p < 0.0001$ ); BMI z-score ( $r_o = 0.642$ ,  $p < 0.0001$ ); body fat mass ( $r_o = 0.724$ ,  $p < 0.0001$ ) and fat mass % ( $r_o = 0.433$ ,  $p = 0.017$ ), HFF ( $r_o = 0.586$ ,  $p = 0.001$ ); HDL-cholesterol ( $r_o = -0.391$ ,  $p = 0.03$ ); ALT ( $r_o = 0.502$ ,  $p = 0.008$ ) and adiponectin ( $r_o = -0.456$ ,  $p = 0.01$ ).

HFF correlated with BMI ( $r_o = 0.518$ ,  $p = 0.003$ ); BMI z-score ( $r_o = 0.466$ ,  $p = 0.009$ ); waist circumference ( $r_o = 0.511$ ,  $p = 0.004$ ); levels of ALT ( $r_o = 0.612$ ,  $p = 0.002$ ), AST ( $r_o = 0.611$ ,  $p = 0.001$ ), adiponectin ( $r_o = -0.426$ ,  $p = 0.019$ ); and HOMA-IR ( $r_o = 0.413$ ,  $p = 0.023$ ).

### 3.3. MRI adiposity, low-grade inflammation and prediction of insulin resistance

Insulin resistant patients were identified at the ROC analysis by EAT (AUC 0.737; 95% CI 0.538–0.936;  $p = 0.03$ ); VAT (AUC 0.772; 95% CI 0.599–0.945;  $p = 0.011$ ); SAT (AUC 0.795; 95% CI 0.628–0.962;  $p = 0.006$ ); and HFF (AUC 0.777; 95% CI 0.607–0.947;  $p = 0.010$ ).

Since EAT correlated significantly with both VAT and SAT, and SAT with HFF, we run stepwise regression models to identify predictors of HOMA-IR among the above mentioned fat depots and adipokines measured in the present series. The model which predicted HOMA-IR in the best possible way? ( $R^2 = 0.71$ ;  $p = 0.001$ ) included epicardial fat ( $\beta = 0.025$ , 95% CI 0.012–0.038,  $p = 0.001$ ) and CRP ( $\beta = 0.154$ , 95% CI 0.069–0.238,  $p = 0.002$ ) as predictors, while the other variables were excluded.

## 4. Discussion

To the best of our knowledge, this is the first report focusing on cross-sectional associations of epicardial fat with metabolically active abdominal fat depots as measured by MRI, insulin resistance and markers of inflammation in pre-pubertal and early pubertal obese children. The main findings of the present investigation are: i) the amount of epicardial fat is highly correlated with visceral and subcutaneous fat (Fig. 1); ii) insulin resistant obese children have higher volumes of epicardial fat than insulin sensitive children, and EAT explains 45% of the variance in insulin resistance when coupled with CRP as marker of low-grade inflammation; iii) EAT performs not differently from VAT, SAT and HFF in identifying patients with insulin resistance.

Taken together, these findings would suggest that ectopic fat accumulates simultaneously at several sites and systemic insulin resistance may promote such accumulation.

Data from the present series confirm the association between EAT and overall general adiposity already described in adult [8,11,12] and pediatric samples [15,23] but, for the first time in a pediatric population, present also findings supporting the significant association of EAT with visceral fat as estimated by MRI. Mazur et al. [23] evaluated epicardial fat thickness by ultrasonography and found a significant association between EAT and waist circumference. Hence, the authors suggested the routine ultrasound measurement of EAT as clinical means to estimate the CVD risk associated with increased visceral adiposity, as previously proposed for adults [8].

In the present series, we took advantage by a high reliable technique, the MRI, for the simultaneous measurements of EAT volume, VAT, SAT and HFF to verify whether EAT behaves as a marker of insulin resistance not different from VAT in pre-pubertal children.

The association of EAT with SAT is also noteworthy. It has been suggested that the relationship between epicardial and pericardial

fat is similar to that between SAT and VAT. As fat accumulation within the different abdominal compartments is related to a different adipocyte size and adipogenic capacity of these tissues during adolescence, a time when the expansion of white adipose tissue results from combined adipocyte hypertrophy and hyperplasia [34], epicardial and pericardial fat may behave similarly and the epicardial fat expansibility may parallel the SAT expansibility.

As to EAT ability to predict insulin resistance, we did not observe a significant correlation between EAT and HOMA-IR, but insulin resistant patients had significantly higher EAT than insulin sensitive mates. In keeping with such findings, Abaci's et al. [15] found no association between epicardial thickness and insulin resistance, but EAT was able to identify insulin resistant patients with good specificity and sensitivity.

To dissect the impact of epicardial fat on insulin resistance despite the cross-sectional design of the present study and overcome the co-linearity expected among estimates of abdominal adiposity, a backward regression approach was used. The results of this analysis showed that EAT was able to explain 45% of the variance in insulin resistance when coupled with CRP. The circulating protein is a marker of inflammation, whose levels have been found to be independent of insulin resistance in young patients [35]. In our series, EAT did not correlate with any marker of inflammation. Hence, epicardial fat may play a role in the pathogenesis of cardiovascular risk associated with obesity but, at least in very young patients, this role is not pivotal and may require external insults promoting local inflammation which develops later than insulin resistance. Insulin resistance itself and free fatty acid turnover, aging, smoking endotoxin may be nominees as insults [36,37]. In the Framingham study, significant correlations were observed between epicardial fat and markers of inflammation (i.e., CRP, IL-6) also after adjusting for the degree of adiposity [14].

It is quite intriguing the strong association between EAT and  $\gamma$ -GT ( $r_o = 0.884$ ), a robust marker of metabolic risk in healthy individuals [38] which, so far, has been found limited to patients with coronary artery diseases? [39].

Serum levels of adiponectin were found to be inversely correlated with the thickness of the subcutaneous fat. This observation is consistent with previous reports in pre-pubertal children [40,41]. In the latter, very similar levels of adiponectin protein concentrations have been found in adipocytes from paired SAT and VAT, suggesting either that the differences observed in adiponectin expression in adults are not translated into differences in protein production or that they develop later in life. Alternatively, large variations in total adiponectin between the two fat compartments may only be observed after the development of an abnormal body composition with an increased proportion of visceral fat. Interestingly, in fat samples taken from normal weight children, those with higher BMI SDS, even within the non-obese range, had lower levels of adiponectin in both SAT and VAT adipocytes compared with leaner individuals, and this negative correlation was statistically significant in the SAT adipocytes. Hence, this suggests that increasing BMI, SDS in childhood is associated with reductions in SAT adiponectin content [41].

We are aware of some limitations of the study. Mainly, our cross-sectional study cannot determine, by design, causal relationships but just describe associations and that are limited to obese patients. Virtually, by having added normal weight control children, some associations (i.e., between epicardial fat and markers of inflammation) might become evident. However, as no direct benefit from participating in the study could be envisaged for normal weight children, the Ethical Committee denied approval to enclose normal weight children as controls for the study. Moreover, we did not estimate the intra-thoracic fat, and, therefore, we could not confute the hypothesis that the intra-thoracic fat is more predictive of CVD risk, inflammation and insulin resistance than the epicardial fat [42].

The strength of the study relies upon the simultaneous measurements of regional adiposity by MRI and the specific age range of the children. In fact, the pre-pubertal age and the successive transition to the puberty identify critical time-windows when the child's body starts to accumulate adiposity. Following the path of associations between the different fat depots (i.e., SAT vs. VAT) may allow tracking the natural history of the CVD risk associated with obesity in childhood.

In conclusion, epicardial fat is positively and highly correlated with increased total body adiposity and, in particular, with abdominal fat accumulation in pre-pubertal and early pubertal obese children. Fat accumulation at the epicardial site is higher in insulin resistant pre-pubertal and early pubertal children.

Longitudinal studies, aimed at investigating the interplay between adiposity make-up and macrophage infiltration of the different fat compartments, are warranted for tracking the natural history of CVD in young patients.

### Disclosure declaration

The authors declare to have nothing to disclose.

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The authors' responsibilities were as follows (the authors were responsible for the following tasks inherent the study): MeM data analysis, drafting of the manuscript; revision for content; AM; MaM; and FR: clinical study and data collection; RM: MRI images acquisition and data analysis; CM: study design, Ethical Committee submission, data analysis, drafting of the manuscript and revision for content.

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### References

- Iacobellis G, Bianco AC. Epicardial adipose tissue: emerging physiological, pathophysiological and clinical features. *Trends Endocrinol Metab* 2011;22:450–7.
- Rosito GA, Massaro JM, Hoffmann U, et al. Pericardial fat, visceral abdominal fat, cardiovascular disease risk factors, and vascular calcification in a community-based sample: the Framingham Heart Study. *Circulation* 2008;117:605–13.
- Campia U, Tesaro M, Cardillo C. Human obesity and endothelium-dependent responsiveness. *Br J Pharmacol* 2012;165:561–73.
- Tesaro M, Cardillo C. Obesity, blood vessels and metabolic syndrome. *Acta Physiol* 2011;203:279–86.
- Djaberi R, Schuijff JD, van Werkhoven JM, Nucifora G, Jukema JW, Bax JJ. Relation of epicardial adipose tissue to coronary atherosclerosis. *Am J Cardiol* 2008;102:1602–7.
- Wang CP, Hsu HL, Hung WC, et al. Increased epicardial adipose tissue (EAT) volume in type 2 diabetes mellitus and association with metabolic syndrome and severity of coronary atherosclerosis. *Clin Endocrinol* 2009;70:876–82.
- Bachar GN, Dicker D, Kornowski R, Atar E. Epicardial adipose tissue as a predictor of coronary artery disease in asymptomatic subjects. *Am J Cardiol* 2012;110:534–8.
- Iacobellis G, Assael F, Ribaldo MC, et al. Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction. *Obes Res* 2003;11:304–10.
- Wang TD, Lee WJ, Shih FY, et al. Relations of epicardial adipose tissue measured by multidetector computed tomography to components of the metabolic syndrome are region-specific and independent of anthropometric indexes and intraabdominal visceral fat. *J Clin Endocrinol Metab* 2009;94:662–9.
- de Vos AM, Prokop M, Roos CJ, et al. Peri-coronary epicardial adipose tissue is related to cardiovascular risk factors and coronary artery calcification in postmenopausal women. *Eur Heart J* 2008;29:777–83.
- Gorter PM, de Vos AM, van der Graaf Y, et al. Relation of epicardial and pericoronary fat to coronary atherosclerosis and coronary artery calcium in patients undergoing coronary angiography. *Am J Cardiol* 2008;15(102):380–5.
- Sicari R, Sironi AM, Petz R, et al. Pericardial rather than epicardial fat is a cardiometabolic risk marker: an MRI vs. echo study. *J Am Soc Echocardiogr* 2011;24:1156–62.
- Sironi AM, Pingitore A, Ghione S, et al. Early hypertension is associated with reduced regional cardiac function, insulin resistance, epicardial, and visceral fat. *Hypertension* 2008;51:282–8.
- Tadros TM, Massaro JM, Rosito GA, et al. Pericardial fat volume correlates with inflammatory markers: the Framingham Heart Study. *Obesity (Silver Spring)* 2010;18:1039–45.
- Abaci A, Tascilar ME, Saritas T, et al. Threshold value of subepicardial adipose tissue to detect insulin resistance in obese children. *Int J Obes (Lond)* 2009;33:440–6.
- Corradi D, Maestri R, Callegari S, et al. The ventricular epicardial fat is related to the myocardial mass in normal, ischemic and hypertrophic hearts. *Cardiovasc Pathol* 2004;13:313–6.
- Kankaanpää M, Lehto HR, Pärkkä JP, et al. Myocardial triglyceride content and epicardial fat mass in human obesity: relationship to left ventricular function and serum free fatty acid levels. *J Clin Endocrinol Metab* 2006;91:4689–95.
- Taguchi R, Takasu J, Itani Y, et al. Pericardial fat accumulation in men as a risk factor for coronary artery disease. *Atherosclerosis* 2001;157:203–9.
- Chaowalit N, Somers VK, Pellikka PA, Rihal CS, Lopez-Jimenez F. Subepicardial adipose tissue and the presence and severity of coronary artery disease. *Atherosclerosis* 2006;186:354–9.
- Iacobellis G, Leonetti F. Epicardial adipose tissue and insulin resistance in obese subjects. *J Clin Endocrinol Metab* 2005;90:6300–2.
- Iacobellis G, Ribaldo MC, Assael F, et al. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. *J Clin Endocrinol Metab* 2003;88:5163–8.
- Iacobellis G, Ribaldo MC, Zappaterreno A, Iannucci CV, Leonetti F. Relation between epicardial adipose tissue and left ventricular mass. *Am J Cardiol* 2004;94:1084–7.
- Mazur A, Ostafski M, Telega G, Malecka-Tendera E. Is epicardial fat tissue a marker of metabolic syndrome in obese children? *Atherosclerosis* 2010;211:596–600.
- Tanner JM, Whitehouse RH, Takaishi M. Standards for birth to maturity for height, weight, height and weight velocity: British children. *Arch Dis Child* 1965;41:454–95.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985;28:412–9.
- American Diabetes Association. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2012 Jan;35(Suppl. 1):S64–71.
- Kuczmarski RJ, Ogden CL, Guo SS, et al. CDC growth charts for the United States: methods and development. *Vital Health Stat* 2000;11(246):1–190.
- Zimmer P, Alberti KG, Kaufman F, et al. IDF Consensus Group. The metabolic syndrome in children and adolescents – an IDF consensus report. *Pediatr Diabetes* 2007;8:299–306.
- Valerio G, Licenziati MR, Iannuzzi A, et al. Insulin resistance and impaired glucose tolerance in obese children and adolescents from Southern Italy. *Nutr Metab Cardiovasc Dis* 2006;16:279–84.
- Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972;18:499–502.
- Maffei C, Manfredi R, Trombetta M, et al. Insulin sensitivity is correlated with subcutaneous but not visceral body fat in overweight and obese prepubertal children. *J Clin Endocrinol Metab* 2008;93:2122–8.
- Johnson D, Dixon AK, Abrahams PH. The abdominal subcutaneous tissue: computed tomographic, magnetic resonance, and anatomical observations. *Clin Anat* 1996;9:19–24.
- Burgert TS, Taksali SE, Dziura J, et al. Alanine aminotransferase levels and fatty liver in childhood obesity: association with insulin resistance, adiponectin, and visceral fat. *J Clin Endocrinol Metab* 2006;91:4287–94.
- Taksali SE, Caprio S, Dziura J, et al. High visceral and low abdominal subcutaneous fat stores in the obese adolescent: a determinant of an adverse metabolic phenotype. *Diabetes* 2008;57(2):367–71.
- Moran A, Steffen LM, Jacobs Jr DR, et al. Relation of C-reactive protein to insulin resistance and cardiovascular risk factors in youth. *Diabetes Care* 2005;28:1763–8.
- Manco M. Endotoxin as a missed link among all the metabolic abnormalities in the metabolic syndrome. *Atherosclerosis* 2009;206:36.
- Manco M, Putignani L, Bottazzo GF. Gut microbiota, lipopolysaccharides, and innate immunity in the pathogenesis of obesity and cardiovascular risk. *Endocr Rev* 2010;31:817–44.
- Ege MR, Guray U, Guray Y, Demirkan B, Kisacik H. Serum  $\gamma$ -glutamyltransferase levels correlate with epicardial adipose tissue thickness in patients with coronary artery disease. *Angiology* 2012 Jan 20 [Epub ahead of print].
- Kozakova M, Palombo C, Paterni Eng M, et al. Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. *Hepatology* 2012;55:1406–15.
- Bacha F, Saad R, Gungor N, Arslanian SA. Adiponectin in youth: relationship to visceral adiposity, insulin sensitivity, and  $\beta$ -cell function. *Diabetes Care* 2004;27:547–52.
- Sabin MA, Holly JMP, Shield JPH, et al. Mature subcutaneous and visceral adipocyte concentrations of adiponectin are highly correlated in prepubertal children and inversely related to body mass index standard deviation score. *J Clin Endocrinol Metab* 2006;91:332–5.
- Gastaldelli A, Basta G. Ectopic fat and cardiovascular disease: what is the link? *Nutr Metab Cardiovasc Dis* 2010;20:481–90.