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ARTICLE

Markers of subclinical vascular damages associate with indices of adiposity and blood pressure in obese children

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Abstract

In this observational study, we aimed at investigating the influence of excess weight and traditional cardiovascular risk factors on vascular structure and function in a cohort of overweight/obese children. Sixty-six obese and 4 overweight children (age 11.5 ± 2.4 years; female *n*: 30) underwent office and ambulatory BP measurements (ABPM); ultrasound was used to measure carotid intima-media thickness (cIMT), endothelial function by Flow-Mediated Dilation (FMD) and carotid distensibility (cDC); and digital photopletismography was used to measure stiffness index (SI_{DVP}). Carotid IMT directly correlated with 24-h and nighttime-systolic blood pressure (SBP); while cDC had inverse correlations with BMI, waist circumference and 24-h BP. Unexpectedly, SI_{DVP} resulted inversely related with several indices of excess weight. Most of these correlations remained significant after adjustment for age, sex, BMI, and BP. In a replication set of 40 obese children, SI_{DVP} but not pulse wave velocity (PWV) remained inversely associated with BMI. These data suggest that arterial structure and elasticity are negatively affected by excess weight and BP levels, even in childhood. Surprisingly, SI may not be a reliable marker of vascular stiffness in obese children, because this measurement is likely confounded by other factors, including vasodilation.

Keywords: Arterial stiffness · Blood pressure · Children · Obesity · Pulse wave velocity

Introduction

Excess weight is a worldwide health problem that has increased in prevalence over the last few decades, even in children [1]. Overweight and obesity are often accompanied by other cardiovascular (CV) risk factors, including high blood pressure, in both children and adults [2]. Obesity and hypertension are some of the main determinants of the onset and the progression of the atherosclerotic process that lead to abnormalities in vascular structure and function, which can already be detected during childhood [3, 4]. The first step in the atherosclerotic process is an impairment of endothelial function, which can be assessed by the flow-mediated dilatation technique. This technique is widely used in the research setting but has never been included in current guidelines due to the difficulties in obtaining reproducible measurements [5].

As atherosclerosis progresses, there are structural and functional changes that can be estimated by several parameters. For example, intima-media thickness (IMT) can be measured at the carotid level and increased IMT is associated with several risk factors for cardiovascular diseases [6, 7]. Furthermore, in adults IMT is an independent predictor of cardiovascular events [8]. Moreover, familial hypercholesterolemia, type 1 diabetes mellitus and metabolic syndrome have been associated with increased cIMT in both adults and children [9].

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Another well-accepted marker of subclinical organ damage is arterial stiffness, and it has been shown to be independently associated with cardiovascular risk in adults [10]. However, in children the clinical relevance is still a matter of debate and current guidelines do not always agree about the assessment of arterial structure and function in children with higher cardiovascular risk [11, 12]. Moreover, different methods and sites of measurement of arterial stiffness can be considered. For example, carotid distensibility (cDC) is a measurement of segmental elasticity in a vessel, which is often affected by the atherosclerotic process, whereas stiffness index (SI_{DVP}) is a row estimate of systemic stiffness as measured by digital volume pulse (DVP). DVP waveform can be recorded at the finger level by photopletismography and is determined by a forward-going pressure wave that travels from the heart to peripheral arteries, and a backward wave that is generated at the small arteries level where the pressure wave traveling along the aorta is reflected back. The DVP waveform, and in particular the backward component, is related to large artery stiffness and pressure wave reflection [13]. Two indeces are derived from the DVP analysis: the stiffness index (SI), which estimates large artery stiffness, and the reflection index (RI), which provides an assessment of the amount of the reflection component. Because of high rate reproducibility, the gold standard to measure aortic elasticity and elasticity of the proximal tract of the vessels directed to the legs is the carotid–femoral pulse wave velocity (cf-PWV). Additionally, validated tools for the assessment of early atherosclerotic vascular disease in children have been developed [9].

Atherosclerosis is a progressive disease resulting from the cumulative effects of individual CV risk factors since childhood. Thus, the aim of our study was to evaluate all the mentioned markers of vascular function and structure in a sample of obese children in relation to traditional CV risk factors, especially BP and fat distribution. This study provides an extensive characterization of several aspects of subclinical vascular organ damage and explores both vascular structure and function, including local and systemic markers of arterial stiffness.

To better interpret an unexpected finding in our main sample, we searched for replication in a subsample of obese children from a school-based survey of children in the 3rd and 4th class of the primary school in South Verona district.

Methods

Obese children referred to a tertiary hospital

Obese children were recruited consecutively from October 2012 to September 2014. This children had been referred to the "Pediatric Obesity Outpatients Unit" of the University Hospital of Verona and of the "Local Health Unit n. 20" of Verona. Inclusion criteria were: children and adolescents aged 5–18 years old; overweight or obesity (BMI ≥ 85th and ≥ 95th percentile according to sex and age, respectively). We excluded children with hepatic or renal chronic diseases, malignancies, diabetes mellitus, lipid-lowering therapy, secondary causes of obesity. Seventy-seven children were enrolled, four (5.2%) children refused the participation to the study, and three (3.9%) children were excluded because of a secondary form of obesity (two (2.6%) children had Prader–Willi Syndrome and 1 (1.3%) child had Cushing disease). Therefore, 70 children were considered for the present study.

Study design

The study was conducted using a cross-sectional observational design. The study was approved by the Ethical Committee of the University Hospital of Verona (CE n. 2218), and written informed consent was obtained from the parents of each participant.

Assessments

Each child was evaluated over a single occasion, between 8 and 9 AM. A questionnaire was administered to the patients and to the parents to collect information about patient medical history, family history, physiological and pathological information and drug use. Then, the participants underwent a physical examination. Subjects were advised not to engage in strenuous exercise and to avoid consuming caffeine within 12 hours before the vascular studies.

During the visit, blood pressure was measured with a semiautomatic oscillometric device (TM-2551, A&D instruments Ltd, Abingdon Oxford, UK) 3 times, 3 min apart. For this procedure, the patient lied supine for at least 10 minutes before the first measurement in a temperature-controlled room (22–24 °C). The mean value of the 3 supine measurements were calculated and is referred to in the text as "office blood pressure". The office blood pressure was then used for z-score and percentile calculations. Then, BP levels were measured in the sitting position by the oscillometric device and by the auscultatory method. Ambulatory blood pressure measurement was recorded with an oscillometric device (Spacelabs 90217; Spacelabs Inc., Issaquah, Washington, USA), which measured BP every 15 minutes during the day and ever 30 minutes through the night. Children and parents recorded physical activities, resting and sleeping time and symptoms on a dedicated diary. After the data were recorded, the daytime and nighttime periods (set to default at 7 A.M. and 10 P.M., respectively) were adapted to "real" awake and sleep times according the

recorded activity data. All of the values derived from BP measurements were transformed into z-scores and percentiles, according to normative values [14, 15].

Body weight, height, and waist and hip circumferences were measured with the patient wearing light clothes. Body weight was measured using a calibrated balance and height was measured using a calibrated stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m); waist/hip ratio was calculated as waist circumference (cm) divided by hip circumference (cm), and waist/height ratio (WHtR) was calculated as waist circumference (cm) divided by height (cm) [16]. Overweight or obesity were defined as BMI ≥ 85th and 95th percentile for sex and age, respectively [17]. The WHO reference for BMI was used to categorize children into the overweight and obese groups [18].

Carotid Intima-media Thickness (cIMT) was assessed by ultrasound of the carotid arteries (LogiQ P5 Pro). The cIMT was estimated by tracking the artery wall in the last centimeter of the common carotid artery and calculated using a dedicated software (Multimedia Video Engine II (MVE2) DSP Lab., CNR, Pisa, Italy). The relative z-scores and percentiles were calculated according to reference values [19].

Endothelial function was assessed by ultrasound of the brachial artery using the Flow-Mediated Dilatation (FMD) technique according to international guidelines and with the aid of a dedicated hardware (Multimedia Video Engine II (MVE2) DSP Lab., Pisa CNR, Italy) [20]. Common carotid artery distensibility (DC) was calculated as: $DC = \Delta A/(A^* \Delta P)$, where A is the diastolic lumen area, ΔA is the stroke change in lumen area, and ΔP is pulse pressure (PP). Changes in diameters were detected using ultrasound B-mode image sequences of the right and left common carotid arteries acquired at different steps. These changes were analyzed by the above mentioned automatic system [21]. The relative z-score and percentile were calculated according to reference values [22].

The Stiffness index (SI_{DVP}) and Reflection Index (RI_{DVP}) were calculated using the digital volume pulse (DVP) method and were obtained using the digital photoplethysmography PulseTracePT1000 (MicroMedical Ltd, Gillingham, Kent, UK) [13]. Laboratory tests, including measurements of plasma cholesterol, triglycerides, glucose and insulin were measured using standard methods. For definition of elevated total cholesterol and elevated triglycerides, we referred to Kwiterovich's work [23].

Obese children in a school-based survey

The second sample set is composed of 40 obese children who were enrolled in a larger observational school-based study that was approved by the Ethical Committee of Verona (CESC n. 375). Children were recruited from the 3rd and 4th classes of three primary schools in the Verona South district. Inclusion criteria were as follows: children in the aforementioned classes who agreed to participate in the study and whose parents gave a written informed consent. Exclusion criteria were either the lack of written informed consent signed by the parents or refusal to participate by the child. As this was the second sample in the present study, we selected only the obese children as a replication sample.

Data about anthropometric features, SI_{DVP} and RI_{DVP} were obtained as previously described for the studied population.

Measures of *pulse wave velocity* were performed by SphygmoCor XCEL (AtCor Medical Pty Ltd; Unit 11, West Ryde Corporate Centre, 1059–1063 Victoria Road, West Ryde, NSW 2114, Australia). To conduct a carotid–femoral PWV measurement, a cuff was placed around the femoral artery of the child to capture the femoral waveform, and a tonometer was used to capture the carotid waveform. The distance between the carotid and femoral arteries was measured, and the velocity automatically determined by dividing the distance by the pulse transit time. The distance between the carotid measurement site and the cuff was calculated by the so-called subtraction method. The distance was calculated from the sternal notch to the top edge of the femoral cuff (distal distance) and from the carotid artery to the sternal notch (proximal distance). To assess the aortic distance, the proximal distance was subtracted from distal distance. The relative z-score and percentile were calculated according to reference values [24].

Statistics

Data are presented as the median and range unless otherwise stated. Statistical analysis were performed using the Statistical Package for Social Sciences software (SPSS/PC for Windows version 23.0). Differences in the measured parameters between normotensive and hypertensive children were analyzed using a nonparametric (Wilcoxon–Mann–Whitney U) tests. Bivariate nonparametric correlations were estimated using Spearman coefficient (r_S). Multiple linear regression analyses were used in the multivariate models with indices of subclinical vascular damage (cIMT, FMD, cDC, SI_{DVP}, PWV) as the dependent variables, and age, sex, BMI (or waist), BP, heart rate when appropriate, total cholesterol, and fasting glucose, as covariates to test for the independent associations. All tests were two-sided and P-values < 0.05 were considered statistically significant.

Results

Sixty-six obese and 4 overweight Caucasian children (males: 40; 57%) were included in the study. Physical and biochemical characteristics and markers of vascular function and

Table 1 General characteristics of the first sample of overweight/obese children, split by gender and by pubertal status

Variable	Male (*n*: 40) Median (range)	Female (*n*: 30) Median (range)	*p*-value*	Prepubertal (*n*: 38) Median (range)	Pubertal (*n*: 32) Median (range)	*p*-value*
Age, y	11 (8–17)	11 (5–15)	0.3	10 (5–15)	13 (9–17)	<0.001
BMI, kg/m^2	28.5 (23–42)	29.3 (24–41)	0.5	28.2 (24–38)	29.8 (23–42)	0.059
BMI percentile	98.3 (93–99)	98.7 (90–99)	0.5	98.9 (95–99)	98 (90–99)	0.027
Waist circumference, cm	96 (79–122)	95.5 (82–119)	0.92	91 (79–122)	97 (83–119)	0.013
Waist percentile	97 (92–99)	98 (91–99)	0.019	98 (92–99)	97 (90–99)	0.006
Waist/height (W/h) ratio	0.6 (0.51–0.76)	0.6 (0.5–0.8)	0.5	0.61 (0.51–0.78)	0.59 (0.49–0.73)	0.2
W/h percentile	95.6 (85–99)	96.7 (69–99)	0.3	96.8 (85–99)	94.8 (69–99)	0.04
Office SBP, mmHg	119 (102–163)	115 (105–143)	0.3	116 (102–163)	122 (107–153)	0.03
Office SBP percentile	85 (45–99)	78 (45–99)	0.9	81 (45–99)	83 (45–99)	0.9
Office DBP, mmHg	68 (52–88)	66 (57–84)	0.4	67 (57–88)	67 (52–83)	0.4
Office DBP percentile	66 (7–98)	65 (23–96)	0.9	66 (19–98)	63 (52–83)	0.3
24-h SBP, mmHg	118 (107–136)	112 (100–130)	<0.001	115 (102–136)	117 (100–130)	0.6
24-h SBP, percentile	74 (26–99)	52 (5–99)	0.09	76 (21–99)	51 (5–99)	0.007
24-h DBP, mmHg	68 (58–79)	64 (55–75)	0.001	67 (55–79)	65 (58–78)	0.2
24-h SBP percentile	57 (4–99)	34 (2–95)	0.003	53 (2–98)	35 (4–98)	0.08
Daytime SBP, mmHg	123 (109–142)	115 (102–137)	0.001	118 (104–142)	120 (102–138)	0.423
Daytime SBP, percentile	72 (14–99)	58 (3–99)	0.052	72 (14–99)	58 (3–99)	0.075
Daytime DBP, mmHg	71 (63–85)	67 (58–80)	0.002	70 (58–84)	68 (61–85)	0.283
Daytime DBP, percentile	42 (6–98)	20 (2–89)	0.005	37 (2–97)	24 (3–98)	0.223
Nighttime SBP, mmHg	108 (100–124)	104 (92–128)	0.053	108 (92–124)	107 (95–128)	0.623
Nighttime SBP, percentile	79 (39–99)	75 (23–99)	0.812	87 (24–99)	71 (24–999	0.018
Nighttime DBP, mmHg	60 (49–73)	57 (48–69)	0.004	59 (51–73)	58 (48–67)	0.138
Nighttime DBP, percentile	76 (9–99)	64 (12–95)	0.068	71 (19–99)	66 (9–95)	0.124
Heart rate, bpm	83 (60–150)	81 (61–109)	0.5	80 (63–150)	82 (60–104)	0.7
cIMT, mm	0.56 (0.32–0.6)	0.42 (0.3–0.55)	0.056	0.46 (0.32–0.55)	0.44 (0.31–0.6)	0.3
cIMT percentile	98.3 (3–99)	84 (1–99)	0.03	98 (1–99)	88 (2–99)	0.04
cDC, 10^{-3}/Kpa	39.7 (19–54)	43.8 (27–63)	0.11	42.3 (20–63)	39.6 (19–56)	0.2
cDC percentile	6.5 (1–50)	13 (1–68)	0.14	12 (1–60)	11 (1–68)	0.5

Table 1 (continued)

Variable	Male (*n*: 40) Median (range)	Female (*n*: 30) Median (range)	*p*-value*	Prepubertal (*n*: 38) Median (range)	Pubertal (*n*: 32) Median (range)	*p*-value*
SI, m/s	6.2 (5–9)	6.1 (4.7–8.4)	0.7	6.1 (4.7–8.4)	6.1 (4.7–9)	0.7
RI	60 (33–83)	58 (20–81)	0.28	63 (37–83)	55 (20–76)	0.004
FMD, %	6.7 (0–14)	6.2 (2.3–14)	0.7	6.7 (0–14)	6.1 (2.4–14)	0.7
Total cholesterol, mg/dL	160 (106–242)	162 (93–216)	0.96	163 (105–213)	153 (93–242)	0.75
HDL-cholesterol, mg/dL	49 (30–77)	52 (37–81)	0.07	50 (33–77)	49 (30–81)	0.91
Triglycerides, mg/dL	79 (28–285)	73 (34–143)	0.43	85 (28–285)	69 (34–208)	0.10
Fasting glucose, mg/dL	88 (81–117)	85 (70–99)	0.01	88 (75–117)	86 (70–108)	0.35
Fasting insulin, uU/mL	19.2 (3.0–62.5)	17.8 (5.3–43.4)	0.79	17.8 (3.0–62.5)	20.7 (6.8–49.8)	0.28
Fasting C-peptide, ng/mL	2.5 (0.8–7)	2.47 (1.3–4.5)	0.7	2.21 (0.8–78)	2.65 (1.5–5.6)	0.08
HOMA-IR	3.9 (0.6–18.0)	4.1 (0.0–8.0)	0.55	4.0 (0.6–18.0)	4.0 (0.0–12.4)	0.74

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *cIMT* carotid intima-media thickness, *cDC* carotid distensibility coefficient, *SI* stiffness index, *RI* reflection index, *FMD* flow-mediated dilation

*Mann–Whitney U Test

Table 2 Correlations between vascular tests and indices of adiposity/ambulatory blood pressure measurements in the first sample of overweight/obese children

	BMI (Kg/m^2)	Waist circumference (cm)	Waist/height ratio	24-h SBP (mmHg)	24-h DBP (mmHg)	Daytime SBP (mmHg)	Daytime DBP (mmHg)	Nighttime SBP (mmHg)	Nighttime DBP (mmHg)
cIMT (mm)	0.131	0.100	−0.071	0.269*	0.116	0.185	−0.026	0.331$_b$**	0.208
z-score cIMT	0.058	−0.018	−0.025	0.220	0.156	0.141	0.020	0.293$_b$*	0.222
cDC (10^{-3}/Kpa)	−0.403$_a$**	−0.346$_a$**	−0.195	−0.449$_b$**	−0.272*	−0.397**	−0.175	−0.490$_b$**	−0.336**
z-score cDC	−0.350$_a$**	−0.245	−0.222	−0.395**	−0.285*	−0.329**	−0.187	−0.464$_b$**	−0.330*
FMD (%)	0.009	0.015	0.057	−0.052	0.009	−0.011	0.049	−0.136	0.021
SI (m/s)	−0.359$_a$**	−0.162	−0.303$_a$*	−0.148	0.120	−0.140	0.099	−0.242	0.025
RI	−0.242	−0.254$_a$*	−0.065	−0.104	0.012	−0.119	−0.044	−0.067	0.070

*Significance below 0.05; **significance below 0.001. In the table r_s (Spearman coefficient of correlation) is reported. The underlined correlations remained significant after adjustment for main confounders as follows: $_a$: age, sex, heart rate, total cholesterol, fasting glucose and DBP; $_b$: age, sex, heart rate, total cholesterol, fasting glucose and BMI.

BMI body mass index, *cIMT* carotid intima-media thickness, *DBP* diastolic blood pressure, *DC* carotid segmental distensibility, *FMD* flow-mediated dilation, *RI* reflection index, *SBP* systolic blood pressure, *SI* stiffness index

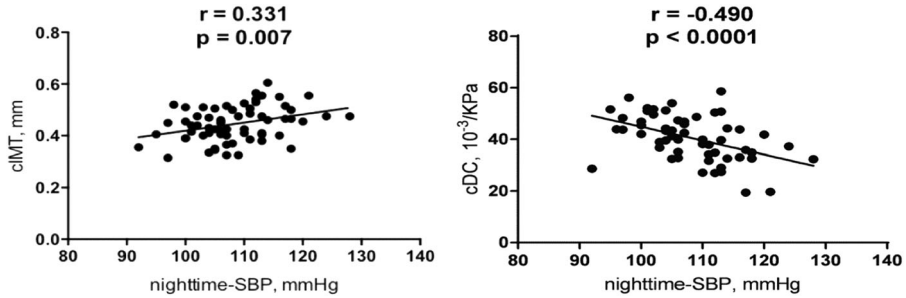

Fig. 1 Correlations between markers of vascular function and nighttime ambulatory BP in the sample of 70 overweight/obese children. In the figures, Spearman coefficients of correlations of nighttime SBP with both cIMT and cDC are presented. cIMT carotid Intima-media thickness, cDC carotid distensibility, SBP systolic blood pressure. For technical reason, data of either cDC or nighttime SBP are missing in seven children

structure for the 70 children are presented (median and range) in Table 1. In Supplementary Table 1, clinical and vascular characteristics of the sample are presented (mean ± SD) after further stratification for both gender and pubertal status. Boys had higher 24-hours SBP and DBP as compared to girls. No major differences were found in vascular tests when comparing males and females, even when the subjects were further divided according to pubertal status. Children with elevated fasting total cholesterol had a higher cIMT compared with subjects with normal cholesterol (Supplementary Table 6).

Correlations

The correlation coefficients and the significance after adjustment for principal confounders are reported in Table 2. We found that cIMT was directly correlated to 24-h SBP and nighttime SBP. Carotid DC, an index of local elasticity of the arterial wall, was inversely correlated with BMI, waist circumference and with 24-h BP. The correlations of cIMT and cDC with BP are depicted in Figure 1.

Conversely, SI_{DVP}, a marker of systemic vascular stiffness, was inversely correlated with BMI and WHtR (Figure 2). Similarly, RI_{DVP} was inversely correlated with waist circumference and with office SBP. Even after adjustment for age, gender, glucose, and lipid profile, and BP or BMI, most of the association remained significant.

Endothelial function, assessed by FMD, was not associated with anthropometric features or with BP. Glucose and lipid profile did not show any significant correlation with the vascular tests (see Supplementary Table 2).

No significant correlations were found within the vascular tests, except the direct association of SI with RI, as detailed in Supplementary Table 4.

Obese children in a school-based survey

To investigate the unexpected inverse association of SI_{DVP} with some anthropometric features in the studied population, we repeated the analysis in another sample of 40 obese children (males *n*: 19, 47.5%). The general characteristics of the second cohort are detailed in Table 3. The correlation coefficients and the significance after adjustment for principal confounders are reported in Supplementary Table 3. In line with the findings in the first sample, in our second sample, we found that SI_{DVP} was inversely correlated with BMI, waist circumference and WHtR. Additionally, RI_{DVP} was inversely correlated with BMI and waist circumference. In contrast, PWV was directly correlated with the indeces of adiposity in the obese children as expected (Figure 2).

No significant correlations were found within the vascular tests, as detailed in Supplementary Table 5.

Discussion

The main finding of the present study is that BP and weight excess independently and negatively affect carotid structure and distensibility in obese/overweight children. In contrast and unexpectedly, SI, which is considered a row marker of systemic vascular stiffness, appeared to be inversely related with the indeces of adiposity. Finally, glucose and lipid profiles were not associated with vascular function.

Carotid IMT is an independent predictor of major cardiovascular events in adults [8, 25, 26]. Increased IMT, when compared to healthy controls, has been associated with several cardiovascular risk factors including obesity, familial hypercholesterolemia, hypertension, and type 1 diabetes mellitus, even in children [27–32]. Moreover, the presence of cardiovascular risk factors during childhood were associated with increased cIMT in young adulthood in longitudinal studies [33, 34]. Our results also showed an increase in cIMT in those children with higher plasma cholesterol.

Carotid IMT reflects morphological changes in the vasculature, whereas functional changes (like an impairment in arterial distensibility) can occur at earlier stage of the

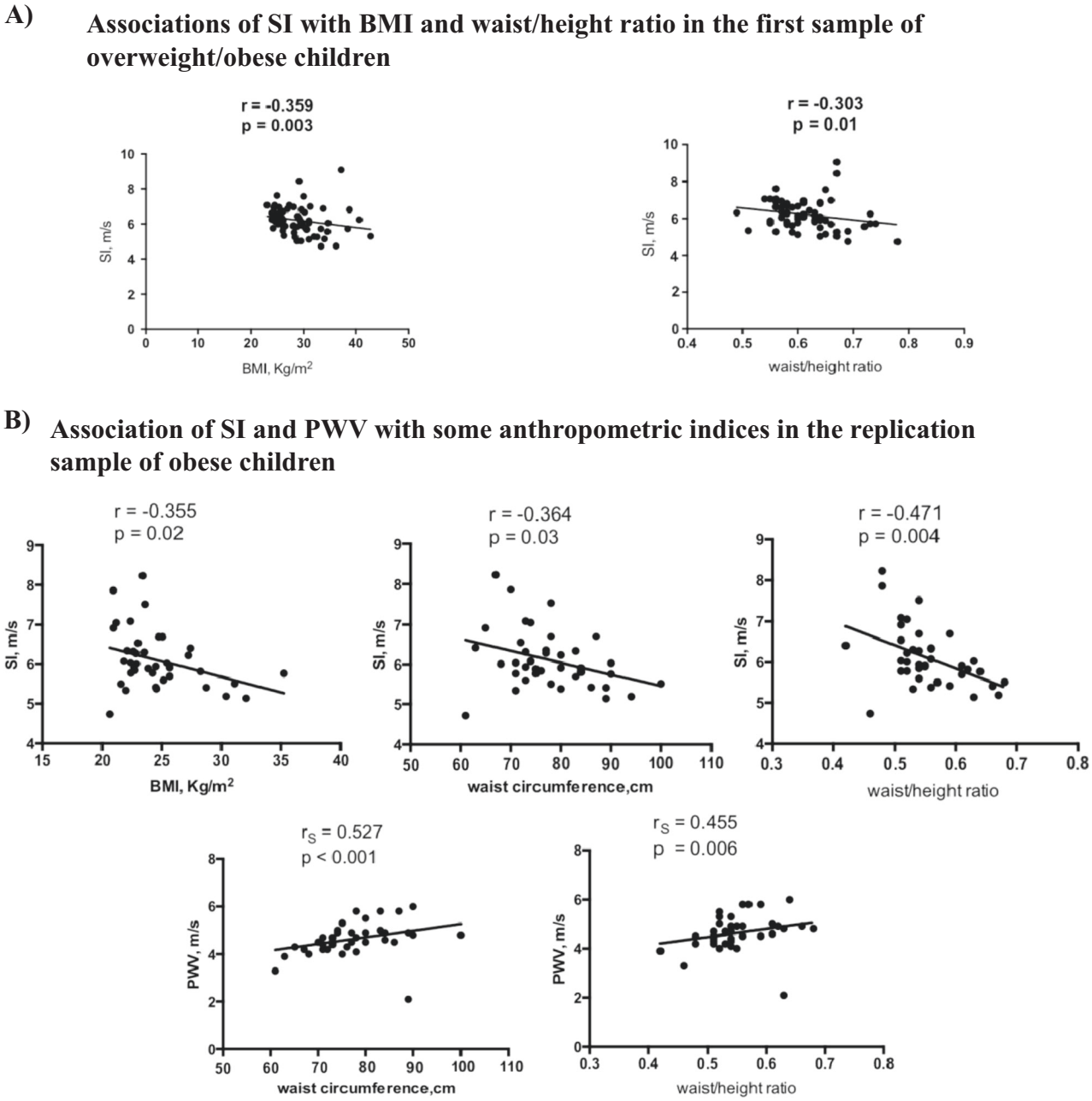

Fig. 2 Correlations between markers of arterial stiffness and markers of adiposity. In the figures, Spearman coefficients of correlations of markers of vascular stiffness and of adiposity are presented. Panel **A** refers to the main sample. For technical reason data of the correlation between SI and anthropometric indices are missing in five children. Panel **B** refers to the replication sample of obese children included in a larger school-based survey. For technical reason, data of PWV are missing in two children. BMI body mass index, PWV pulse wave velocity, SI stiffness index

atherosclerotic process. In adults, traditional CV risk factors such as hypertension and altered glucose homeostasis have been associated with decreased carotid distensibility [35, 36]. Even in children, an increased carotid stiffness was found in subjects with familial hypercholesterolemia and obesity [6, 7]. In accordance with previous studies, the results of this observational study confirm the harmful association of BP with carotid structure and distensibility. We also confirm the association between obesity with local carotid distensibility even during childhood [37–39]. Moreover, our results highlight a stronger association of nocturnal BP with IMT and DC, in comparison with daytime BP. Previous studies in adults have suggested that nighttime BP has a stronger predictive value for CV outcomes than daytime BP, and subjects with a less drastic drop in nighttime BP have a higher incidence of CV events;

Table 3 General characteristics, anthropometric indices and markers of vascular function in the replication cohort of obese children, split by gender

	Males (*n*: 19)	Females (*n*: 21)	*p*-value*
Age, years	8 (8–10)	8 (8–9)	0.81
BMI, Kg/m^2	22.69 (20.63–32.09)	24.49 (20.94–35.26)	0.17
BMI percentile	97.48 (95.81–99.54)	97.37 (95.39–99.72)	0.70
Waist circumference, cm	77 (61–94)	75 (63–100)	0.40
Waist percentile	96.1 (67.57–99.13)	94.91 (64.57–99.4)	0.40
Waist/height (W/h) ratio	0.55 (0.46–0.67)	0.54 (0.42–0.68)	0.45
W/h percentile	92.47 (56.93–99.04)	89.79 (16.69–99.28)	0.33
SI, m/s	6.03 (4.74–15.6)	5.94 (5.38–8.23)	0.70
RI, %	76.5 (50.5–94)	68 (36–86.5)	0.18
PWV, m/s	4.9 (2.1–5.8)	4.5 (3.9–6)	0.17
PWV, percentile	78.01 (0–97.76)	56.29 (17.08–99.2)	0.14
Office SBP, mmHg	115 (101–124)	118.5 (97–130.5)	0.40
Office SBP percentile	85 (37–98)	90 (34–99)	0.18
Office DBP, mmHg	68 (56.5–87)	67.5 (56.5–93)	0.97
Office DBP percentile	83 (40–99)	77 (35–99)	0.83

BMI body mass index, *SBP* systolic blood pressure, *DBP* diastolic blood pressure, *cIMT* carotid intima-media thickness, *cDC* carotid distensibility, *SI* stiffness index, *RI* reflection index, *FMD* flow-mediated dilatation

*Mann–Whitney U test

moreover, nocturnal BP was associated with subclinical organ damage [40–43]. In line with our findings, several studies reported similar results for subclinical vascular damages even in children [44, 45].

Unexpectedly, in our study, SI, a marker of systemic stiffness, was inversely associated with several indeces of excess weight, even after adjustment for other confounders. Most of the previous studies and a meta-analysis have shown a direct association between an increase in different indices of adiposity (often the BMI) and impaired arterial elasticity [39, 46, 47]. However, some studies have reported a "paradoxical" effect of BMI on vascular stiffness in children, in particular the increase excess weight was associated with a lower PWV [48–50] and with higher large vessel elasticity, investigated by pulse wave analysis [51, 52]. Moreover, weight reduction was not associated with an improvement in PWV in a longitudinal study conducted in a sample of obese children and adolescents [53]. A possible alteration of pulse waveform due to volume overload was suggested as a potential explanation for the unexpected association between increased BMI and decreased PWV [50, 54]. Additionally, muscle strenght could be a confounder, as it has been shown to be inversely related to aortic stiffness [55]. Another hypothesis is that obese children have an increased overall vasodilation and therefore, a consequent reduction in total peripheral resistance, as suggested in obese adults [56]. This hypothesis seems to be, at least indirectly, supported by the results of our study. SI_{DVP} is influenced by the distensibility not only of the aorta but also of the distal muscular arteries, which are less affected by the stiffening process and are determinants of total peripheral resistance. Thus, the interpretation of its apparently beneficial association with markers of adiposity could be due to other haemodynamic factors in obese children. Furthermore, we found an inverse correlation between weight and reflection index, which reflects the height of the diastolic component of the digital pulse contour. RI is influenced by pressure wave reflection, which in turn depends on the vasodilating status of small muscular arteries [13]. The results of the replication sample, where PWV and SI_{DVP} were associated in opposite directions with adiposity support this hypothesis.

In previous studies, SI_{DVP} was directly correlated to PWV [57] but it is recognized to be influenced by other factors such as peripheral resistance sites and left ventricular ejection [13]. We hypothesize that digital photoplethysmography is likely influenced by vasodilation in obese children and cannot be considered an accurate index of arterial stiffness.

Moreover, sex-specific differences in cardiovascular disease and in vascular aging have been shown, with a putative hormonal influence [58]. It would be reasonable to expect differences in vascular structure and function in relation to gender and pubertal status. Our data do not support this hypothesis, as vascular characteristics were not different between boys and girls, even when the groups were further stratified for pubertal development. However, we performed only an exploratory analysis to answer this question and due to a small sample size of the subgroups, this conclusion should be interpreted with caution. In

contrast, in our population, blood pressure may be influenced by sex and pubertal status; there was a slightly but significant difference in BP when comparing boys and girls and a more drastic difference in BP when comparing pubertal and prepubertal girls. Moreover, the interaction between blood pressure and vascular features is complex and likely bidirectional, and BP may be considered as a component of early vascular aging [59, 60]. Our results raise the question of whether gender and pubertal status in addition to the interaction between blood pressure and vascular function could influence the vascular changes that were observed in our sample. However, the observational design of the study prevents obtaining clear conclusions and suggest the need for further longitudinal investigations.

Our study has some limitations: the cross-sectional design of the study does not allow for any conclusion about a possible causal link between the analyzed variables. However, we found meaningful correlations between several markers of vascular structure and function with BP and excess weight that strengthen the concept of an early onset of vascular damage and rise some questions about haemodynamic mechanisms in obese children. The small sample size and the lack of a control group of normal-weight children suggest caution in the interpretation of the results and further studies are needed to confirm these findings and to explore the underlying pathophysiological mechanisms. Moreover, this study was conducted in subjects with European ancestry, so results may not be directly relatable to subjects with other ethnic backgrounds. Furthermore, BP measurements were conducted by devices without a specific validation in children. Finally, in the first population we could not measure PWV, which is considered the gold standard method for arterial stiffness.

The strengths of our study include the exploration of this hypothesis in children, where there were only a few previous studies, and the use of multiple vascular techniques for an in-depth characterization of early subclinical damage. The multivariate analysis allowed us to verify the independent contribution of CV risk factors in children.

The associations between anthropometric indeces, especially central distribution of fat, with BP and arterial elasticity, call for an action to modify unhealthy behavior already in children to prevent a possible organ damage progression over time.

In conclusion, subclinical organ damage linked to excess weight and increase in BP is detectable even in children. SI_{DVP} could not be used as a reliable marker of vascular stiffness in obese children, because of the influence of other haemodynamic conditions, particularly the vascular tone of small muscular arteries.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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