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CLINICAL RESEARCH

Prevention and epidemiology

Impaired endothelial and smooth muscle functions and arterial stiffness appear before puberty in obese children and are associated with elevated ambulatory blood pressure

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Aims

To determine whether impaired brachial endothelial (flow-mediated dilation, FMD) and smooth muscle function (nitroglycerin-mediated dilation, NTGMD), and remodelling of the common carotid artery (CCA) develop before puberty in obese children.

Methods and results

Arterial intima–media thickness (IMT), FMD and NTGMD were measured by high-resolution ultrasound in 48 obese and 23 lean pre-pubertal children (8.8 ± 1.5 years old). We assessed central pulse pressure, incremental elastic modulus (Einc), casual and ambulatory systolic (SBP) and diastolic blood pressure (DBP), and body fatness by DXA. Obese children had significantly lower FMD (4.5 ± 4.0 vs. $8.3 \pm 1.7\%$), NTGMD (19.0 ± 9.0 vs. $25.8 \pm 6.1\%$), and increased Einc (13.9 ± 5.2 vs. 10.4 ± 5.2 mmHg/10²), ambulatory SBP (121.3 ± 12.6 vs. 106.6 ± 7.1 , mmHg), and DBP (69.1 ± 5.7 vs. 63.7 ± 4.5) than lean subjects, whereas IMT was not augmented. Ambulatory systolic hypertension was present in 47% of obese subjects. FMD, NTGMD, and Einc were correlated with body fatness, body mass index, and blood pressure (BP).

Conclusion

Impaired endothelial and smooth muscle functions and altered wall material develop before puberty in obese children, however remodelling of the CCA is not yet present. Arterial dysfunction may be considered as the first marker of atherosclerosis and is associated with elevated BP. Ambulatory blood pressure monitoring may be a potential tool to improve risk stratification in obese children.

Keywords

Obesity • Endothelium • Arteries • Hypertension • Cardiovascular disease • Child • Paediatrics

Introduction

The prevalence of childhood obesity is increasing rapidly in developing countries,¹ resulting in increased risk of cardiovascular disease (CVD).² Atherosclerosis is a complex multifactorial disease, the earliest stage of which is known to commence in childhood.^{3,4} Impaired endothelial function is recognized as a marker of arterial damage that precedes plaque formation, and it is associated with abnormal coronary angiography in adults.⁵ The evaluation of

brachial artery endothelial function (flow-mediated dilation, FMD) and carotid artery intima–media thickness (IMT) by high-resolution ultrasound is now increasingly used for paediatric cardiovascular risk evaluation.⁶ In obese children and adolescents, reduced FMD and elastic mechanical properties of the carotid artery have been previously described.^{7–12} The early activation of vascular endothelial cells and platelets observed in this population contributes to increased CVD risk later in life.¹³ In addition, few studies reported reduced smooth muscle cell function

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assessed by nitroglycerin-mediated dilation (NTGMD),^{7,10} which is recognized as an independent risk factor for atherosclerosis in adults, but is related to FMD.¹⁴

However, previous studies have been performed without differentiating the pubertal stage. Indeed, puberty is defined as a period of intense hormonal change and increased insulin resistance.^{15,16} Obese children have generally accelerated growth and early pubertal development,¹⁷ that may play a detrimental role in the progression and deterioration of arterial function. In children with type 1 diabetes, puberty modulates endothelial function and antioxidant mechanisms,¹⁸ however there is no information in obese children.

The primary purpose of this study was to measure viscoelastic parameters of the common carotid artery as well as endothelial and smooth muscle function of the brachial artery in obese children before the onset of puberty, using non-invasive ultrasound method that we previously applied in the paediatric population.⁷ We aimed also to determine the relationships between arterial function parameters, systemic blood pressure, and biological markers. It is tempting to hypothesize that arterial dysfunction is already present in obese children before puberty, suggesting that adiposity is a major determinant of premature development of CVD.

Methods

Study design and subjects

This was a cross-sectional study including 71 pre-pubertal children (68% female) aged 6–11 years, in two groups: obese ($n = 48$) and lean children ($n = 23$). Obese children were recruited among the patients visiting the Obesity Clinic of the Children's Hospital of Geneva. Fifty obese children aged 6–11 years were initially assessed for the inclusion in the study, two of them were not eligible, and 48 gave their consent. Obesity was defined as body mass index (BMI) above the 97th age- and gender-specific percentiles.¹⁹ Body fatness by DXA was $>25\%$ in boys and $>30\%$ in girls.²⁰ All obese subjects were considered as overweight according to the IOTF definition, and 67% of them were obese.²¹ None of the obese was born prematurely or small-for-gestational age (SGA), but four of them were large-for-gestational age (LGA).

Lean children were recruited in local schools. Teachers distributed the information sheet to all students in the first to fourth elementary school year (165 children). Twenty-three volunteered for the study, however two of them were not eligible for inclusion (overweight). We recruited two more lean children among peers of obese patients. They had height and weight within the normal range (± 2 DS) and BMI <90 th age- and gender-specific percentiles. None of them was premature or SGA, and only one child was LGA at birth.

Subjects were excluded from the study if they had: (i) a family history of dyslipidaemia or hypertension; (ii) taken any medications or hormones, which might influence cardiovascular function, body composition, lipid, or glucose metabolism in the preceding 6 months; (iii) a genetic disorder or a chronic disease.

We assessed the pubertal stage by clinical examination according to methods of Tanner. All obese and lean subjects were at the pre-pubertal Tanner Stage 1. With these restrictive inclusion criteria, we do not have to consider the effect of gender on body fat gain at the time of puberty, generally observed in adolescent girls.²²

The Mother and Child Ethics Committee of the University Hospitals of Geneva approved this study and informed written consent was obtained from both parent and child.

Anthropometric measurement

We measured body weight to the nearest 0.1 kg using an electronic scale (SecaTM, Germany), height to the nearest 0.1 cm using a Harpenden stadiometer, waist circumference to the nearest 0.1 cm using a non-elastic flexible tape at the mid-point between the lower ridge of the ribs in the mid-axillary line and the top of the iliac crest, at minimal respiration. BMI was calculated as weight/height squared (kg/m^2). We assessed whole body fatness (%) using dual-energy X-ray absorptiometry (DXA—GE Lunar ProdigyTM, Lunar Corp., USA).²³ The intra-class correlation for repeated measurements of body fat mass was 0.998 in our laboratory.

Systemic blood pressure

Casual blood pressure (BP) was measured after 10 min of rest and in recumbence using a standard oscillometric method (ColinTM Press-Mate BP 8800C, USA). The cuff covered two-thirds of the length of the upper arm, with the length of the bladder covering the arm circumference. The left brachial BP was measured three times at a 2 min interval, and the average was taken as baseline. Hypertension was defined as BP >95 th gender-, age-, and height-specific percentiles.²⁴ The pulse pressure (PP) was the difference between systolic (SBP) and diastolic blood pressure (DBP).

We performed an ambulatory blood pressure monitoring (ABPM) during 24 h using an automatic monitor in oscillatory mode and a position captor (Dyasis Integra IITM, Physicor S.A., France). The accuracy of this device was previously validated by the British Hypertension Society and Association for the Advancement of Medical Instrumentation.²⁵ The cuff was placed on the left arm at the end of the initial visit and covered two-thirds of the length of the upper arm, with the length of the bladder covering the arm circumference. During the recordings, subjects were asked to follow their ordinary daily activities and to relax the arm by the side of the body. Measures were taken every 30 min during daytime and every 60 min during nighttime to the nearest 1 mmHg. Measurements were repeated twice at a 2 min interval if SBP or DBP was >95 th percentiles,²⁴ or in case of artefactual readings, identified according to predetermined editing criteria. Only ABPM profiles with at least 10 valid recordings during daytime (7 a.m. to 8 p.m.) and five during nighttime (midnight to 7 a.m., in supine position) were accepted. Thirty-nine obese and 15 lean children had valid recordings. We calculated mean SBP and DBP during 24 h, daytime and nighttime; 24 h BP z-scores and BP load (percentage of BP readings >95 th percentiles), based on the normative data of the German Working Group on Pediatric Hypertension.²⁶ Hypertension stage 2 was defined as mean ambulatory SBP >95 th percentile with an SBP load $\geq 25\%$, and hypertension stage 3 if SBP load $\geq 50\%$.^{27,28}

Arterial function and mechanical indices

Non-invasive measurements of arterial geometry and function were performed with a real-time B-mode ultrasound imager (VingmedTM CFM800C system Ltd, Norway) using a 10 MHz linear high-resolution vascular probe as previously described.^{29,30} Imaging of the IMT was performed in the far wall of the right common carotid artery (CCA) 2–3 cm proximal to the bifurcation. The two parallel echogenic lines (double-line pattern), corresponding to the lumen–intima and media–adventitia interfaces defining the IMT, were obtained in the right carotid artery in all subjects. The correct IMT image was 'frozen' in end-diastole by ECG R-triggering, transferred to

a computer, digitized into 640 × 580 peak cells with 256 grey levels, and stored for off-line analysis. All off-line measurements of IMT were performed by the same reader (Y.A.) without knowing subject group assignment and using an automated computerized program (Iotec System™, Iodata Processing™, France).²⁹ Average IMT was calculated as the mean value of a great number of local IMT measurements performed every 100 μm along at least 1 cm of longitudinal length of the artery. The measurement field included specifically the far wall IMT and drew automatically a rectangle of at least 1 cm in length in the longitudinal axis of the vessel and of at least 0.3 cm in width, perpendicular to the wall. The computerized program of measurement located the two interfaces (lumen–intima and media–adventitia) by discriminating changes in grey levels inside the rectangle. The same software was used to measure automatically the end-systolic (Ds) (T wave triggering) and end-diastolic diameter (Dd) (R wave triggering) of the CCA.

Non-invasive assessment of endothelium-dependent dilation (flow-mediated dilation, FMD) and endothelium-independent dilation (in response to 300 μg sublingual nitroglycerin, NTGMD) of the right brachial artery (RBA) were performed by the same echographic vascular linear probe as previously described.⁷ After baseline measure, we assessed the dilation of the RBA in response to increased flow and NTG, and FMD and NTGMD were calculated as absolute and percentage maximum increase in vessel size from baseline.

We determined the pulse wave using applanation tonometry probe (Sphygmocor™; Atcor Medical Ltd, Australia) that was applied to the surface of the skin overlying the radial artery and the peripheral radial pulse wave was recorded continuously. For accurate measurement, the micromanometer was applied with high pressure to flatten the vessel walls, so that transmural forces were perpendicular to the arterial surface. The mean values of at least 20 pulse waves were used for analyses. The Sphygmocor™ system incorporates the pulse recorded at the radial artery and the properties of the transfer function between the aorta and the radial artery to estimate central aortic pressure non-invasively.^{31,32} The waveform was calibrated using the diastolic and mean pressure values from the brachial oscillometric measurement, and Sphygmocor™ derived a complete waveform for the whole cardiac cycle for the aortic pulse. An average waveform was calculated from a series of contiguous pulses. Once the aortic pulse was derived, a number of data were extracted to enable calculations, which could not be made from the peripheral pulse or the conventional measures of brachial blood pressure. The non-invasive assessment of central pulse pressure (CPP) was derived and used to calculate mechanical indices of the CCA.

Cross-sectional compliance (CSC) and cross-sectional distensibility (CSD) were determined according to the following formulas:

$$\text{CSC} = \pi(D_s^2 - D_d^2)/4 \cdot \text{PP}(\text{mm}^2 \cdot \text{mm Hg}^{-1}), \text{ and } \text{CSC} \\ = (D_s^2 - D_d^2)/(D_d^2 \cdot \text{PP}), \text{ mm Hg}^{-1} \cdot 10^{-2}$$

These parameters provide information about 'elasticity' of the artery as a hollow structure.

Incremental elastic modulus was calculated using the following formula:

$$\text{Einc} = (3 \cdot (1 + \text{LCSA} \cdot \text{WCOSA}^{-1})) \cdot \text{CCA distensibility}^{-1}, \text{ mm Hg} \\ \cdot 10^3$$

This parameter provides information on the properties of the wall material, independent of its geometry, where lumen cross-sectional area (LCSA, mm²) is calculated as $\pi(D_d^2)/4$, and wall cross-sectional area (WCOSA, mm²) is calculated as $\pi(\pi D_d/2 + \text{IMT})^2 - \pi(D_d/2)^2$.

Biological markers

Blood samples were collected via venipuncture following a 10 h overnight fast. Total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), triglycerides (TG) levels [mmol·l⁻¹] were determined using a standard automotive techniques (SYNCHRON LX20®). Plasma insulin concentrations were measured by radioimmunoassay (Access® ultrasensitive insulin, Beckman Coulter Ireland Inc.) and insulin resistance was assessed by using the homeostasis model assessment (HOMA-IR),³³ according to the equation: $\text{HOMA-IR} = \text{fasting insulin } [\mu\text{U}\cdot\text{ml}^{-1}] \times \text{fasting glucose } [\text{mmol}\cdot\text{l}^{-1}]/22.5$.

Statistical analysis

Data were screened initially for normalcy, using skewness and kurtosis tests. The following arterial function variables were transformed and successfully normalized: CSC and NTGMD (1/square-root); CSD and FMD (square-root); Einc (log). Data were expressed as mean ± standard deviation or median ± interquartile range (IQ range, 25–75), when appropriate. We compared the means of groups by independent *t*-tests (two-sided) and analysis of covariance (ANCOVA), including age and gender as covariates, as well as Mann–Whitney *U* tests to compare the shapes of distributions. As there were no age and gender effects in ANCOVA, and Mann–Whitney *U* tests yield similar results, we report only independent *t*-test results. Differences among groups were highly significant and we did not have to correct for the inflation of the Type I error due to multiple testing. We also evaluated relationships between dependent and independent variables using Pearson's correlations, univariate and multivariate regression analysis. We used the statistical software program SPSS version 11.0 (Chicago, IL) and Stata release 9.2 (College Station, TX). Differences were considered significant if $P < 0.05$.

Results

Physical and haemodynamic characteristics of subjects are shown in Table 1. Body weight, BMI, waist circumference, and percentage of body fat were significantly higher in obese compared with lean children. There were no significant differences in casual SBP, DBP, MBP, PP, and CCP. Obese subjects had significantly higher 24 h, daytime and nighttime ambulatory SBP and DBP than lean children. Mean 24 h SBP load (47.7 ± 26.0 vs. $17.2 \pm 12.8\%$, $P < 0.001$) and DBP load (25.2 ± 15.9 vs. $14.2 \pm 9.2\%$, $P = 0.003$) were increased in children with obesity compared with lean controls. Forty-seven percent (18/38) of obese subjects had 24 h SBP above the ABPM 95th percentile,²⁶ and 15% (6/38) of them had both SBP and DBP hypertension. Of 18 hypertensive children detected by ABPM, five had also casual SBP above the 95th percentiles,²⁴ whereas 13 had normal casual SBP (masked hypertension). All of them had 24 h SBP load above 50% (stage 3 hypertension, mean load $77.2 \pm 15.7\%$). White coat hypertension (high casual BP but normal ambulatory BP) was present in 3% (1/38) of obese children. None of the lean subjects had elevated casual or ambulatory BP.

Blood lipids and insulin resistance indices are shown in Table 2. Obese children had significantly lower HDL-cholesterol and higher fasting insulin and HOMA-IR than lean controls.

Arterial geometry, mechanical properties, and vascular reactivity results are shown in Table 3. The systolic diameter, IMT, CSC, and CSD of the CCA were not different between groups. Obese children had significantly increased diastolic diameter, resulting in

Table 1 Physical characteristics and blood pressure of obese and lean children

Parameters	Obese, n = 48	Lean, n = 23	P-value
Gender (% female)	67	69	0.69
Age (years)	8.9 ± 1.5	8.3 ± 1.5	0.57
Height (cm)	137.6 ± 9.4	134.3 ± 9.6	0.67
Body weight (kg)	47.8 ± 13.3	28.2 ± 5.4	<0.0001
BMI (kg/m ²)	24.9 ± 4.7	15.5 ± 1.5	<0.0001
Waist circumference (cm)	79.0 ± 7.6	55.4 ± 3.8	<0.0001
Body fat (%)	42.0 ± 7.0	19.2 ± 6.3	<0.0001
Casual SBP (mmHg)	109.2 ± 10.8	106.7 ± 6.9	0.33
Casual DBP (mmHg)	55.1 ± 9.0	52.4 ± 5.2	0.19
Casual pulse pressure (mmHg)	54.0 ± 10.4	54.5 ± 8.4	0.87
Central pulse pressure (mmHg)	39.4 ± 14.7	36.2 ± 11.1	0.24
24 h SBP (mmHg)	124.1 ± 14.3	104.7 ± 9.2	<0.0001
24 h DBP (mmHg)	72.5 ± 7.4	62.1 ± 4.3	<0.0001
24 h SBP Z-scores	1.9 ± 1.6	-0.4 ± 1.2	<0.0001
24 h DBP Z-scores	1.2 ± 1.3	-0.7 ± 0.8	<0.0001
Daytime SBP (mmHg)	126.4 ± 13.9	106.6 ± 11.1	<0.0001
Daytime DBP (mmHg)	75.9 ± 8.8	65.1 ± 4.7	0.001
Nighttime SBP (mmHg)	118.0 ± 17.5	101.0 ± 9.3	0.004
Nighttime DBP (mmHg)	64.4 ± 9.1	56.6 ± 5.8	0.011

Values are shown as mean ± standard deviation. BP, blood pressure; S, systolic; D, diastolic.

Table 2 Blood lipids and insulin resistance indices of obese and lean children

Parameters	Obese, n = 48	Lean, n = 23	P-value
Total cholesterol (mmol/L)	4.37 ± 0.78	4.30 ± 0.42	0.71
LDL-cholesterol (mmol/L)	2.96 ± 0.76	2.64 ± 0.39	0.065
HDL-cholesterol (mmol/L)	1.09 ± 0.23	1.39 ± 0.27	<0.0001
Triglycerides (mmol/L)	0.66 ± 0.29	0.56 ± 0.21	0.15
Fasting glucose (mmol/L)	4.80 ± 0.38	4.74 ± 0.39	0.52
Fasting insulin (mU/L)	12.26 ± 6.28	4.83 ± 3.26	0.001
HOMA-IR	2.62 ± 1.36	1.05 ± 0.80	0.001

Values are shown as mean ± standard deviation. LDL, low-density protein; HDL, high-density protein; HOMA-IR, homeostasis assessment model of insulin resistance.

augmented CSA, WCSA, Einc, and increased change in RBA diameter between baseline and post-dilation hyperaemia (Δ RBA diameter). The FMD and NTGMD, expressed as % dilation of the RBA, were significantly lower in obese compared with lean subjects.

Inverse correlations between body fatness and FMD or NTGMD in lean and obese children ($n = 71$) are shown in Figure 1A and B, respectively. Body fatness by DXA was also correlated with casual SBP ($r = 0.24$, $P < 0.040$) and DBP

Table 3 Mechanical properties of the common carotid artery and vascular reactivity of the brachial artery of obese and lean children

Parameters	Obese, n = 48	Lean, n = 23	P-value
Systolic diameter of CCA (mm)	5.82 ± 0.50	5.85 ± 0.30	0.16
Diastolic diameter of CCA (mm)	5.06 ± 0.50	4.78 ± 0.52	0.03
Intima-media thickness of CCA (mm)	0.49 ± 0.03	0.48 ± 0.02	0.29
LCSA (mm ²)	20.34 ± 4.02	18.14 ± 3.98	0.04
WCSA (mm ²)	8.49 ± 1.10	7.87 ± 0.79	0.022
Cross-sectional compliance (mm ² /mmHg) ^a	0.12 ± 0.11	0.10 ± 0.13	0.11
Cross-sectional distensibility (mmHg ⁻¹ /10 ²) ^a	0.64 ± 0.10	0.84 ± 0.50	0.23
Incremental elastic modulus (mmHg/10 ²) ^a	13.89 ± 5.19	10.43 ± 5.16	0.009
Δ RBA diameter (mm)	0.14 ± 0.01	0.21 ± 0.01	0.0007
Flow-mediated dilation (%) ^a	4.50 ± 4.01	8.30 ± 1.70	<0.0001
NTG-mediated dilation (%) ^a	19.02 ± 9.04	25.80 ± 6.06	0.0014

Values are shown as mean ± standard deviation. CCA, common carotid artery; LCSA, lumen cross-sectional area; WCSA, wall cross-sectional area; RBA, right brachial artery; Δ RBA, difference RBA hyperaemia—baseline diameter; NTG, nitroglycerin.

^aMedian ± interquartile range.

($r = 0.28$, $P < 0.012$), ambulatory SBP ($r = 0.49$, $P < 0.0001$), and DBP ($r = 0.52$, $P < 0.0001$), HDL-cholesterol ($r = -0.42$, $P = 0.0004$), triglycerides ($r = 0.25$, $P = 0.039$), insulin ($r = 0.65$, $P < 0.0001$), HOMA-IR ($r = 0.64$, $P < 0.0001$), LCSA ($r = 0.33$, $P < 0.006$), WCSA ($r = 0.29$, $P < 0.007$), and Einc ($r = 0.34$, $P < 0.005$). Correlations between BMI and the preceding parameters were similar and all significant. Neither body fatness nor BMI were associated with IMT, CSC, or CSD. When the correlations were carried out in the two groups separately, relationships were no longer present in the lean group, except between body fatness and Einc ($r = 0.48$, $P = 0.03$).

Flow-mediated dilation and NTGMD were negatively correlated with ambulatory SBP ($r = -0.38$, $P = 0.002$ and $r = -0.31$, $P = 0.014$), and DBP ($r = -0.31$, $P = 0.015$ and $r = -0.26$, $P = 0.04$), respectively. They were not related to age, gender, or other mechanical indices, though correlations coefficients between FMD, NTGMD, and Einc were close to significance ($r = -0.21$, $P = 0.08$ and $r = -0.23$, $P = 0.054$, respectively). Ambulatory SBP ($r = 0.47$, $P = 0.004$ and $r = 0.48$, $P = 0.0008$), DBP ($r = 0.45$, $P = 0.003$ and $r = 0.46$, $P = .002$), as well as FMD ($r = -0.41$, $P = 0.004$ and $r = -0.43$, $P = 0.030$) were significantly correlated with insulin or HOMA-IR, respectively, however these associations disappeared when adjusted for body fatness.

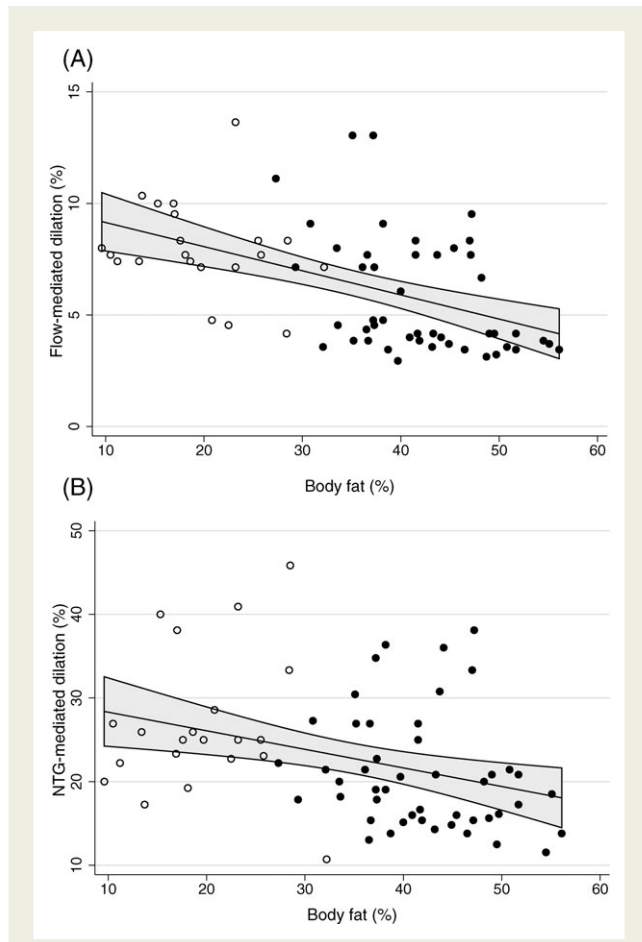


Figure 1 (A) Inverse relationship between flow-mediated dilation (FMD, %) and percentage of body fat by DXA in obese and lean children ($n = 71$, $r = -0.52$, $R^2 = 0.27$, $P < 0.0001$). \circ , Lean; \bullet , Obese. (B) Inverse relationship between nitroglycerin-mediated dilation (NTGMD, %) and percentage of body fat by DXA in obese and lean children ($n = 71$, $r = -0.40$, $R^2 = 0.15$, $P = 0.0008$). \circ , Lean; \bullet , Obese

Discussion

Impaired endothelial and smooth muscle cell functions, as well as arterial stiffness have been previously described in children and adolescents,^{7–10} however these studies did not take into account the stage of puberty. We demonstrate that impaired brachial endothelial (FMD) and smooth muscle (NTGMD) functions, as well as alteration of wall material of the carotid artery, appear before puberty in obese children, without concomitant increase of the carotid IMT. We also report elevated ambulatory systolic and diastolic blood pressure in obese compared with lean subjects, whereas casual blood pressures remain within the normal range. Reduction of FMD and NTGMD may be considered as the first markers of atherosclerosis and a consequence of obesity even in this very young population. Our findings suggest that early functional changes in the vessel wall are not limited to the endothelium.

In this present study, both FMD and NTGMD were inversely related to BMI and body fatness, in accordance with previous

publications in obese children and adolescents.^{7–9,34} We also found that insulin resistance is not an independent determinant of endothelial dysfunction in this population.^{7–9}

Some authors reported also that IMT of the CCA was augmented in obese children and adolescents and was associated with high peripheral blood pressure at rest, compared with lean subjects.^{2,9,35–37} Remodelling of the arterial wall can be a matter of debate. Indeed, studies that showed increased carotid IMT in obese youth^{4,8,9,35,36,38} included subjects with severe hypertension^{4,35,39} or with BP values significantly higher when compared with normative data.³⁵ On the opposite, a recent publication did not observe any increase of IMT in severe obese adolescents with a mean age of 12,^{5,40} in accordance with our previous findings in a similar population.⁷ In this present study, we do not demonstrate any significant increase of IMT, or any association with body fatness, using a validated computerized ultrasonic procedure previously described. This method automatically calculates the IMT of the far wall of the CCA and the arterial lumen diameter with high repeatability,³⁰ avoiding observer bias, and is likely more precise than measurements using a caliber.

Mechanical indices of the CCA were derived from non-invasive assessment of CPP. Our study shows that incremental elastic modulus (Einc) is significantly increased in pre-pubertal obese children compared with lean subjects, suggesting early alterations of wall material. However, these changes appear to be insufficient to induce a decrease of cross-sectional compliance or distensibility. As we aimed to detect early modification of the morphology and function of the CCA, it was necessary to have the nearest estimate of the blood pressure in this artery. The tonometry of applanation, a technique described by O'Rourke *et al.*, is widely used and highly reproducible when compared with direct central aortic measurements in adults.^{31,32} This non-invasive method has been applied in children and adolescents,^{7,41,42} although it has not been specifically validated for this population. We calculated the mechanical indices of the CCA using the CCP that depends on blood flow, peripheral vascular resistance, and elasticity of the aorta and large central elastic arteries. Indeed, blood pressure is a well-defined stress to the arterial wall and increase of IMT is an adaptive response to tensile stress. Pressurizing a vessel distends the wall; if we consider only the circumferential stress (CS), as in previous studies, this parameter is comparable with the wall tension given by the law of Laplace but accounts for the finite wall thickness of arteries. The law of Laplace states that the tension (T), force per unit length in the wall of a very thin cylindrical shell, is related to transmural pressure (P) and radius r as $T = P \cdot r$. The circumferential stress on the wall depends on T and the wall thickness ($CS = T \cdot IMT^{-1}$) so that the increase of the IMT compensates for a constant wall stress when the tension increases. The increase of the IMT is considered as a consequence of a remodelling process of the arterial wall, induced by atherosclerosis, but also by an increase of the CCP.

To our knowledge, this is the first study reporting that both CCP and IMT of the CCA are not modified in pre-pubertal children with obesity, though the augmentation of the incremental elastic modulus reflects changes of the arterial wall properties independently of geometry. Arterial wall remodelling may be incipient in pre-pubertal obese children, being sufficiently advanced to

increase vessel stiffness but not enough to increase IMT. We hypothesize that the persistence of adiposity during puberty may reach the state of arterial thickening, as previously described.^{2,9,35–37} The long-term impact of obesity on morbidity and mortality is in fact well demonstrated.⁴

Twenty-four hour ABPM is considered as the method of choice for the diagnosis and therapeutic monitoring of arterial hypertension in paediatric population.⁴³ In adults, ABPM has a much higher correlation with cardiovascular outcomes than do casual BP measurements.⁴⁴ Several studies reported that ABPM was related to BMI and body fat distribution in children and adolescents,^{26,45} as well as insulin resistance,⁴⁶ though the later study did not compare BP with lean children or normative references. Lurbe *et al.*⁴⁵ found increased ambulatory mean BP and BP load in obese children aged 9.5 ± 2.5 years, using the Task Force on Blood Pressure Control in Children Tables.²⁴ However, their subjects were not hypertensive using ABPM normative data.²⁷ We show that pre-pubertal children with obesity have significantly higher ambulatory mean SBP and SBP than lean children, or normative data of the German Working Group on Pediatric Hypertension.²⁶ Higher prevalence of hypertension in our study might be explained by the use of different normative data, as well as higher age-specific BMI in our cohort.

We show that only few subjects with obesity had systolic hypertension at rest, whereas the prevalence of ambulatory systolic hypertension was high (47%). One-third of our obese subjects had masked hypertension, defined as elevated ABPM without concomitant elevation of casual BP. Indeed, normal casual BP described in children with obesity is not a contradictory finding with high BP measured by ABPM.⁴³ Lurbe *et al.*⁴⁷ reported that 7.6% of healthy subjects aged 6–18 years had masked hypertension, and it was associated with obesity. Though the prevalence of hypertension by ABPM in obese children was not reported. Indeed, several pediatric studies demonstrated that casual BP does not reflect the real hypertension state and may miss the diagnosis in about 60% of children and adolescents.^{28,45} Our findings increase the awareness of masked hypertension in pre-pubertal obese children, a less appreciated and more dangerous issue than white-coat hypertension. Of particular importance is the documentation that elevated BP in childhood often correlates with hypertension in early adulthood, thereby supporting the need to track BP in obese children.⁴⁸ Ambulatory blood pressure monitoring may therefore be a potential tool to improve risk stratification in young patients with obesity, in reference to normative data taking into account the role of body dimensions.²⁶

Several factors may explain the development of systemic hypertension in obese children. Reduced activity of the parasympathic nervous system, manifested as a depressed heart rate response to deep breathing, in the presence of an unaltered reactivity of the sympathetic nervous system, has been observed in this population.⁴⁹ High systemic blood pressure is also associated with increased cardiac output in children and adolescents with excessive weight gain.⁵⁰ In our study, reduced FMD and NTGMD could also play a role in the progression of systemic hypertension in young obese children. We demonstrated a significant inverse relationship between ABPM and FMD, or NTGMD. Impairment of endothelial and smooth muscle functions contributes to

reduce the dilation of peripheral arteries in daily activities, which can explain elevated ABPM and normal BP at rest. Normal CCP is not a contrary finding with elevated peripheral BP, if dynamic viscoelastic parameters of the CCA are not yet altered, as observed in this study. Further investigations should clarify the clinical value of ABPM by comparing a similar group of obese children in different conditions of daily activities.

In conclusion, to our knowledge, this is the first study that demonstrates impaired endothelial and smooth muscle cell functions and increased local arterial stiffness before puberty in obese children, without significant evidence of arterial remodelling. These findings may be considered as the first sub-clinical signs of atherosclerosis and are associated with elevated ambulatory blood pressure. This study gives essential information to understand the mechanisms of the development of vascular disease associated with childhood obesity. Ambulatory blood pressure monitoring may be a useful tool to detect cardiovascular risk factors for the long-term in this population. Indeed, accurate diagnosis in children is particularly important as the complications of hypertension are strongly related to its longevity. Future studies will be required to demonstrate that early intervention in this population can improve vascular function and preserve the mechanical properties of large elastic artery during puberty.

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