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# Determinants of carotid-femoral pulse wave velocity in prepubertal children☆

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ABSTRACT

*Background:* Pulse wave velocity (PWV) is a noninvasive technique to evaluate arterial stiffness, a dynamic property of the vessels, reflecting their structure and function. Childhood obesity is associated with several cardiovascular comorbidities and to the progression of atherosclerosis. We aimed to compare carotid-femoral PWV between normal weight and overweight/obese prepubertal children and to quantify its association with other cardiovascular risk factors.

*Methods:* Cross-sectional study of 315 children aged 8–9 years. Anthropometrics, 24-h ambulatory blood pressure (BP) and carotid-femoral PWV were measured. Classification of obesity was according to World Health Organization (WHO) body mass index (BMI)-for-age reference values.

*Results:* Compared to normal weight children, overweight and obese children presented significantly higher levels of PWV (4.95 (P25–P75: 4.61–5.23), 5.00 (4.71–5.33), 5.10 (4.82–5.50) m/s, respectively; $p_{trend} < 0.001$). Significant positive correlations were found between PWV and total cholesterol, LDL cholesterol, triglycerides, fasting insulin and insulin resistance levels (HOMA-IR) and with high-sensitivity C-reactive protein (hs-CRP). In a multivariate linear regression model adjusted for sex, age, height and 24-h systolic blood pressure z-score, the independent determinants of PWV were BMI, HOMA-IR and the absence of dipping.

*Conclusions:* The association between PWV and the loss of dipping and insulin resistance levels, independently of the BMI, reinforces the contribution of these comorbidities to vascular injury in early life.

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## 1. Introduction

Obesity is a prominent public health problem worldwide with a growing prevalence in both adults and children [1]. Childhood obesity is known to be associated with the premature development of several cardiovascular (CV) risk factors, such as dyslipidemia, hypertension and insulin resistance [2], and with the development and progression of atherosclerosis [3]. In fact, the process of atherosclerosis, a common denominator in CV diseases, was demonstrated to begin in early life, with preclinical vascular wall abnormalities being already present in childhood [4,5].

Arterial stiffness is a dynamic property of the vessels which reflects changes in the arterial structure and function [6]. In adults, several promising techniques for large arterial function evaluation continue to emerge, such as wave intensity analysis [7,8]. Arterial stiffness is an indirect marker of subclinical atherosclerosis [6] that has proved to be an accurate CV risk predictor in adults [9]. In the pediatric population its usefulness is also being increasingly recognized [6]. Pulse wave velocity (PWV) can be used to assess arterial stiffness by a noninvasive and high-resolution technique [10] that has become one of the primary methods for evaluation of vascular damage in childhood [6,11]. PWV, the pulse pressure's velocity during its propagation along the arterial tree [12], depends on geometric and elastic properties of the arterial wall [11] and is influenced by various factors such as age, height, gender, race and blood pressure [11,13,14].

Several CV risk factors can accelerate the age-related natural process of atherogenesis [12,15]. Specifically in children some studies reported lower values of PWV in the obese [16,17], but the overall evidence is

☆ All authors listed above takes responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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in favor of obese children having higher levels of arterial stiffness and PWV compared to non-obese controls [18]. At the same time, contradictory evidence still exists concerning the relationship between pulse wave velocity and classical CV risk factors, such as dyslipidemia [19–21]. Higher levels of blood pressure (BP) are usually positively associated with parameters of arterial stiffness [13,14,16] and the association of the latter with insulin resistance levels has also been reported [22]. The differentiation between the natural process of vessels' development and aging from the pathological influence of CV risk factors on the vascular tree is quite challenging, particularly during child's growth [23]. For this reason, we consider particularly important to evaluate the association of PWV values with obesity and CV risk factors in young children, avoiding the issue of secondary pathology related to target-organ damage and comorbidities often encountered in older populations.

Our main objective was to compare carotid-femoral PWV between normal weight and overweight/obese 8–9-year-old children. Secondarily, we aimed to identify if an association between other cardiovascular risk factors and pulse wave velocity could be found.

## 2. Methods

### 2.1. Study design and sample

We studied children aged 8–9 years that have been followed since birth in a previously established cohort study (Generation XXI, Porto-Portugal) [24]. From the original cohort (n = 8647), 4590 children attended a face-to-face follow-up visit at 7 years, thus being eligible for the ObiKid project — a specific project aiming to clarify the impact of childhood obesity and associated comorbidities on the kidney. To obtain a minimum sample of 300 children for the ObiKid project, we consecutively screened 463 children, according to the date of their 7 years old evaluation: 16 could not be contacted, 32 refused to participate, 23 unable to schedule the study visit during the recruitment period and 68 met exclusion criteria (4 chronic diseases, 1 chronic usage of medication, 51 with residence >30 km away from the study site and 6 pairs of twins). We enrolled 324 participants, between August 2013 and August 2014, but for the present analysis we additionally excluded 9 children due to incomplete evaluation, such as absence of PWV assessment.

A total of 315 children were included, which provides a statistical power above 95% to detect a difference in PWV levels between nonoverweight and overweight/obese children of at least 1.2 m/s (assuming a standard deviation (SD) of 1.0 in each group) [25].

### 2.2. Data collection and variables definition

The study visits took place at the Department of Clinical Epidemiology, Predictive Medicine and Public Health, Faculty of Medicine of University of Porto. Anthropometric and general physical examination were performed, according to standard procedures and as previously reported [26]. Waist circumference was indexed to height (waist-to-height ratio, WHtR in cm/m) for statistical analysis. Body mass index (BMI) was calculated and BMI-for-age values were classified according to the World Health Organization (WHO) reference data for BMI z-score into the following categories: nonoverweight (≤ + 1 SD, including 1 children with thinness) and overweight/obesity (> + 1 SD) [27]. Body fat percentage was assessed by foot-to-foot bioelectrical impedance analysis (Tanita®, model TBF-300, Arlington Heights, Illinois).

Ambulatory BP monitoring (ABPM) for 24 h was performed with a portable non-invasive oscillometric BP recorder (Spacelabs Healthcare®, model 90,207, Snoqualmie, Washington). The non-dominant arm was used in all children with a cuff size appropriate to the child's arm circumference. BP measurements were taken automatically at 20-min intervals during the daytime and at 30-min intervals during the nighttime. A minimum monitoring duration of 24 h with gaps of less than 2 h was required for acceptance; 5 exams were excluded of the ABPM analysis due to insufficient readings. The readings were used to calculate mean 24 h, day and night mean arterial pressure (MAP), systolic (SBP) and diastolic BP (DBP), with the SpaceLabs® software. Standard deviation scores for BP values were calculated (by the least mean square method) and hypertension was defined using the published reference values of the German Working Group on Pediatric Hypertension [28]. The absence of dipping was considered as a fall in the MAP during nighttime of less than 10% of the corresponding daytime MAP.

The carotid-femoral PWV analysis was performed by a single trained cardiopneumology technician with a portable device (Micro Medical®, model PulseTrace PWV PT4000, Kent, UK). Children were placed in a supine position. After 5 min of rest, the carotid-femoral distance was assessed as the distance of suprasternal notch to the umbilicus and from there to the measuring point at the femoral artery minus the suprasternal notch to the measuring point at the carotid artery. Electrocardiogram registry was performed simultaneously, allowing the software to calculate the time from the peak of the R-wave to the foot of the pulse wave at the carotid and femoral arteries, respectively. The digital volume pulse waveform had to fill 2/3 of the display with little or no noise and artifact to be considered and 3 measurements of PWV were performed and averaged for analysis.

### 2.3. Laboratory procedures

A venous blood sample was collected from all the participants, after an overnight fast of at least 8 h and analyzed for glucose, insulin, triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol, high-sensitivity C-reactive protein (hsCRP) and interleukin-6 (IL-6). Insulin resistance was determined using the homeostasis model assessment index (HOMA-IR). All the standard laboratory analyses were performed in the Clinical Pathology Department of Centro Hospitalar São João, Porto — Portugal. hsCRP was tested by immunonephelometric assay with CardioPhase® hsCRP (Siemens Healthcare Diagnostics®, Munich, Germany) and IL-6 was tested by immunoassay (Cobas Integra 700 Autoanalyzer, Roche®, Basel, Switzerland).

### 2.4. Ethics

The ObiKid study was approved by the Ethics Committee of Centro Hospitalar São João, Porto – Portugal and Faculty of Medicine of the University of Porto and by the Portuguese Data Protection Authority. It complies with the Helsinki Declaration and the current national legislation. Written informed consent from parents (or their legal substitute) and verbal assent from children was obtained regarding the collection of information and biological samples.

### 2.5. Statistical analysis

Data are presented as mean and standard-deviation (SD) or, if skewed, as median and percentiles 25 and 75 (P25-P75). The tertiles of WHtR and % of body fat mass were defined based on all enrolled participants. Bivariate associations were assessed by Spearman correlation. A multivariate linear regression model was fitted to identify independent determinants of carotid-femoral PWV. Sex, age (in months), height (in cm) and 24-h systolic BP, factors known to affect PWV [11,13,14], were forced into the model. Additionally, all variables with significant correlations were initially included in the linear regression model as covariates and a backward stepwise approach was performed to fit the final model.

## 3. Results

A total of 315 children (53% male) with a mean (SD) age of 8.8 (0.2) years were included in the present analysis. General characteristics, ABPM values and laboratorial parameters are shown in Table 1, by classes of BMI (normal weight, n = 165; overweight, n = 89; obese, n = 61). Regarding ABPM values, a trend towards higher z-score values across BMI classes was evident for 24-h, daytime and nighttime systolic BP. No differences were found between the groups in the diastolic BP z-score values, percentage of children presenting hypertension and non-dipping pattern. Overweight and obese children presented significantly higher levels of triglycerides, fasting insulin and HOMA-IR and hs-CRP.

**Table 1**
Characteristics and laboratorial parameters, in non-overweight, overweight and obese children.

| | WHO BMI z-score classification | | | p |
|---|---|---|---|---|
| | Normal weight | Overweight | Obese | |
| | n = 165 | n = 89 | N = 61 | |
| *Demography and anthropometry* | | | | |
| Age (months) | 105.0 ± 2.9 | 105.3 ± 2.8 | 105.6 ± 2.8 | 0.394 |
| Male sex | 84 (50.9%) | 43 (48.3%) | 40 (65.6%) | 0.084 |
| BMI z-score | −0.01 ± 0.74 | 1.56 ± 0.30 | 2.70 ± 0.5 | <0.001 |
| WHtR (cm/m) | 44.8 ± 2.5 | 50.2 ± 3.3 | 56.6 ± 4.5 | <0.001 |
| % body fat mass | 11.0 ± 7.2 | 20.1 ± 7.9 | 28.0 ± 9.4 | <0.001 |
| *24-h ambulatory blood pressure* | | | | |
| 24-h systolic BP (mm Hg) | 111.6 ± 7.0 | 113.2 ± 7.2 | 114.5 ± 8.8 | 0.026 |
| z-Score | 0.48 ± 0.97 | 0.93 ± 0.99 | 0.78 ± 0.98 | 0.002 |
| 24-h diastolic BP (mm Hg) | 67.0 ± 4.4 | 67.1 ± 5.2 | 66.5 ± 6.0 | 0.783 |
| z-Score | 0.06 ± 0.94 | 0.29 ± 1.05 | 0.06 ± 1.10 | 0.204 |
| Daytime systolic BP (mm Hg) | 115.7 ± 7.3 | 116.9 ± 7.9 | 118.2 ± 9.0 | 0.083 |
| z-Score | 0.35 ± 1.01 | 0.72 ± 1.04 | 0.57 ± 1.14 | 0.023 |
| Daytime diastolic BP (mm Hg) | 71.1 ± 4.6 | 70.6 ± 5.6 | 70.0 ± 6.3 | 0.387 |
| z-Score | −0.26 ± 0.88 | −0.14 ± 0.92 | −0.31 ± 1.06 | 0.456 |
| Nighttime systolic BP (mm Hg) | 102.9 ± 6.9 | 104.8 ± 6.6 | 106.0 ± 8.6 | 0.007 |
| z-Score | 0.54 ± 0.93 | 0.99 ± 0.84 | 0.87 ± 0.73 | <0.001 |
| Nighttime diastolic BP (mm Hg) | 58.4 ± 5.2 | 58.7 ± 5.7 | 58.7 ± 6.0 | 0.884 |
| z-Score | 0.23 ± 0.98 | 0.42 ± 0.97 | 0.24 ± 0.99 | 0.319 |
| Hypertension | 12 (7.5%) | 8 (9.0%) | 10 (16.7%) | 0.116 |
| Absence of dipping pattern (mm Hg) | 35 (21.7%) | 30 (33.7%) | 17 (28.3%) | 0.113 |
| *Laboratorial parameters* | | | | |
| Total cholesterol (mg/dL) | 156.5 ± 24.7 | 162.7 ± 28.1 | 161.7 ± 24.7 | 0.131 |
| LDL cholesterol (mg/dL) | 91.7 ± 19.9 | 96.5 ± 23.1 | 95.9 ± 21.9 | 0.157 |
| HDL cholesterol (mg/dL) | 54.8 ± 10.1 | 53.7 ± 10.4 | 51.9 ± 8.5 | 0.150 |
| Triglycerides (mg/dL) | 53.1 ± 19.6 | 62.1 ± 29.8 | 69.3 ± 32.8 | <0.001 |
| Fasting glucose (mg/dL) | 85.7 ± 5.5 | 85.4 ± 5.3 | 87.3 ± 5.0 | 0.078 |
| Fasting insulin (μIU/mL) | 5.7 ± 2.4 | 7.2 ± 2.9 | 9.5 ± 5.1 | <0.001 |
| HOMA-IR | 1.12 (0.83–1.96) | 1.46 (1.07–1.84) | 1.68 (1.30–2.63) | <0.001 |
| High sensitivity C-reactive protein (mg/L) | 0.0 (0.0–0.40) | 0.50 (0.20–1.18) | 0.70 (0.35–1.80) | <0.001 |
| Interleukin-6 (pg/mL) | 0.75 (0.75–2.43) | 1.61 (0.75–3.24) | 1.98 (0.75–3.01) | 0.107 |

The values presented are mean ± standard deviation, median (interquartile range) or n (%). The BMI classes are according to the WHO classification for BMI z-score values [27]. BMI, body mass index; WHtR, Waist to height ratio; BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR — homeostasis model assessment of insulin resistance.

The distribution of PWV levels by obesity indexes (BMI classes and tertiles of WHtR and body fat mass) is presented in Fig. 1. Considering BMI classes, compared to normal weight children, the overweight and obese children presented significantly higher levels of PWV (4.95 (P25–P75: 4.61–5.23), 5.00 (4.71–5.33), 5.10 (4.82–5.50) m/s, respectively; $p_{trend} < 0.001$). Concordantly, higher median PWV values were found in the highest tertile of WHtR (5.00 (4.60–5.32), 4.97 (4.67–5.20), 5.07 (4.78–5.35) m/s, $p_{trend} = 0.040$) and % body fat mass (4.88 (4.60–5.23), 4.98 (4.68–5.26), 5.13 (4.85–5.45) m/s, $p_{trend} < 0.001$).

The correlations between PWV and CV risk variables is shown in Table 2. Significant positive correlations were found between PWV and WHtR and body fat mass. Additionally, PWV was positively correlated with total cholesterol, LDL cholesterol and triglycerides, with fasting insulin and HOMA-IR levels and with hsCRP. No significant correlation was detected between PWV and z-score values of BP.

In a multivariate linear regression model adjusted for sex, age, height and 24-h systolic BP (z-score), the independent determinants of PWV identified were BMI, HOMA-IR and the absence of dipping pattern (Table 3). PWV was 0.18 (95% CI: 0.01 to 0.35) m/s higher in the obese group (compared to the normal weight group), 0.16 (0.03 to 0.28) m/s higher in children without a dipping pattern, and increased by 0.11 (0.03 to 0.19) m/s per unit of HOMA-IR.

## 4. Discussion

Carotid-femoral PWV was evaluated in a large sample of healthy prepubertal children as an indirect marker of arterial stiffness. We found higher levels of this marker in overweight and obese children, and also in those with highest WHtR and body fat mass. Moreover, PWV was significantly correlated with several CV risk determinants, namely blood lipids, insulin and HOMA-IR levels and hs-CRP. In a final multivariate model, BMI, HOMA-IR and the absence of dipping were independently associated with PWV.

We report a trend for increasing carotid-femoral PWV levels across BMI classes and other obesity indexes. Several studies reported a greater arterial stiffness in children with obesity but contradictory results have been described, with some studies reporting decreased arterial stiffness [16,17] or increased arterial compliance [29,30] among obese children. One of these studies that reported lower PWV levels in obese children, interpreted those findings as a consequence of overall vasodilatation, possibly secondary to a physiological adaptation to the higher metabolic and hemodynamic demands in the obese group [17]. Interestingly, 5 years later, in the same sample, obese children presented higher PWV levels and a steeper change of arterial stiffness was observed, with an increase of 25% in PWV levels in the obese group (vs. only 3% in normal weight children) [31]. Moreover, our findings are in agreement with the only systematic review and meta-analysis in children on this topic, that reported greater arterial stiffness in children with obesity and a consistency between different types of measures, such as intimae-media thickness and arteries flow-mediated dilatation [18].

In 2010, reference values for PWV were published based on a sample of about 1000 European and northern African healthy children [14]. Although the mean values of carotid-femoral PWV in our sample were below the 95th percentile of those reference values, we believe that the observed trends probably translate some degree of early vascular impairment. Given the expected variations among populations, defining the most appropriate cut-offs of PWV reference levels for prediction of CV events requires a longitudinal follow-up of the subjects into adulthood [14].

Aortic PWV is known to vary during growth. Arteries widen with age and growth, thus causing a natural augmentation in arterial compliance [32]. It has also been reported that healthy children present stable values until the age of 8, when a gradual increase in PWV values usually starts [33]. The process of atherogenesis is believed to begin early in

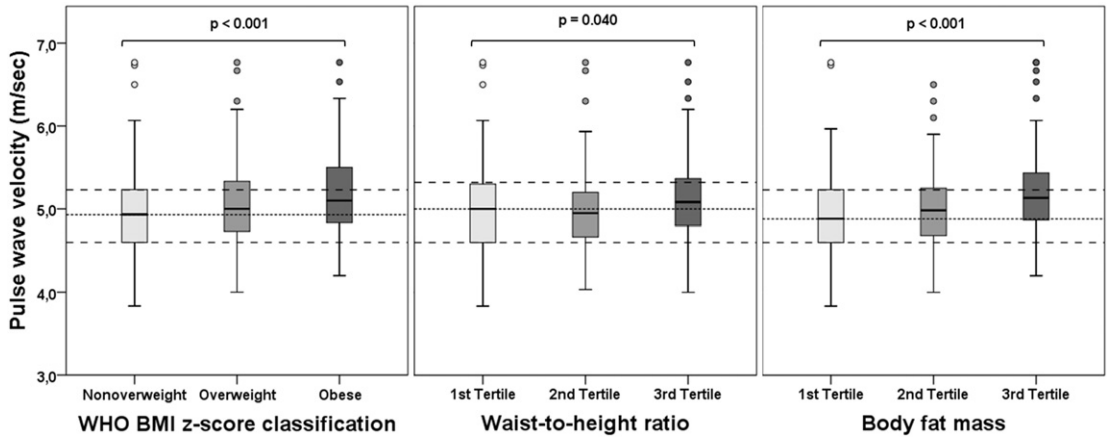


**Fig. 1.** Distribution of carotid-femoral pulse wave velocity levels by classes of body mass index (BMI) and tertiles of waist-to-height ratio and percentage of body fat mass. The normal weight, overweight and obese group classification is according to the WHO classification for BMI z-score [27]. The tertiles of waist-to-height ratio (≤45.77; 45.77–50.00; >50.00) and % of body fat mass (≤11.50; 11.50–20.70; >20.70) were defined based on all enrolled participants. The pulse wave velocity data is expressed as medians and percentiles 25 and 75. P values for linear trend across groups were calculated by linear regression, adjusting for age (in months) and sex.

childhood, with some autopsy studies describing vascular lesions in children as young as 2 years old [5]. Previous studies have reported that childhood obesity is associated with the development and progression of atherosclerosis [3]. Thus, we interpret the higher levels of PWV found in overweight and obese children in our sample of prepubertal children as evidence that obesity, along with other associated comorbidities, is contributing to the acceleration of the age-related process of atherosclerosis. Several mechanisms have been proposed for the pathogenesis of vascular damage in the setting of obesity, leading to endothelial dysfunction and loss of its physiologic properties. In children, studies exploring this topic found obesity-related inflammation [34], oxidative stress [35] and the status of insulin sensitivity [36] to be associated with markers of arterial stiffness.

One of the most interesting results of our study was the finding that PWV levels were independently associated by the absence of dipping. The loss of the dipping pattern represents an alteration in the circadian BP variation, in which the physiological decrement of nocturnal BP is absent, and the prevalence and physiological mechanisms of loss of dipping BP pattern in children, before the onset of hypertension, are largely unknown. In adults, it has been reported that the loss of dipping pattern was associated with PWV, in untreated hypertensive adults [37] and in diabetic patients [38]. Indeed, the non-dipping pattern increases the risk of BP-related complications and overall mortality [39]. In children, although ABPM is the recommended method for BP evaluation, being the most strongly related to target organ damage and CV risk [40], few studies addressed the association of the dipping pattern with PWV or other arterial stiffness markers and none in healthy children. A study in type 1 diabetic children found that systolic dipping was a significant predictor of intimae-media thickness [41] and another study in kidney-transplanted children found that the ambulatory arterial stiffness index, derived from ABPM, was associated with the dipping state but no associations were reported between PWV and BP or dipping [42]. Thus, we believe that our novel finding in healthy children expands the existent evidence in children, and supports the importance of loss of dipping as an early marker of vascular dysfunction and possibly as a surrogate marker of CV risk. In our study, we could not find an association between PWV and z-score BP levels, despite several previous studies reporting it in children and BP being generally considered a major determinant of arterial stiffness [6,13,14]. Considering the established modulating effect of age, height, gender and BP on PWV levels, all these variables were included in the final multivariate model of carotid-femoral PWV determinants.

We also report an association between PWV and HOMA-IR and, more importantly, this association held after adjustment for BMI. Other previous studies reported that children with stiffer vessels presented higher HOMA-IR levels [22,43,44]. The finding of HOMA-IR as an independent determinant of PWV has been described before, although in an older sample of male adolescents [45]. Furthermore, insulin resistance before puberty was also shown to be a risk factor for

**Table 2**
Correlations between carotid-femoral pulse wave velocity (PWV) and cardiovascular risk determinants.

| | PWV (m/s) | |
|---|---|---|
| | Spearman's correlation | *p* |
| Age (months) | −0.247 | <0.001 |
| BMI z-score | 0.102 | 0.070 |
| WHtR (cm/m) | 0.132 | 0.019 |
| % body fat mass | 0.207 | <0.001 |
| 24-h systolic BP (z-score) | −0.106 | 0.062 |
| 24-h diastolic BP (z-score) | −0.001 | 0.986 |
| Daytime systolic BP (z-score) | −0.089 | 0.118 |
| Daytime diastolic BP (z-score) | −0.009 | 0.875 |
| Nighttime systolic BP (z-score) | −0.100 | 0.079 |
| Nighttime diastolic BP (z-score) | 0.020 | 0.727 |
| Total cholesterol (mg/dL) | 0.166 | 0.003 |
| LDL cholesterol (mg/dL) | 0.160 | 0.005 |
| HDL cholesterol (mg/dL) | 0.064 | 0.258 |
| Triglycerides (mg/dL) | 0.116 | 0.040 |
| Fasting glucose (mg/dL) | 0.030 | 0.593 |
| Fasting insulin (μIU/mL) | 0.125 | 0.027 |
| HOMA-IR | 0.124 | 0.028 |
| High sensitivity C-reactive protein (mg/L) | 0.111 | 0.048 |
| Interleukin-6 (pg/mL) | 0.011 | 0.841 |

The values presented are Spearman's ρ and p value.
BMI, body mass index; WHtR, waist to height ratio; BP, blood pressure; LDL, low density lipoprotein; HDL, high density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance.

**Table 3**
Independent determinants of carotid-femoral pulse wave velocity.

| | Carotid-femoral pulse wave velocity (m/s) | |
|---|---|---|
| | Adjusted β (95% CI) | *p* |
| BMI classes | | |
| Overweight | 0.053 (−0.079 to 0.185) | 0.427 |
| Obese | 0.180 (0.014 to 0.347) | 0.034 |
| HOMA-IR (per unit) | 0.109 (0.030 to 0.189) | 0.007 |
| Absence of dipping (vs. dipping) | 0.157 (0.034 to 0.279) | 0.012 |

The values presented are adjusted linear regression coefficients (β) and 95% confidence intervals, estimated with pulse wave velocity as dependent variable. The adjusted model is adjusted for all variables in the table and additionally for sex, age (in months), height (in cm) and 24-h systolic blood pressure (z-score).
BMI, body mass index; HOMA-IR, homeostasis model assessment of insulin resistance.

premature atherosclerosis, independently of BMI [44]. The chronic state of hyperglycemia is believed to induce atherogenesis by increasing oxidative stress, leading to increased LDL oxidation and decreased nitric oxide bioavailability, causing endothelial dysfunction [46]. Thus, our findings of an early association of PWV and HOMA-IR at such a young age, along with the existent literature and as suggested by other authors [44], reinforce the importance of early management of insulin resistance, as a way to alter future CV risk.

At last, the association between lipids and PWV, although not holding after full adjustment, is also a relevant finding. Dyslipidemia is considered a CV risk factor for atherosclerosis and CV disease in both children and adults, playing an important role in endothelial injury and atheromatous plaques development [47]. Although the relation between lipids disorders and arterial stiffness has been extensively explored in adults [6], conflicting evidence exists concerning this topic in the pediatric population. Nonetheless, some studies also found a positive association between PWV and blood lipids [19–21]. Our findings support the hypothesis of blood lipids contributing to higher values of PWV in children and, accordingly, recently published recommendations for dyslipidemia recommend general screening in children, starting at 9 years of age, in order to identify those at risk for early accelerated atherosclerosis [48].

### 4.1. Strengths and limitations

Our study covers an expanding area of investigation and reinforce that the association between obesity and arterial stiffness is present even at very young ages. A major strength of our study lies in the fact that we included a large sample of prepubertal children, homogenous in terms of age, and with an extensive evaluation of CV risk markers. As stated above, the study of CV markers in young ages naturally precludes the establishment of associations with CV events but enabled us to evaluate the consequences of adiposity on intermediate vascular phenotypes avoiding indirect and confounding effects that the emergence of target-organ damage and comorbidities might conceal. The main limitation of our study relates to the cross-sectional design of our analysis, since only a long-term evaluation of these children could warrant certainties on the direction and prognostic value of the associations found. We also have to acknowledge that for PWV analysis some variability might exist in the determination of length of the carotid-femoral segment, especially in the setting of obesity when overestimation might occur. Nonetheless, the fact that all measurements were performed by a single trained cardiopneumology technician helps to minimize this possible bias and PWV has been accepted as a gold standard validated method for arterial stiffness measurement, especially due to its low cost, feasibility and reproducibility, with proved association with cardiovascular outcomes.

### 4.2. Conclusions

Our study extends the evidence available on the association of carotid-femoral PWV with obesity and CV risk factors. The finding of an association between this marker of arterial stiffness and the loss of dipping and insulin resistance levels, independently of the BMI, reinforces the contribution of obesity-related comorbidities to the vascular injury in early life. The longitudinal follow-up of high risk children until adulthood, observing the occurrence of CV events, is the only way to strengthen the role of PWV as a valid tool for risk stratification and clinical management guidance.

## Contributors' statement

Ana Luísa Costa and Liane Correia-Costa conceptualized and designed the study, analyzed the data and drafted the initial manuscript, and approved the final manuscript as submitted.

Alberto Caldas Afonso and José Carlos Areias conceptualized and designed the study, participated in the initial analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted.

Franz Schaefer, António Guerra and Tiago Guimarães critically reviewed the manuscript, and approved the final manuscript as submitted. Tiago Guimarães was also responsible for all laboratory measurements.

Cláudia Moura and Cláudia Mota supervised data collection of 24-h ambulatory blood pressure monitoring and carotid-femoral pulse wave velocity, critically reviewed the manuscript, and approved the final manuscript as submitted.

Henrique Barros and Ana Azevedo conceptualized and designed the study, supervised data collection, participated in and supervised data analyses, reviewed and revised the manuscript, and approved the final manuscript as submitted.

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## Disclosure statement

The authors have nothing to disclose.

## Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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