Comorbidities, complications and treatment of childhood obesity

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Chapter 5

Increased arterial stiffness in adolescents with obesity

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ABSTRACT

Increased arterial stiffness (AS) is an early sign of cardiovascular disease. Influence of weight, puberty, and insulin resistance (IR) on AS in adolescents is unclear. Therefore this study compared AS, assessed with pulse wave velocity (PWV) and augmentation index (AIx), of adolescents with and without obesity and evaluated the influence of puberty and IR on AS.

Sixty-two lean and 61 adolescents with obesity were included. Significantly higher PWV was observed in adolescents with obesity (4.1 ms^{-1} (2.4 - 5.6 ms^{-1}) vs. 3.6ms^{-1} (0.4 - 6.1ms^{-1}) p=0.01), while AIx was not significant different. However, significantly higher AIx was observed in adolescents with obesity and IR (3.0 (-17.5 - 28.5%) vs. -3.0 (-19.0 - 13.0%); p=0.01). For Tanner stages no differences were observed.

The higher PWV in adolescents with obesity, and higher AIx in adolescents with obesity and IR both indicate an increased AS. Consequently measurement of AS should be considered in adolescents with obesity and IR as part of cardiovascular risk assessment.

INTRODUCTION

Metabolic and cardiovascular complications are more frequently seen in pediatric populations due to the increasing incidence of obesity and shift of obesity towards a younger age [1-3]. Since cardiovascular complications are the leading cause of death worldwide, awareness for cardiovascular risk assessment during childhood is important [4]. However, measurements for early cardiovascular changes in children with obesity are rarely performed during routine clinical care, potentially causing underestimation and undertreatment of obesity-related cardiovascular complications [5]. However, this is essential as it is known that early detection, evaluation and treatment of cardiovascular complications can result in reversibility of cardiac abnormalities [5].

Arterial stiffness (AS) is an early sign of cardiovascular disease [6-8]. Stiffening of the arteries is a physiological process as a result of aging [9]. It is the result of decreasing amount of elastin fibers and an overproduction of abnormal collagen in the arterial wall induced by an inflammatory milieu [8]. In addition, other factors such as hypertension and dyslipidemia may accelerate this process [10]. It has also been suggested that AS is influenced by gender and puberty, caused by differences in sex steroids [11]. Estrogens are assumed to decrease AS while androgens are proposed to promote it, causing higher AS in boys compared to girls [11]. Ethnicity might also influence AS, since it has been shown that South-Asians and Africans have higher AS in comparison with Caucasians [12]. Moreover, weight and insulin resistance (IR) are suggested to have an influence on AS, although literature shows conflicting results [10,13].

AS can be measured using pulse wave velocity (PWV) and augmentation index (AIx), which are increased in case of atherosclerosis [14,15]. Applanation tonometry is considered to be the gold standard for measuring PWV and AIx [10]. PWV can be determined at multiple sites of the arterial system. However, central measurements from carotid to femoral artery are preferred, since this has the strongest correlation with cardiovascular morbidity and mortality [16,17].

As there is limited and conflicting evidence on the influence of weight, puberty, and IR on AS in adolescents, the aim of this study is to compare AS of adolescents with obesity with lean adolescents and to determine the influence of puberty and IR**.**

METHODS

Population

For this cross-sectional study data were obtained through two separate study protocols, and both were approved by the Medical Ethical Committee of the St. Antonius Hospital, Nieuwegein/Utrecht, the Netherlands (lean participants: NL39370.100.12; participants

with obesity: NL34811.100.11). All study procedures were in accordance with the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO) of the Netherlands. Written informed consent was obtained from all participants and their parents/caregivers.

Lean participants (hereafter referred to as controls) were recruited between June 2012 and February 2016 during preoperative assessment at the outpatient clinic of the anesthesia department of the St. Antonius Hospital. Controls were eligible for inclusion if they were 10 to 20 years of age, were lean (body mass index standard deviation score (BMI-sds) > -1.1 and ≤1.1) [18,19], of Caucasian descent, and scheduled for minor surgery. Exclusion criteria were syndromal disorders and chronic somatic diseases.

Participants with obesity (hereafter referred to as patients) were participating in a randomized controlled trial (RCT) on metformin versus placebo [20], in which PWV and AIx measurements were performed using applanation tonometry between August 2011 and January 2017. Only the first PWV and AIx measurement, before start of any study medication, was used in the current study. They were eligible for inclusion if they were 10 to 16 years of age, suffered from obesity (BMI-sds >2.3) [18,19], and were of Caucasian descent. Patients with syndromal disorders and/or chronic somatic diseases (especially diabetes mellitus type 2) were excluded.

Measurements

During a physical examination by the attending physician the following measurements were performed: assessment of pubertal stage using Tanner stage classification, measurement of weight, height, waist circumference, and blood pressure. Pubertal stage was categorized in prepubertal (T1), pubertal (T2-4), and postpubertal (T5). Weight and height were measured using calibrated measuring equipment with an accuracy of 0.05kg and 0.1cm respectively. BMI was calculated as weight divided by height-squared (kg/m^2), BMI-sds and height-sds were calculated using the TNO growth calculator for professionals [21]. Blood pressure was measured in supine position from the right arm.

Bio-impedance measurements were obtained with the bio-impedance scale (Tanita Body Composition Analyser BC-420MA, Tanita Corporation, Tokyo, Japan). This noninvasive measurement is determining in a single frequency mode the total body water (kg), fat mass and fat free mass.

Blood samples were taken for fasting plasma glucose, fasting plasma insulin, total cholesterol, High-density lipoprotein, Low-density protein, and triglyceride. IR was calculated using Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) (fasting plasma glucose (mmol/L) x fasting plasma insulin (mU/L))/22.5) [22] and defined IR as HOMA-IR ≥3.4 [23].

AS was measured non-invasively using the SphygmoCor (Model SCOR-Px, Software version, 7.01; AtCor Medical Pvt. Ltd, Sydney, Australia), measuring both the carotid-femoral PWV and the AIx with applanation tonometry. For this measurement, pressure is applied to applanate (flatten) the artery using a special probe creating a signal that approximates arterial pressure [10,24]. For the PWV, waveforms of the right carotid artery and the right femoral artery were obtained. The waveforms of the carotid and femoral artery were recorded sequentially gated by the R-wave on a simultaneously recorded electrocardiogram. In addition, the distance from the carotid artery to femoral artery was measured to the nearest 0.1cm using a tapeline. PWV was subsequently calculated by the software as the distance from the carotid-to-distal path length divided by the lapsed time between the "feet" of the 2 waveforms (carotid and femoral artery) reported in ms^{-1} [10,25,26]. A higher PWV indicates higher AS, since this method is based on the theory that a pulse wave generated by contraction of the left ventricle travels at a speed determined by the size, shape and properties of the artery, which is altered by stiffening of the arteries [10,25]. AIx was measured at the right radial artery. It represents the pulse wave reflection, which is derived from the difference between the main outgoing wave and the reflected wave of the central arterial waveform (i.e. augmentation pressure). The AIx is calculated by dividing the augmentation pressure by the pulse pressure (systolic blood pressure minus diastolic blood pressure) and expressed as percentage [10,25]. A higher AIx indicates stiffer arteries. Since the AIx is influenced by heart rate, all values were automatically adjusted to a heartrate of 75/min.

Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics, version 24 (IBM SPSS Statistics, Chicago, IL, USA). Continuous parameters were reported as median with range. Categorical data were expressed as frequencies with percentage. The students t test or Mann-Whitney U test and χ^2 test were used to compare controls and patients. As it can be assumed that most participants with IR are part of the patient subgroup, the analysis with respect to the influence of IR on AS was limited to the patient subgroup, so that BMI can be excluded as a potential confounder. The students t test or Mann-Whitney U test and χ^2 test was used to compare the no-IR and IR subgroup. The Kruskal-Wallis test and χ^2 test were used to compare the different Tanner stages. Correlations were determined using the Spearman R test. An α-level of 5% was considered significant for all statistical tests.

RESULTS

Table 1 shows the population characteristics of the study population. Patients and controls were comparable regarding pubertal stage. Patients were significantly younger, smaller and less frequently of the male sex (p=0.01; p=0.02; p=0.02). In addition, patients had by definition a significant higher weight, BMI and BMI-sds (all p<0.01). Moreover, all metabolic parameters were significantly higher in the patients compared to controls (all p<0.01) (Table 1).

Table 1. Population characteristics

	Patients (n=61)	Controls (n=62)	P
Age (years)	13.4 (10.2-16.8)	15.0 (10.1-19.5)	**0.01**
Male n (%)	17 (27.9)	30 (48.4)	**0.02**
Height (cm)	162.6 (145.7-179.2)	166.5 (143.0-192.0)	**0.02**
Height-sds	0.2 (-1.90-2.90)	0.24 (-1.36-1.71)	0.71
Weight (kg)	82.7 (58.6-117.8)	54.0 (31.5-86.2)	**<0.01**
BMI (kg/m2)	30.8 (25.3-44.1)	18.6 (14.8-24.0)	**<0.01**
BMI-sds	3.3 (2.4-4.4)	0.1 (-1.0-1.0)	**<0.01**
Fat (%)	41.2 (27.1-54.7)	17.6 (5.5-33.6)	**<0.01**
Tanner stage n (%)			0.30
-Prepubertal	9 (14.8)	12 (19.4)	
-Pubertal	40 (65.8)	42 (67.7)	
-Postpubertal	11 (18.0)	6 (9.7)	
SBP (mmHg)	120 (92-135)	114 (92-153)	**<0.01**
DBP (mmHg)	67 (46-84)	66 (40-84)	0.41
FPG (mmol/l)	4.8 (4.0-5.5)	4.7 (3.1-6.5)	0.62
FPI (mmol/l)	19.0 (1.0-48.0)	6.8 (0.8-26.1)	**<0.01**
HOMA-IR	4.1 (0.2-11.5)	1.4 (0.2-6.6)	**<0.01**
IR n (%)	39 (63.9)	4 (6.5)	**<0.01**
Cholesterol (mmol/l)	4.7 (2.8-7.8)	3.9 (2.3-5.5)	**<0.01**
LDL (mmol/l)	2.8 (1.6-5.8)	2.1 (0.8-3.9)	**<0.01**
HDL (mmol/l)	1.20 (0.70-1.80)	1.45 (0.89-2.18)	**<0.01**
Triglyceride (mmol/l)	1.4 (0.5-3.4)	1.0 (0.3-3.0)	**<0.01**

Data presented as median with range or frequency with percentage. BMI= Body mass index, sds = standard deviation score, SBP = systolic blood pressure, DBP = diastolic blood pressure. HOMA-IR = Homeostatic Model Assessment for Insulin Resistance. FPG = fasting plasma glucose. FPI = fasting plasma insulin. LDL = low density lipoprotein. HDL = high density lipoprotein. P values represents the differences between healthy controls and study participants (student's t test). Bold represent significant p-values.

As shown in Figure 1, PWV was significantly higher in patients compared to controls (4.1 ms^{-1} (2.4 - 5.6 ms^{-1}) vs. 3.6ms^{-1} (0.4 - 6.1ms^{-1}); p=0.01). For AIx, no significant difference was observed (1.0% (-19.0 - 28.5%) vs. -1.0% (-33.5 - 29.0%); p=0.38).

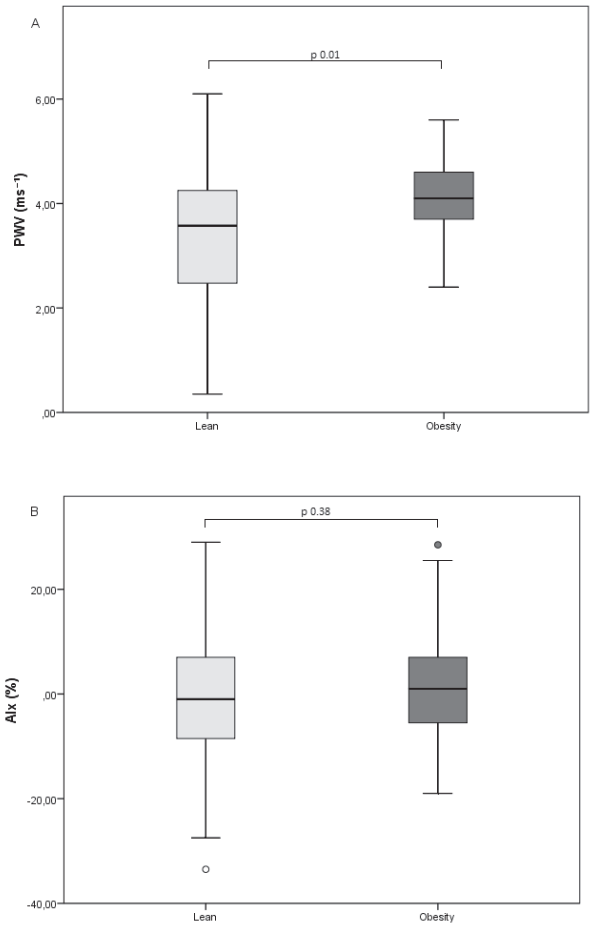

Figure 1. Boxplot with median and interquartile range of (A) PWV and (B) AIx of controls (light grey) compared to patients (dark grey).

When stratifying the total population according to Tanner stage, significant differences were observed for sex, age, height, weight, and BMI, while no differences were observed for BMI-sds and metabolic parameters, which is shown in supplementary Table 1. In the prepubertal subgroup, participants were significant more frequently male (p<0.01). And at the successive puberty stages, participants had a significant higher age, height, weight, and BMI (all p<0.01) (Supplementary Table 1).

With regard to the PWV and AIx, no significant differences between the Tanner stages were observed in the total population (PWV: prepubertal 3.8 ms^{-1} (0.4 - 5.2 ms^{-1}) vs. pubertal 4.2 ms^{-1} (0.6 - 6.1 ms^{-1}) vs. postpubertal 3.9 ms^{-1} (1.4 - 4.9 ms^{-1}); p=0.17); (AIx prepubertal -1.0% (-18.25 - 23.5%) vs. pubertal 1.0% (-33.5 - 29.0%) vs. postpubertal -2.5% (-27.5 - 4.5%); p=0.12) which is shown in Figure 2. When stratifying the controls and patients separately according to Tanner stage, all results with respect to population characteristics and PWV and AIx measurements were comparable to the results observed in the total population and therefore only shown in Supplementary Table 1.

Patients with obesity, with and without IR were comparable regarding age, sex, pubertal stage, weight, and all of the laboratory values except triglyceride and by definition

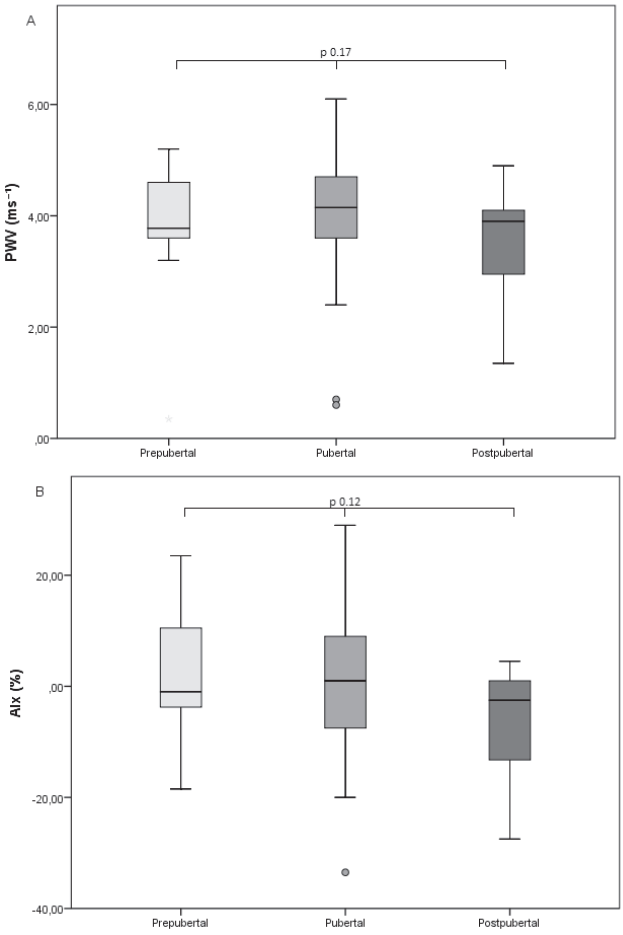

Figure 2. Boxplot with median and interquartile range of (A) PWV and (B) AIx of the total population stratified by pubertal stage; prepubertal (light grey; n=21, pubertal (medium grey; n=83), and postpubertal (dark grey n=17) participants.

HOMA-IR (Table 2). Patients with IR had a significantly higher AIx (3.0% (-17.5 - 28.5%) vs. -3.0% (-19.0 - 13.0%); p=0.01), while no difference was observed for PWV (4.1ms^{-1} (2.4 - 5.6ms^{-1}) vs. 4.2ms^{-1} (2.5 - 5.5ms^{-1}); p=0.64) between patients with and without IR as shown in Figure 3.

Table 2. Characteristics of patients with obesity stratified by presence of IR

	No IR n=22	IR n=39	p
Age (years)	14.2 (10.2-16.6)	12.7 (10.3-16.8)	0.16
Male (%)	8 (36.4)	9 (23.1)	0.27
Tanner stage n (%)			0.32
-prepubertal	3 (14.3)	6 (15.4)	
-pubertal	12 (57.1)	28 (71.8)	
-postpubertal	6 (28.6)	5 (12.8)	
Height (cm)	163.0 (145.7-177.6)	161.5 (147.4-179.2)	0.56
Height-sds	-0.08 (-1.86-2.21)	0.23 (-1.40-2.94)	0.19
Weight (kg)	82.6 (53.2-117.8)	82.4 (58.6-114.4)	0.96
BMI (kg/m^2)	31.46 (23.46-44.09)	31.11 (25.39-42.22)	0.29
BMI-sds	3.38 (2.42-4.15)	3.25 (2.39-4.42)	0.72
Fat (%)	39.0 (28.1-51.0)	41.5 (33.6-54.7)	0.05
SBP (mmHg)	118 (109-135)	120 (92-135)	0.83
DBP (mmHg)	67 (46.83)	65 (46-85)	0.74
FPG (mmol/l)	4.6 (4.0-5.0)	4.8 (4.4-5.5)	**<0.01**
FPI (mmol/l)	11.0 (1.0-16.0)	25.0 (16.0-48.0)	**<0.01**
HOMA-IR	2.3 (0.2-3.3)	5.3 (3.4-11.5)	**<0.01**
Cholesterol (mmol/l)	4.5 (2.8-7.2)	4.5 (3.2-7.8)	0.60
LDL (mmol/l)	2.7 (1.6-5.3)	2.7 (1.6-5.8)	0.92
HDL (mmol/l)	1.13 (0.70-1.77)	1.14 (0.80-1.80)	0.82
Triglyceride (mmol/l)	1.1 (0.5-3.4)	1.5 (0.6-2.3)	**0.04**

Data presented as median with range or frequency with percentage. IR = Insulin resistance. BMI = Body mass index, sds = standard deviation score, SBP = systolic blood pressure, DBP = diastolic blood pressure. HOMA-IR = Homeostatic Model Assessment for Insulin Resistance. LDL = low density lipoprotein. HDL = high density lipoprotein. P values represents the differences between the no IR and IR subgroup (student's t test). Bold represent significant values.

For the total populations significant correlations were observed between PWV and BMI, BMI-sds, and Fat percentage (r=0.22, p=0.04; r=0.31, p<0.01; r=0.24, p=0.03). No correlation was observed between PWV and HOMA-IR r=0.16, p=0.17; age r= -0.13, p=0.26; Tanner stage r=-0.10, p=0.39, nor for blood pressure or lipid spectrum. Significant correlations were observed between AIx and Tanner stage r=-0.26, p=0.01; and HOMA-IR r=0.23, p=0.02. No correlation was observed between AIx and BMI r=0.09, p=0.37, BMI-sds r=0.10, p=0.34; age r=0.17, p=0.08; fat% r=0.16, p=0.12, nor for blood pressure or lipid spectrum.

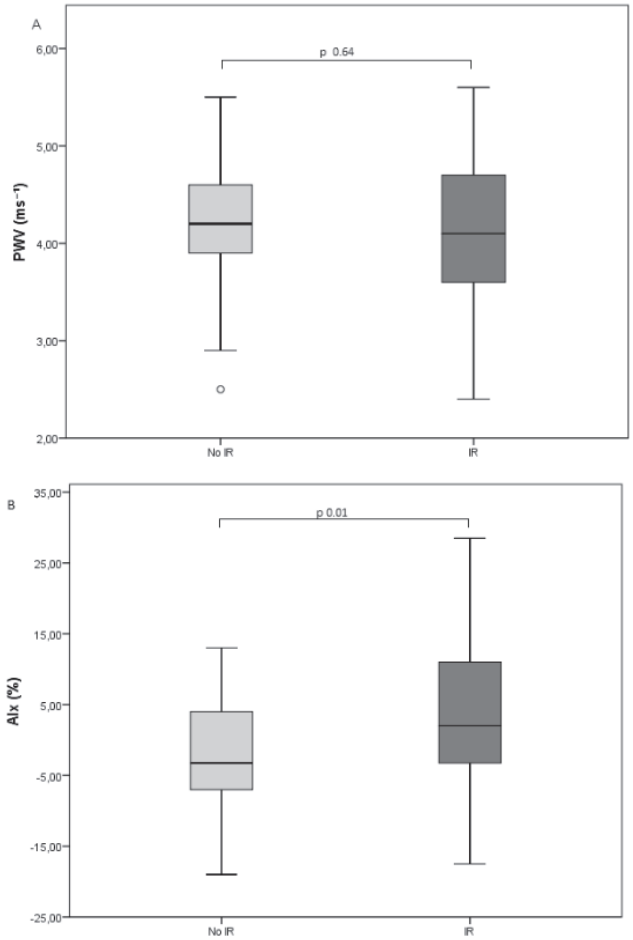

Figure 3. Boxplot with median and interquartile range of (A) PWV and (B) AIx of patients with obesity with IR (light grey; n=22) compared to obesity without IR (dark grey; n=39).

DISCUSSION

Arterial stiffness is considered an early sign of cardiovascular disease [6-8], which can be measured non-invasively using PWV and AIx. It has been suggested that AS in pediatric patients is influenced by sex steroids, weight, and IR [10,11,13]. However, in pediatric populations literature upon these topics is scarce and conflicting [10,11,13]. Therefore, AS of lean and obese adolescents of Caucasian descent was compared in this study and the influence of puberty and IR on AS evaluated.

In the current study, a significant higher PWV was observed in adolescents with obesity. In contrast, no significant difference in AIx was observed between patients and controls. Our findings regarding the effect of obesity on PWV are in line with some

previous studies in adolescents (aged 10 to 24) and adults [10,27-31]. However, other studies reported a lower PWV in children/adolescents with obesity compared with lean controls [10,32-34]. It is known that that the arterial diameter and compliance increases physiologically with growth and development, resulting in a decreased peripheral resistance [5]. It has been suggested that these adaptions are greater in children with obesity resulting in a lower PWV in comparison with those with normal weight [5,10]. However, obesity is also associated with a higher arterial pressure caused by an increased preload and afterload due to increased metabolic demand [5]. Consequently, it can be anticipated that PWV is higher in populations with obesity if the arterial pressure surpasses the (greater) physiological adaptations [5,10]. The higher PWV (and thus AS) observed in our patients with obesity, implicates that the physiological adaptations have been exceeded, indicating that they may already be exposed to an increased risk to develop cardiovascular diseases. This observation is strengthened by the fact that the patients subgroup consisted of significantly more girls, and was significantly younger, both factors that normally will be "protective", as it is known that PWV is higher in boys and increases with age [11,17,35]. The significant higher systolic blood pressure and disturbed lipid spectrum in our population of obese adolescents (Table 1) may also explain the current findings and support the hypothesis that our patients are already at higher risk to develop cardiovascular disease. The difference with studies reporting a lower PWV in children/adolescents with obesity might be explained by a lower age and blood pressure in comparison with our patients, so that the physiological adaptations were still sufficient to mask the negative consequences of obesity [32-34].

With respect to AIx and obesity, literature is conflicting, as some did show an association whereas others did not [10,13,25,34]. As we did not observe a difference for AIx nor a correlation between BMI and AIx, it might be suggested that AIx is not a discriminating measurment to determine AS in children with obesity. As it has been described that estrogens might decrease AS and that androgens might promote AS, differences between Tanner stages were expected as sex steroids are changing during puberty [11]. However, in the current study no significant differences for PWV nor AIx were observed between Tanner stages in the entire population nor for the lean and obese populations separately (Figure 2, Supplementary Table 1). It is known that girls with obesity have relativily higher androgen levels and are consequently prone for an increased AS, which might have influenced the results [36]. However, we could only speculate on the theory, since no analysis stratified by sex was performed, since the number of participants assigned to a subgroup, stratified by sex and Tanner stage was too small to analyze. In addition, the observed difference in BMI may have limited the influence of Tanner stage on AS. Since BMI increases physiologically with age in children, this factor could also not be excluded in analysis performed solely in controls or patients. To the best of authors knowledge only one study has been performed with specific interest into the effect of

Tanners stage on AS [11]. This study only included prepubescent and postpubescent subjects and focused on differences in sex; therefore comparison with the results of the current study was not possible [11]. In the current study, no differences were observed for Tanner stage, which might suggest that AS is not influenced by puberty.

IR is associated with obesity and usually the first sign of a disturbed glucose metabolism [37]. Apart from being a risk factor for development of type 2 diabetes mellitus, IR has also been suggested as an independent risk factor for development of cardiovascular diseases by potentiating the onset of dyslipidemia [37,38]. Therefore, an additional analysis into the influence of IR in patients with obesity was performed in the current study. A significant higher AIx but not PWV was observed in patients with IR, and only AIx was correlated with IR. Several studies investigated this association between IR and AS [10,13,25,39-42]. Some observed an association between PWV and IR [10,39,40,42] while others did not [10,13,25,41]. One of the studies incorporate AIx in their analysis and showed an association between IR and AIx [13]. However, the association between IR and AIx disappeared after adjustment for other cardiovascular risk factors such as age, gender, BMI, and blood pressure [13]. From the above, it can be concluded that IR has a role in increasing AS. However, the association might be influenced by the duration and severity of IR besides other factors such as obesity. Nevertheless, based on the results of the current study, measurement of AS in pediatric populations with obesity and IR should be considered, in order to evaluate early signs of cardiovascular diseases. This is important as cardiovascular diseases are the leading cause of death worldwide [4], and especially since it is known that early detection, evaluation and treatment of cardiovascular complications can result in reversibility of cardiac abnormalities [5]. However, prospective trials have to be performed to evaluate the predictive value of measurement of AS with PWV and AIx, the ability to use this tool in routine clinical care as well as the cost-effectiveness.

To the best of authors' knowledge this is the first study concerning the influence of weight, Tanner stage, and IR on AS, measured with PWV and AIx using applanation tonometry, in a population of lean and obese adolescents of Caucasian descent. Consequently, there is no influence of ethnic background as confounder in the current study. However, certain limitations should be mentioned. First, applanation tonometry used to measure PWV and AIx requires a skilled operator and can be difficult to measure in subjects with obesity. To overcome this limitation, measurements of all participants were performed by a trained staff. Second, the influence of Tanner stage on AS was not observed, which might have been caused by the limited number of participants and/or the fact that no analysis stratified by sex could be performed.

CONCLUSION

Higher PWV in adolescents with obesity and higher AIx in adolescents with obesity and IR were observed, both indicate an increased AS. Consequently, measurement of AS should be considered in adolescents with obesity and IR as part of cardiovascular risk assessment.

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CONFLICT OF INTEREST

The Author(s) declare(s) that there is no conflict of interest

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Supplementary Table 1. Population stratified by Tanner stage (total population, and stratified for lean controls and patients with obesity).

	Total population				**Controls**				**Patients**			
	Prepubertal n=21	**Pubertal n=82**	**Postpubertal n=17**	**p**	**Prepubertal n=12**	**Pubertal n=42**	**Postpubertal n=6**	**p**	**Prepubertal n=9**	**Pubertal n=40**	**Postpubertal n=11**	**p**
Demographics and anthropometric measurements												
Age (years)	11.6 (10.1-13.9)	14.1 (10.2-18.3)	16.5 (13.9-19.5)	**<0.01**	11.4 (10-13.9)	15.3 (10.8-18.3)	17.1 (16.3-19.5)	**<0.01**	12.0 (10.2-13.6)	12.9 (10.2-16.8)	15.7 (13.1-16.2)	**<0.01**
Male (%)	15 (71.4)	25 (30.5)	6 (35.3)	**<0.01**	10 (83.3)	15 (35.7)	4 (66.7)	**<0.01**	5 (55.6)	10 (25.0)	2 (18.2)	0.132
Height (cm)	154.4 (143.0-171.0)	165.0 (145.0-192.0)	168.0 (161.2-190.6)	**<0.01**	153.5 (143.0-171.0)	167.0 (145.0-192.0)	181.5 (168.0-190.6)	**<0.01**	158.6 (150.1-169.0)	162.5 (145.7-179.2)	165.0 (161.2-176.7)	**0.02**
Height-sds	0.24 (-0.88-1.71)	0.18 (-1.86-2.94)	-0.08 (-1.21-1.41)	0.80	0.20 (-0.88-1.71)	0.10 (-1.36-1.58)	0.47 (-0.51-1.41)	0.56	0.37 (-0.75-0.79)	0.21 (-1.86-2.94)	-0.14 (-1.21-1.17)	0.52
Weight (kg)	47.5 (31.5-92.4)	68.1 (35.6-114.4)	87.0 (35.6-114.4)	**<0.01**	42.0 (31.5-54.0)	57.7 (35.6-72.0)	70.7 (60.0-86.2)	**<0.01**	75.2 (58.6-92.4)	81.2 (59.7-114.4)	96.7 (70.5-117.8)	**<0.01**
BMI (kg/m^2)	18.16 (14.78-36.89)	24.00 (15.24-41.23)	30.25 (18.26-44.09)	**<0.01**	17.54 (14.78-19.83)	19.10 (15.24-24.00)	20.60 (18.26-23.70)	**<0.01**	29.75 (25.28-36.89)	30.81 (25.39-41.23)	34.99 (27.13-44.09)	**0.04**
BMI-sds	0.78 (-1.00-4.26)	0.99 (-1.03-4.42)	2.87 (-0.76-4.39)	0.40	0.45 (-1.00-0.92)	-0.13 (-1.03-0.99)	0.29 (-0.76-0.93)	0.74	3.32 (2.49-4.26)	3.27 (2.45-4.42)	3.23 (2.39-4.39)	0.96
Fat (%)	18.7 (7.2-54.1)	31.0 (6.8-54.7)	40.1 (5.5-51.0)	0.37	15.6 (7.2-18.7)	19.2 (6.8-31.8)	11.7 (5.5-33.6)	0.20	38.6 (27.1-54.1)	41.4 (28.1-54.7)	41.8 (36.3-51.0)	0.61
SBP (mmHg)	115 (102-153)	117 (92-140)	118 (109-134)	0.30	111 (102-153)	114 (92-140)	120 (110-125)	0.29	119 (109-133)	120 (92-135)	118 (109-134)	0.85
DBP (mmHg)	65 (40-80)	65 (47-85)	68 (52-85)	0.29	62 (50-80)	65 (47-84)	70 (68-74)	0.06	68 (46-78)	66 (52-85)	64 (52-85)	0.94

Supplementary Table 1. Population stratified by Tanner stage (total population, and stratified for lean controls and patients with obesity). (*continued*)

	Total population				**Controls**				**Patients**			
	Prepubertal n=21	**Pubertal n=82**	**Postpubertal n=17**	**p**	**Prepubertal n=12**	**Pubertal n=42**	**Postpubertal n=6**	**p**	**Prepubertal n=9**	**Pubertal n=40**	**Postpubertal n=11**	**p**
Laboratory measurements												
FPG (mmol/l)	4.8 (3.6-5.6)	4.8 (3.1-6.5)	4.6 (3.8-5.7)	0.70	4.7 (3.6-5.6)	4.7 (3.1-6.5)	4.2 (3.8-5.7)	0.52	4.9 (4.0-5.5)	4.8 (4.3-5.4)	4.6 (4.3-4.8)	**0.02**
FPI (mmol/l)	9.6 (0.8-40.0)	11.5 (2.0-48.0)	13.4 (1.0-40.0)	0.43	5.0 (0.8-13.9)	7.0 (2.0-16.5)	9.2 (2.0-26.1)	0.16	18.0 (10.0-40.0)	21.5 (2.0-48.0)	15.0 (1.0-40.0)	0.36
HOMA-IR	1.8 (0.2-9.8)	2.3 (0.4-11.5)	2.4 (0.2-8.2)	0.47	0.9 (0.2-3.5)	1.5 (0.4-4.3)	1.7 (0.5-6.6)	0.15	4.2 (1.8-9.8)	4.1 (0.4-11.5)	3.0 (0.2-8.2)	0.33
Cholesterol (mmol/l)	4.3 (3.4-7.8)	4.2 (2.3-6.1)	4.2 (2.8-7.2)	0.85	3.8 (3.4-4.6)	3.9 (2.3-5.5)	3.7 (2.8-4.2)	0.62	4.7 (3.8-7.8)	4.5 (2.8-6.1)	4.8 (3.2-7.2)	0.45
LDL (mmol/l)	2.2 (0.8-5.8)	2.3 (0.8-4.3)	2.4 (1.0-5.3)	0.98	2.2 (0.8-2.6)	2.1 (0.8-3.9)	1.9 (1.0-2.4)	0.50	2.9 (1.8-5.8)	2.7 (1.6-4.3)	2.9 (1.6-5.3)	0.54
HDL (mmol/l)	1.39 (0.92-2.18)	1.23 (0.80-1.23)	1.28 (0.70-1.74)	0.10	1.58 (1.29-2.18)	1.43 (0.89-1.14)	1.36 (0.91-1.49)	0.13	1.16 (0.92-1.77)	1.14 (0.80-1.80)	1.09 (0.70-1.74)	0.67
Triglyceride (mmol/l)	1.0 (0.3-3.0)	1.0 (0.3-3.4)	1.4 (0.6-2.1)	0.24	0.60 (0.3-3.0)	0.8 (0.3-2.1)	1.4 (0.8-2.0)	**0.03**	1.5 (0.5-1.9)	1.4 (0.6-3.4)	1.4 (0.6-2.1)	0.85
Arterial stiffness												
PWV (ms^{-1})	3.8 (0.4-5.2)	4.2 (0.6-6.1)	3.9 (1.4-4.9)	0.17	3.85 (0.4-5.10)	3.73 (0.6-6.1)	2.15 (1.4-3.4)	0.17	3.7 (3.2-5.2)	4.3 (2.4-5.6)	4.0 (2.5-4.9)	0.65
AIx (%)	-1.0 (-18.3 -23.5)	1.0 (-33.5-29.0)	-2.5 (-27.5-4.5)	0.12	-2.25 (-18.5-23.5)	0.50 (-33.5-29)	-6.5 (-27.5-1.00)	0.44	4.0 (-6.0-17.5)	1.5 (-16.0-28.5)	-2.5 (-19.0-4.5)	0.17

Data presented as median with range or frequency with percentage. IR = Insulin resistance. BMI = Body mass index, sds = standard deviation score, SBP = systolic blood pressure, DBP = diastolic blood pressure. HOMA-IR = Homeostatic Model Assessment for Insulin Resistance. LDL = low density lipoprotein. HDL = high density lipoprotein. PWV = pulse wave velocity. AIx = augmentation index. P-values represents the differences between the Tanner stages Kruskal-Wallis test. Bold represent significant values.