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Clinical Research Article

Glucose Alterations, Insulin Resistance, Arterial Hypertension, and Renin are Strictly Associated in Pediatric Obesity

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Abstract

Context: Insulin resistance, glucose alterations, arterial hypertension (HTN), and the renin–angiotensin–aldosterone system (RAAS) are related in adult obesity. This crosstalk is still unexplored in childhood.

Objective: Characterize the relationships of fasting and postload glucose and insulin levels with new American Academy of Pediatrics classification of HTN and RAAS in pediatric obesity.

Methods: This was a retrospective observational study; 799 pediatric outpatients (11.4 ± 3.1 years) at a tertiary center who were overweight or obese and not yet on diet were included. The main outcome measures were mean and correlations among parameters of a complete clinical and metabolic screening (body mass index, blood pressure, and glucose and insulin levels during an oral glucose tolerance test, and renin and aldosterone levels and their ratio).

Results: 774 subjects had all the parameters, of whom 87.6% had HTN (5% elevated blood pressure, 29.2% stage I HTN, and 53.4% stage II HTN). Eighty subjects had 1 or more glucose alterations, and more frequently presented HTN. Blood pressure levels were higher in subjects with glucose alterations than in those with normal glucose levels. Fasting and stimulated glucose and insulin levels were directly related to the HTN stages, and insulin sensitivity was lower in HTN than in normal blood pressure. Aldosterone, renin, and aldosterone–renin ratio (ARR) were similar in sexes, whereas aldosterone was higher in prepubertal individuals. Subjects with impaired glucose tolerance (IGT) had higher renin and lower ARR. Renin was positively correlated with postload glucose, and ARR was negatively correlated with the Homeostatic Model Assessment for Insulin Resistance index.

Conclusion: A close relationship exists among insulin resistance, glucose alterations, HTN, and renin in childhood obesity. Specific categories of risk could provide indicators for strict clinical surveillance.

Key Words: obesity, glucose tolerance, insulin resistance, hypertension, aldosterone, renin

Abbreviations: ARR, aldosterone–renin ratio; BMI, body mass index; BP, blood pressure; DI, disposition index; HOMA-IR, Homeostatic Model Assessment for Insulin Resistance; HTN, hypertension; IGF, impaired fasting glucose; IGT, impaired glucose tolerance; IOTF, International Obesity Task Force; ISI, Matsuda Insulin Sensitivity Index; OGTT, oral glucose tolerance test; QUICKI, Quantitative Insulin Sensitivity Check Index; RAAS, renin–angiotensin–aldosterone system; SPISE, single point insulin sensitivity estimator; T2D, type 2 diabetes mellitus.

Childhood obesity is a worldwide epidemic and its prevalence is growing [1]. Obesity is the main cause of insulin resistance, a common denominator for developing many metabolic and cardiovascular complications. Among them, arterial hypertension (HTN) is not an uncommon clinical condition, and, although often underestimated, the prevalence ranges from 3% to 5% [2].

HTN is not only a comorbidity of obesity, but a component of a cluster of metabolic disorders with a common origin, including insulin resistance and inflammation among the main studied mechanisms. In obese adult subjects, the local production of proinflammatory cytokines into the adipose tissue microenvironment and the presence of insulin resistance and hyperinsulinemia seem to be 2 master regulators of blood pressure (BP) disruption [3]. Animal models have revealed that hyperglycemia and hyperinsulinemia induce inflammation, oxidative stress, vascular dysfunction, sodium retention, sympathetic excitation, renin–angiotensin–aldosterone system (RAAS) activation, and kidney damage, leading to elevated BP [4]. Moreover, insulin resistance and hyperinsulinemia

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are activators of the sympathetic nervous system. This mechanism causes vasoconstriction and reduces renal blood flow, with RAAS activation leading to sodium and water retention, which raise BP [5].

In adults, high BP and type 2 diabetes mellitus (T2D) are very common pathologic entities that often coexist and are both associated with increased cardiovascular morbidity and mortality [6]. Several studies have indicated an increased risk of developing cardiovascular diseases and HTN in subjects with impaired glucose tolerance (IGT) and impaired fasting glucose (IFG). In fact, dysglycemia and HTN show substantial similarities in pathophysiologic mechanisms (ie, inflammation, oxidative stress, obesity, and insulin resistance) [6, 7]. Less is known about pediatric obesity. Recent research on 534 subjects aged 10-18 years with BMI (body mass index) Z-score ≥2, demonstrated higher fasting insulin levels, Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) index, and postload glucose levels in hypertensive patients than in normotensive ones [4].

In turn, RAAS seems to be involved in the pathogenesis of obesity, inflammation, oxidative stress, and insulin resistance [8]. Recent evidence has demonstrated that most RAAS determinants could be produced in various tissues, such as adipose tissue and pancreas. In particular, angiotensin II is a main proinflammatory adipokine produced in adipose tissue, and it can be fundamental in identifying the relationship between obesity, inflammation, and insulin resistance [9]. Thus, plasma levels of angiotensinogen and angiotensin II are correlated with adiposity, being regulated by energy state, glucose, and free fatty acids. Furthermore, the components of RAAS directly impact the physiology of adipocytes [8], as they can directly influence the size of adipocytes by affecting differentiation and lipid metabolism, and modulating lipid storage capacity, inflammatory phenotype, and, consequently, insulin sensitivity [10]. Considering this evidence, in children with obesity the marked activation of the RAAS could contribute to the development of insulin resistance [11].

Based on the above, the study's primary outcome was to define the association between glucose alterations, insulin resistance, and arterial HTN; the second outcome was to describe renin and aldosterone levels in overweight and obese pediatric subjects and the relationship with arterial HTN, glucose alteration, and insulin resistance.

Subjects and Methods

We studied subjects recruited in a cross-sectional and retrospective study on pediatric obesity from 2008 to 2020 at the Division of Pediatrics of Maggiore Hospital, Novara, Italy. The study was approved by the Ethics Committee of Novara (protocol number 95/12) and conducted in accordance with the principles of the Declaration of Helsinki. Before the start of any procedures, written informed consent was obtained from all parents or legally acceptable representatives.

To be included, an oral glucose tolerance test (OGTT) of 5 points was required; glucose levels should be available at 0, 60, and 120 minutes, and insulin at fasting. We definitively selected 799 subjects; the flow chart of the study is shown in Fig. 1.

We included children and adolescents (<18 years) who were overweight or obese, according to the International Obesity Task Force (IOTF) curve [12] naive to dietary lifestyle. Subjects with genetic or endocrine obesity, intellectual disability, secondary HTN, or under treatments influencing glucose metabolism or BP were excluded. When glucose alterations at fasting or after an OGTT were detected, we excluded patients with type 1 diabetes, according to positive islet autoantibodies [13]. Cushing syndrome or maturity-onset diabetes of the young, if family history for young diabetes was detected and type 1 diabetes was negative, were excluded. More details were previously described [14, 15].

Anthropometric measurements were collected. Height was calculated with a Harpenden stadiometer to the nearest 0.5 cm and classified according to Italian growth curves [16]. Weight was measured (with patients only in underwear) using a manual scale with an accuracy of 0.5 kg. Then BMI was calculated by dividing the weight of the subject expressed in kilograms by the height expressed in square meters (kg/m^2) and classified according to IOTF curves [12], and also BMI standard deviation score (SDS) through the lambda-mu-sigma (LMS) method [16]. Waist circumferences were measured to the nearest 0.1 cm with a flexible meter, applied in the area between the ribs and the iliac crest at the lowest horizontal circumference, in a standing position, at the end of a normal exhalation. Puberty stages were defined according to Tanner stage [17]. Measurements (3 consecutive times) of systolic and diastolic BP were assessed in a sitting position after at least 5 minutes of rest using a sphygmomanometer with an appropriately sized cuff for the left arm.

Overweight and obesity were classified according to IOTF charts [12].

HTN was defined following the latest definition by the National High Blood Pressure Education Program Working Group of American Academy of Pediatrics (AAP). In detail, subjects were divided into 4 stages: normal BP, elevated BP, stage I HTN, and stage II HTN [18]. IFG and IGT were defined according to the American Diabetes Association. Insulin resistance was assessed with the HOMA index (HOMA-IR) at fasting [19], and basal and stimulated insulin sensitivity were assessed with the Quantitative Insulin Sensitivity Check Index (QUICKI) [20] and Matsuda Insulin Sensitivity Index (ISI) [17].

The single point insulin sensitivity estimator (SPISE) was also calculated for insulin sensitivity, considering triglycerides, high-density lipoprotein cholesterol, and BMI [21].

β-Cell response to changes in plasma glucose level was calculated using the insulinogenic index and β-cell compensatory capacity was evaluated by the disposition index (DI) both through HOMA and ISI [22, 23]. All index formulas are summarized in Table 1.

Subjects underwent an oral glucose load curve (OGTT: 1.75 g/kg glucose, with a maximum of 75 g at time 0′). Blood glucose and insulin levels were evaluated every 30 minutes for 120 minutes. Serum blood glucose levels were expressed in mg/dL, insulin levels were expressed in μUI/mL; both were measured with Siemens Advia 2400 with an analytical variability coefficient of 2.74% for blood glucose and 7.94% for insulin [15]. In subjects with 2 serum or plasma samples (10 μL) stored at −80 ° C, aldosterone (DiaSorin Cat# 310450, RRID:AB_2889867) and renin (DiaSorin Cat# 310470, RRID:AB_2889866) were measured with a chemiluminescence technique using LIAISON kits. The normal ranges for aldosterone are 2.52 to 39.2 ng/dL in orthostatism and 1.76 to 23.2 ng/dL in clinostatism; for renin the ranges are 4.4 to 46.1 μU/mL in orthostatism and 2.8 to

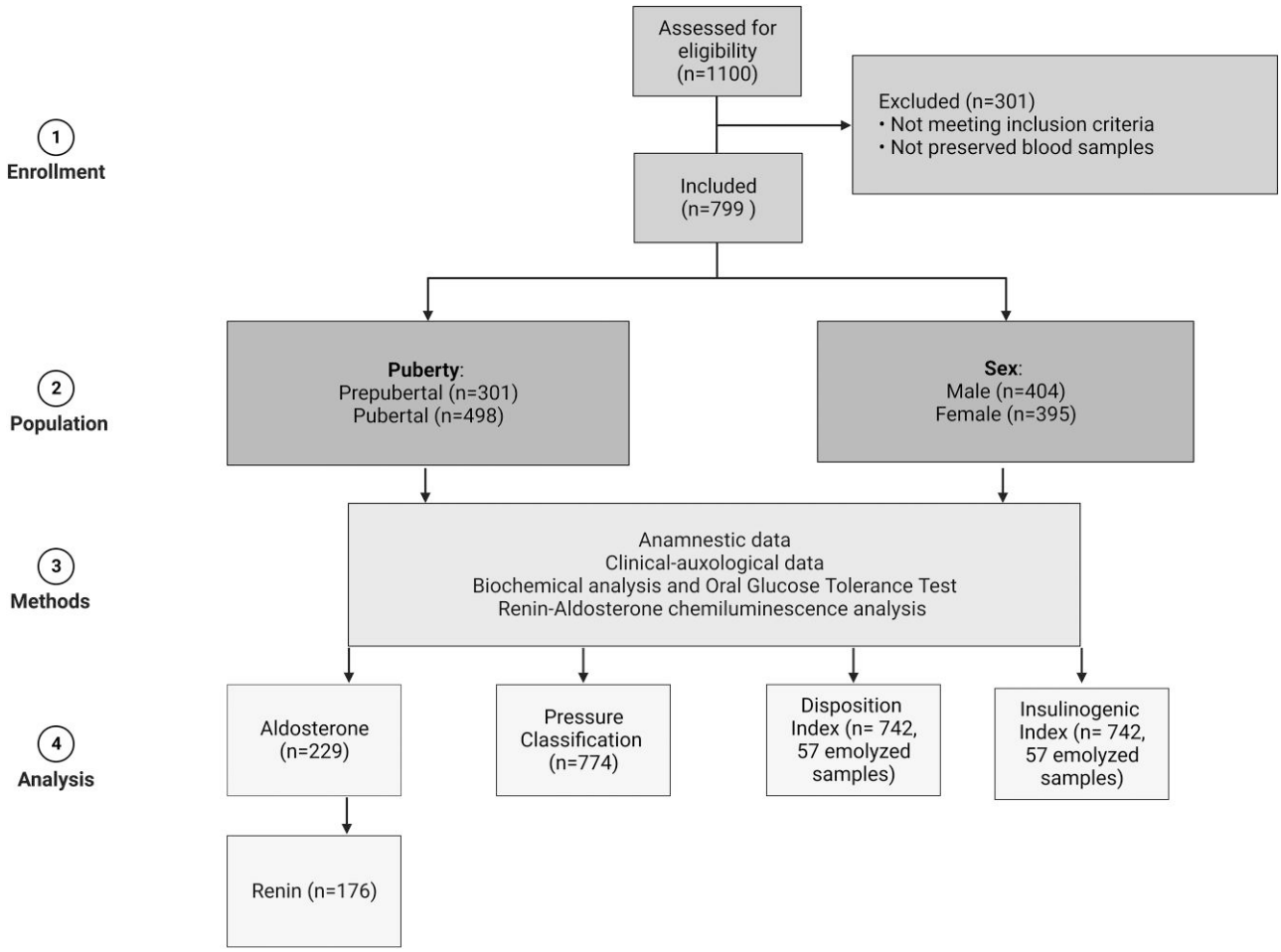

Figure 1. Flow chart of the study. Created with BioRender.com.

Table 1. Formulas of calculated indices

Index	Formula
HOMA-IR	Fasting glucose [mg/dL] × fasting insulin [mU/L]/22.5
HOMA-β	360 × fasting insulin [mU/L]/(fasting glucose [mg/dL] – 63)
QUICKI	log (fasting glucose [mmol/L]) × log (fasting insulin [mU/L])
ISI	(10.000/square root of (fasting glucose [mg/dL] × fasting insulin [mU/L]) × (mean glucose [mg/dL] × mean insulin [mg/dL] during OGTT))
SPISE	$600 \times \text{HDL-C [mg/dL]}^{0.185}/\text{TG [mg/dL]}^{0.2}/\text{BMI [kg/m}^2\text{]}^{1.338}$
INS	Increment of insulin [mU/L]/increment of glucose [mg/dL] during first 30 minutes of 75 g OGTT
DI	INS/HOMA-IR or AUCinsulin/AUCglucose × Matsuda index.

Log is the natural logarithm.
Abbreviations: AUC, area under the curve; DI, disposition index; HOMA-β, Homeostasis Model Assessment of Beta-cell function; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; INS, Insulinogenic Index; QUICKI, Quantitative Insulin Sensitivity Check Index; ISI, Matsuda Insulin Sensitivity Index; SPISE, Single Point Insulin Sensitivity Estimator.

40.0 μU/mL in clinostatism. The aldosterone–renin ratio (ARR) was calculated and cut-offs greater than 3.7 and 1.0 ng/dL were evaluated for suspected hyperaldosteronism according to the Endocrine Society Guidelines [24] and age-related evidence, respectively [25].

Statistical Analysis

Data are expressed as mean ± SD, median and interquartile range (IQR), absolute values, or percentages. The distribution of all continuous variables was analyzed for normality using the Kolmogorov–Smirnov test and skewed variables were logarithmically transformed. Patients were grouped according to puberty, sex, HTN stage, or presence of glucose alterations. In the case of more than 1 glucose alteration (IFG + IGT or T2D), subjects were evaluated in any of the categories or altogether. Differences between groups were assessed firstly with univariate analysis of variance or the Kruskal–Wallis test. Multivariate analysis correcting for puberty and BMI SDS was performed as a second step and post hoc comparisons with Tukey's test were done for multiple groups. The association between the variables was evaluated according to the Pearson test after logarithmic transformation of the parameters when necessary. Regression analysis was conducted for a restricted portion of the studied variables by creating regression models through a stepwise linear regression method. The best association between variables was evaluated by studying the curves. Statistically significant correlations are expressed as $P < .05$. Since the prevalence of glucose alterations is relatively low in pediatric obesity, a sample of 59 individuals is estimated to be enough to demonstrate a reduction of 33% in HOMA-IR with SD 1.8 and a power of 90%, based on our previous papers [14, 15, 26]. A sample of 26

individuals is estimated to be enough to demonstrate a reduction of 33% in aldosterone with SD 4.0 ng/dL, according to previous evidence [26]. All analyses were performed using the SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

Results

We examined 799 subjects (age 11.4 ± 3.1 years; 404 M/395 F) who were overweight or obese, of whom 301 (37.7%) were prepubertal and 498 (62.3%) pubertal. Individuals in pubertal Tanner stages 2-5 were distributed as follows: 135 (16.9%), 67 (8.4%), 49 (6.1%), and 247 (30.9%). Most patients were obese (50.9% moderate obesity, 37.6% severe obesity). Clinical and metabolic data of the population divided by sex and puberty are represented in Table 2. Females had a BMI slightly higher than males but not significantly, with a similar waist circumference. At fasting and the end of the OGTT, glucose levels were lower in females, whereas insulin, in contrast, was slightly higher in females than males at fasting and after OGTT. Pubertal subjects were less obese in terms of BMI SDS but had higher waist circumference than prepubertal ones. As expected, puberty increased insulin resistance, and decreased insulin sensitivity and DI (Table 2). By analyzing data according to the 5 Tanner stages, insulin at fasting, HOMA-IR, and SPISE were lower in prepubertal and early pubertal subjects than the other subgroups ($P < .001$) but were similar in mid-pubertal (Tanner 3 and 4 stages) individuals. ISI and QUICKI had similar inverse relationships. DI HOMA and DI ISI were higher in Tanner 1 than Tanner 5 ($P < .03$), but they did not reach significance for comparisons with the other groups. Glucose levels were not markedly influenced by puberty. Results were not influenced by correction for sex.

Blood Pressure

Based on the classification of HTN in the 2017 AAP guidelines [16], subjects were classified into 4 different categories based on BP values found at the time of measurement. Twenty-five subjects were excluded because of transcription errors in the values. Most patients had higher BP levels than expected (87.6%; χ^2: 430.8, $P < .0001$); of these, 29.2% were in HTN stage I, while 53.4% were in HTN stage II. A significant similar distribution was also observed in any group if we evaluated males (χ^2: 232.8, $P < .0001$) and females alone (χ^2: 199.6, $P < .0001$), or prepuberal (χ^2: 124.9, $P < .0001$) and puberal individuals (χ^2: 344.0, $P < .0001$). No differences were detected in BP categories between sexes, but differences according to puberty were observed with higher BP levels in puberal individuals ($P < .001$), as shown in Table 3. Individuals in Tanner stage 5 had a relatively prevalent distribution of HTN stage II compared with other pubertal individuals (56.5% in Tanner 2 vs 60.2% in Tanner 5, $P < .001$). Correcting for BMI SDS did not modify the results, although it significantly influenced subjects classified as HTN stage II (unstandardised coefficient B: 1.186 ± 0.182; $P < .001$).

Glucose Alterations and Blood Pressure

Individuals were subsequently analyzed for the presence of any glucose changes, identifying 41 subjects (5.1%) with IFG, 51 (6.4%) with IGT, and 1 (0.1%) with T2D. In general, 80 (10.2%) patients had at least 1 glucose impairment (classified as "glucose alteration"). Thirteen subjects with IFG also presented with IGT or T2D (1 subject). Subjects with glucose alterations more frequently had HTN than those with normal glucose levels ($P < .02$; Table 4). Correcting for puberty and BMI SDS did not undo the association with IFG or glucose alterations as a whole ($P < .05$), although these variables were also significant per se, with IFG and glucose alterations more prevalent in pubertal and heavier individuals. However, the association disappeared for subjects with IGT. Furthermore, they had higher systolic pressure levels than normoglycemic subjects (IFG: 130.0, 120.0, 140 mmHg $P < .02$; IGT: 130.0, 120.0, 141.0 mmHg, $P < .04$ vs normoglycemic 125.0, 120.0, 135.0 mmHg). Significance was also maintained when corrected for puberty and BMI SDS ($P < .02$).

Glucose levels during OGTT and fasting insulin were available in all 799 subjects. Insulin levels during OGTT were available for 742 out of 774 patients with BP measurements. In addition, fasting and stimulated glucose and insulin levels were directly related to increased BP. Fasting and post-OGTT glucose and insulin levels were higher in subjects with HTN as a whole (elevated BP, HTN I, and II stages) and also according to HTN I and II stages than in those with normal BP levels, except the 30-minute peak for both (Fig. 2). Significances at univariate analysis were maintained when corrected for puberty and BMI SDS for all the glucose and insulin points. Furthermore, puberty, but not BMI SDS, also increased glucose and insulin levels during OGTT.

Accordingly, subjects with HTN had higher insulin resistance at fasting (HOMA-IR; $P < .002$) and consensually lower insulin sensitivity both at fasting (QUICKI; $P < .002$; SPISE; $P < .0001$) and after OGTT (ISI; $P < .007$) than subjects with normal BP.

At multivariate analysis, puberty and BMI SDS did not undo significance for HOMA-IR ($P < .02$) and SPISE ($P < .001$).

Evaluating the different classes of HTN, HOMA-IR increased (beta: 0.281; $P < .001$), and QUICKI (beta: −0.078; $P < .03$) and ISI (beta: −0.092; $P < .01$) decreased with the stages of HTN compared with normal levels (beta: 0.281; $P < .001$), independently of puberty and age. The curves for QUICKI and ISI were nonlinear. The model with the best association was that with SPISE, which decreased with the increase in HTN, independently of puberty and BMI SDS ($r^2 = 0.6176$, $P < .001$) (Table 5).

Renin and Aldosterone

We were able to measure aldosterone levels in 229 stored samples (110 males and 119 females), of which 176 were also suitable for renin measurements. None had primary hyperaldosteronism according to ARR.

Aldosterone, renin, and ARR were similar in males and females, but aldosterone was higher in prepubertal than in pubertal individuals (16.8 ± 9.8 vs 13.3 ± 8.3 ng/dL; $P < .002$), decreasing progressively from Tanner 1 to Tanner 5 stage.

Aldosterone, renin, and ARR were similar in the 24 pediatric subjects with normal BP levels compared with the other grouped pressure categories. One hundred and fifteen subjects classified as stage 2 HTN had lower aldosterone levels (12.9 ± 9.8 vs 16.6 ± 10.4 ng/dL, $P < .009$) than the other categories together. However, this difference disappeared when weighted for puberty or puberty and BMI SDS.

Table 2. Clinical and metabolic characteristics of patients divided by sex and puberty

Variables		Patients (n = 799)	Males (n = 404)	Females (n = 395)	*P* value	Prepuberty (n = 301)	Puberty (n = 498)	*P* value
Weight	Overweight	92 (11.5%)	39 (9.7%)	16 (5.3%)	ns	77 (15.5%)	53 (13.5%)	ns
	Moderate obesity	406 (50.9%)	205 (50.7%)	131 (43.5%)	ns	275 (55.2%)	201 (51%)	ns
	Severe obesity	300 (37.6%)	160 (39.6%)	154 (51.2%)	ns	146 (29.3%)	140 (35.5%)	ns
Height (cm)		152.4 (139.8; 161.8)	150.1 (139.4; 161.8)	154.4 (140.2; 161.8)	ns	138.5 (128.0; 146.8)	158.8 (151.6; 164.7)	ns
Waist circumference (cm)		92.0 (84.0; 101.0)	92.0 (84.5; 100.8)	92.8 (84.0; 101.0)	ns	85.0 (79.0;91.5)	97.0 (90.0; 104.0)	<.0001
SBD (mm/Hg)		125.0 (120.0; 140.0)	125.0 (120.0; 140.0)	125.0 (120.0; 135.0)	ns	120.0 (110.0;130.0)	130.0 (120.0; 140.0)	<.0001
DBP (mm/Hg)		80.0 (75.0; 90.0)	80.0 (75.0; 90)	80.0 (72.0; 90.0)	ns	80.0 (70.0; 85.0)	81.0 (75.0; 90.0)	<.0001
BMI (kg/m^2)		28.3 (25.9; 31.8)	28.0 (25.9; 31.1)	28.6 (25.9; 32.3)	ns	26.1 (24.1; 28.4)	29.9 (27.3; 32.2)	<.0001
BMI SDS		2.1 (1.9; 2.5)	2.1 (1.9; 2.4)	2.2 (1.9; 2.5)	ns	2.07 (1.87; 2.28)	2.2 (1.9; 2.6)	<.0001
Glucose 0 min (mg/dL)		87.0 (82.0; 92.0)	87.0 (83.0; 93.0)	86.0 (81.0; 91.0)	<.01	87.0 (82.0; 92.0)	87.0 (82.0; 92.0)	ns
Glucose 120 min (mg/dL)		108.0 (96.0; 120.0)	109.0 (97.0; 121.0)	106.0 (95.0; 120.0)	<.05	109.0 (96.5; 119.5)	108.0 (96.0; 120.0)	ns
Insulin 0 min (µU/mL)		13.8 (8.9; 19.3)	12.8 (8.7; 17.7)	14.5 (9.5; 21.6)	<.01	11.8 (7.6; 16.9)	14.7 (10.1; 20.9)	<.0001
Insulin 120 min (µU/mL)		56.9 (34.5; 97.5)	51.7 (33.2; 85.1)	64.9 (38.0; 113.4)	<.0001	51.7 (33.6; 86.8)	61.8 (35.9; 103.4)	<.01
HOMA-IR		2.9 (1.9; 4.3)	2.9 (1.8; 3.8)	3.1 (1.9; 4.7)	<.01	2.5 (1.5; 3.7)	3.2 (2.1; 4.6)	<.0001
ISI		3.5 (2.4; 5.2)	3.6 (2.5; 5.6)	3.3 (2.2; 4.9)	<.01	3.8 (2.5; 5.7)	3.3 (2.2; 4.8)	<.0001
QUICKI		0.32 (0.30; 0.34)	0.32 (0.31; 0.34)	0.32 (0.30; 0.34)	<.01	0.33 (0.31; 0.35)	0.32 (0.30; 0.34)	<.0001
INS		3.50 (1.45;7.54)	3.70 (1.72; 8.09)	3.25 (1.14; 6.70)	<.02	3.98 (1.94; 8.77)	3.22 (1.17; 6.68)	<.003
DI HOMA		1.27 (0.46; 3.07)	1.49 (0.61; 3.28)	1.05 (0.37; 2.68)	<.001	1.73 (0.82; 3.80)	1.06 (0.32; 2.51)	<.0001
DI ISI		11.77 (5.06; 27.35)	14.25 (7.18; 29.16)	9.80 (4.02; 24.20)	<.001	14.69 (7.41; 32.64)	10.52 (3.89; 22.74)	<.001
SPISE		5.74 (4.74; 6.77)	5.85 (4.84; 6.79)	5.63 (4.68; 6.72)	<.05	6.44 (5.59; 7.49)	5.26 (4.46; 6.23)	<.0001

Data are expressed as percentages or median and 95% interquartile range (IQR).
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; DI HOMA, Disposition Index calculated by HOMA-IR; DI ISI, disposition index calculated by Metsuda-ISI; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; INS, Insulinogenic Index; ISI, Matsuda Insulin Sensitivity Index; ns, not significant; QUICKI, Insulin Sensitivity Check Index; SBD, systolic blood pressure; SDS, standard deviation score; SPISE,

Table 3. Blood pressure levels according to puberty

Blood pressure	Prepuberty	Puberty
Normal BP	41 (42.7%; 95% CI 33.3-52.7%)	55 (57.3%; 95% CI 47.3-66.7%)
Elevated BP	11 (28.9%; 95% CI 17.0-44.8%)	27 (71.1%; 95% CI 55.2-83.0%)*
Stage I HTN	107 (47.3%; 95% CI 40.9-53.8%)	119 (52.7%; 95% CI 46.2-59.1%)
Stage II HTN	127 (30.7%; 95% CI 26.4-35.3%)	287 (69.3%; 95% CI 64.7-73.6%)**

Abbreviations: BP, blood pressure; HTN, hypertension. Percentages are calculated on the total sample of individuals in any category.
*$P < .01$; **$P < .001$.

Aldosterone, renin, and ARR were similar in subjects with or without IFG, but renin was higher (59.7 ± 41.6 vs 38.5 ± 25.5 µUI/mL; $P < .05$) and ARR lower (0.32 ± 0.14 vs 0.61 ± 0.54; $P < .04$) in the 10 individuals with IGT than the others.

Aldosterone levels positively correlated with QUICKI, and negatively with age, systolic BP, diastolic BP and its percentiles, and fasting glucose levels.

Renin levels positively correlated with all glucose levels during OGTT, glucose area under the curve, and insulin sum during OGTT, but negatively with diastolic BP and its percentiles.

Finally, ARR positively correlated with QUICKI and the insulinogenic index, but negatively with fasting insulin, and HOMA index. Correlations are reported in Table 6.

Discussion

The prevalence of pediatric obesity and its related diseases is growing [1, 27, 28]. Here, we demonstrate that insulin resistance and/or glucose alterations are strictly and progressively associated with higher BP, mainly systolic, levels. Aldosterone secretion is puberty dependent; however, high renin and low AAR have a precocious relationship with glucose homeostasis and RAAS in childhood.

We observed a consistent prevalence of HTN (87.6%) with 53.4% of subjects in stage II, mainly in puberty, according to the latest AAP classification [29]. These findings are in line with recent evidence underlining that the new AAP cut-off values increase HTN prevalence in all age groups and in overweight/obesity [30-33]. The increased detection rate seems to be best correlated with organ damage and metabolic alterations, as also shown by our data [30, 33]. Furthermore, because our study was conducted in a referral center for pediatric obesity, the likelihood of visiting and enrolling outpatients with comorbidities is higher than in other clinical settings.

Besides, glucose alterations were frequent, accounting for about 10% of our population, with a relatively higher prevalence of IGT than IFG because of more numerous pubertal subjects. These results are in line with those of 2 European studies, 1 conducted in Rome, the "Bambino" meta-cohort [34], and the other in German-speaking children, the Adipositas Patienten Verlaufsbeobachtung registry [35], as well as the latest report from the 2005 to 2016 National Health and Nutrition Examination Survey (NHANES) [36]. These findings suggest that in the presence of similar rates of pediatric overweight and obesity in several countries, the contribution of food habits, lifestyle, and pollutants to complications of obesity is similar in high- and upper-high–income countries [1, 27, 28].

Most importantly, subjects with any glucose alteration had a major prevalence of HTN at any stage. Similar data were reported in the "Bambino" meta-cohort, although we could not perform a detailed comparison with it, since they used the previous cut-off ranges of the AAP for pediatric HTN [34]. All these results strengthen parallel evidence in adult obesity [37-39].

These findings suggest that obesity complications are already strictly related to each other in childhood and adolescence and that the presence of 1 is a red flag to screen the patient's phenotype in depth.

Interestingly, we observed a close association between glucose alterations and high systolic BP. This result agrees with both previous observational and derived data by Mendelian randomization studies which demonstrated that genetically high systolic BP increases the risk of T2D [38]. Furthermore, we showed that glucose and insulin at fasting and during OGTT were progressively higher according to the stages of HTN. Insulin resistance and sensitivity at fasting and during OGTT were also related to BP. Another study of the pediatric age group has recently demonstrated a close relationship between HTN, postload glucose levels, and insulin resistance at fasting, in agreement with our results. However, they failed to show different postload insulin levels at 120 minutes, likely

Table 4. Distribution of HTN in glucose alterations

Blood pressure	IFG	IGT	T2D	GA	NOT GA
Normal BP	5 (4.9%; **12.2%**)	2 (2%; **3.9%**)	0	7 (6.9%; **8.75%**)	88 (86.3%; **12.7%**)
Elevated BP	1 (2.4%; **2.4%**)	2 (4.8%; **3.9%**)	0	3 (7.1%; **3.75%**)	36 (85.7%; **5.2%**)
Stage I HTN	5 (2%; **12.2%**)	15 (6%; **28.8%**)	0	18 (7.3%; **22.5%**)	210 (84.7%; **30.2%**)
Stage II HTN	30 (6.3%; **73.2%**)	33 (6.9%; **63.4%**)	1 (0.21%; **100%**)	52 (10.9%; **65.0%**)	360 (75.3%;**51.9%**)

Abbreviations: BP, blood pressure; HTN, hypertension; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2D, type 2 diabetes; GA, glucose alterations; NOT GA, patients with no glucose alterations.
Percentages are calculated on the total sample of individuals in any category of BP; second percentage (in bold) is calculated on the total sample of individuals in any category of glucose levels. IFG (unadjusted $P < .01$; adjusted for standardised BMI (BMISDS) and puberty $P < .05$) and GA altogether (unadjusted $P < .02$; adjusted for BMISDS and puberty $P < .05$) had more HTN than not GA. IGT had more HTN than not GA only in the unadjusted analysis (crude $P < .03$).

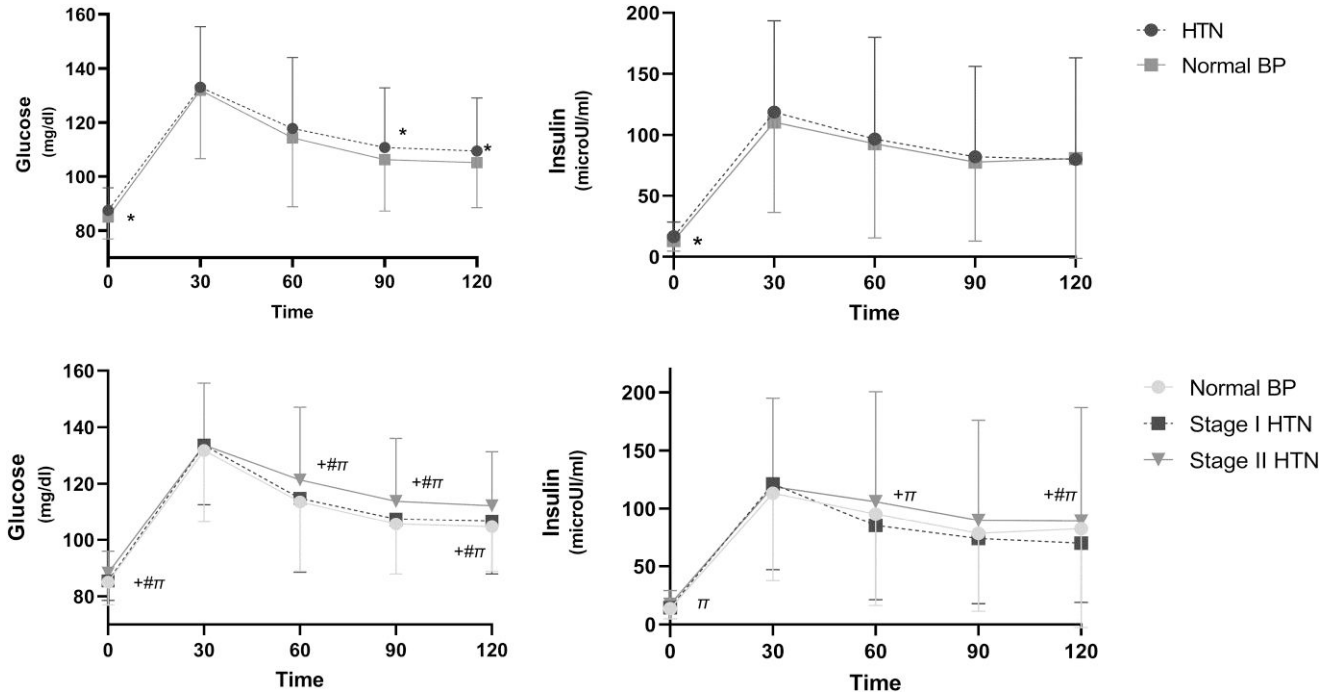

Figure 2. Glucose and insulin levels during OGTT in different blood pressure stages. BP, blood pressure; HTN, hypertension. (A, B) Subjects with normal BP vs the other 3 categories together (elevated BP, HTN stages I and II). (C, D) Post hoc comparisons between groups, in which significant differences were observed between patients in HTN stage I and HTN stage II and patients with normal BP. Lines representing elevated BP are not represented in the figures to make them clearer, since patients with elevated BP did not reach significance. *Significant at multivariate analysis in A and B (glucose: fasting $P < .01$, 90 minutes $P < .05$, 120 minutes $P < .01$; insulin: fasting $P < .04$). (C, D) At multivariate analysis, + means significant in normal BP vs HTN stage I (glucose: fasting $P < .02$, 60 minutes $P < .05$, 90 minutes $P < .02$, 120 minutes $P < .02$; insulin: 60 minutes $P < .05$, 120 minutes $P < .03$); #normal BP vs HTN stage II (glucose: fasting $P < .03$, 60 minutes $P < .05$, 90 minutes $P < .04$, 120 minutes $P < .03$; insulin: 120 minutes $P < .03$); πHTN stage I vs HTN stage II (glucose: fasting $P < .01$, 60 minutes $P < .05$, 90 minutes $P < .02$, 120 minutes $P < .02$; insulin: fasting $P < .03$, 60 minutes $P < .05$, 120 minutes $P < .03$).

due to a cohort that was almost half the size of ours, HTN was diagnosed with other criteria without clustering patients in stages, and insulin levels during the OGTT were not measured at any time [4]. Our data increase the evidence and suggest that the relationship among the 3 actors is progressive. Furthermore, multivariate analyses did not modify the significance of our results for puberty and BMI grade. This suggests that insulin resistance or glucose alterations have an additive impact on HTN, separate from that of obesity per se and physiological pubertal insulin resistance.

The crosstalk among glucose alterations, insulin, and HTN opens several mechanistic hypotheses. Hyperglycemia and hyperinsulinemia induce inflammation, oxidative stress, including the production of advanced glycation end products, immune alterations, vascular dysfunction and remodeling, sodium retention, sympathetic activation, and organ damage, all of which could contribute to the elevation of systemic BP [4, 40, 41].

One other suggestion is that HTN, mainly systolic, results in vasoconstriction, impaired peripheral blood flow, and glucose disposal in tissues critical for glucose metabolism, resulting in prediabetes and T2D. Antihypertensive treatments acting on RAAS have been demonstrated to decrease HTN, potentially blunting the effects described above on metabolism and protecting against T2D [3, 42]. Our study shows that RAAS modulation is precocious in childhood obesity, reflecting whether treatment tailored to RAAS should be considered as soon as the dietary treatment fails in the presence of HTN and insulin resistance.

First, we observed no primary hyperaldosteronism using 2 cut-off values for ARR, since ARR seems lower in childhood than in adulthood [24-26]. This suggests that aldosterone hypersecretion is a rare occurrence in HTN related to obesity in pediatrics, and when suspected a genetic cause has a high likelihood at this age [43]. Furthermore, also in adults, obesity has been demonstrated as a critical confounder in primary hyperaldosteronism diagnosis [44]. However, we did not perform adrenal imaging, and confirmatory studies with this information are needed in pediatric age groups.

Physiological aldosterone and renin secretion have been poorly investigated in children and adolescents, with contrasting evidence. Some authors have observed similar aldosterone levels across puberty stages and renin levels that decreased during puberty only in females [36] or in both sexes [45], others have shown low renin levels up to 12 years of age, with the following surge more pronounced in females than in males [46] in cohorts with fewer than 300 individuals mainly of normal weight. We failed to observe any gender difference in renin and aldosterone levels; furthermore, a progressive decrease in aldosterone levels from prepuberty to puberty was detected. These differences could be related to the size of our cohort, obesity, and prevalence of HTN and insulin resistance, as suggested by the correlations. It could be speculated that visceral adiposity and changes in body composition due to pediatric obesity affect gender and puberty regulation of the RAAS, since previous papers included few subjects who were overweight or obese [26, 45, 46]. Because we did not measure salt intake due to the

Table 5. Multiple regression analysis of log SPISE (as independent variables) on blood pressure levels controlled for BMI SDS, and puberty

Dependent variable	Significant effects	B (95% CI)	β	*P* value
Model 1				
Log SPISE	Log BMI SDS	−.686 (−.732; −.640)	−0.669	<.001
	Puberty	−.074 (−.084; −.064)	−0.317	<.001
	HTN	−.009 (−.014; −.004)	−0.078	<.001
R:0.786				<.001
R2: 0.616				
				<.001
Model 2				
Log SPISE	Log BMISDS	−.651(−.696; −.606)	−0.635	<.001
	Tanner	−.024 (−.027; −.021)	−0.360	<.001
	HTN	−.009 (−.013; −.004)	−0.076	<.001
R:0.803				<.001
R2: 0.644				<.001

Model 1: independent variables were Log BMI SDS (including sex and age), puberty (codes: prepubertal 1; pubertal 2), HTN (codes: normal BP 1, elevated BP 2, HTN stage I 3, HTN stage 2 3) LogAge, gender (male:1; female:2).
Model 2: independent variables were those of Model 1 but puberty was classified as Tanner stages (codes, prepubertal 1, puberty from 2 to 5).
Abbreviations: BMI, body mass index; BMISDS, standardised BMI; HTN, hypertension; SPISE, Single Point Insulin Sensitivity Estimator.

Table 6. Correlation among aldosterone, renin, ARR, and clinical parameters

	Aldosterone	*P* value	Renin	*P* value	ARR	*P* value
Age	−0.125	.05	−0.055	ns	−0.071	ns
BMI	0.097	ns	−0-091	ns	0.007	ns
SBP	−0.169	<.01	−0.111	ns	−0.036	ns
DBP	−0.225	<.001	−0.177	<0.02	0.071	ns
Percentile SBP	−0.033	ns	−0.059	ns	−0.067	ns
Percentile DBP	−0.201	<.001	−0.229	<.003	−0.089	ns
Fasting glucose	−0.164	<.01	−0.036	ns	−0.066	ns
Glucose 30 min	0.059	ns	0.197	<.009	−0.073	ns
Glucose 60 min	−0.007	ns	0.188	<.01	−0.060	ns
Glucose 90 min	0.011	ns	0.160	<.03	−0.049	ns
Glucose 120 min	−0.103	ns	0.151	<.04	−0.093	ns
Glucose AUC	−0.003	ns	0.216	<.004	−0.085	ns
Fasting insulin	−0.068	ns	−0.026	ns	−0.183	<.03
Insulin 30 min	0.069	ns	0.095	ns	−0.057	ns
Insulin 60 min	0.014	ns	0.203	<.009	−0.106	ns
Insulin 90 min	0.096	ns	0.168	<.03	−0.093	ns
Insulin 120 min	−0.076	ns	0.133	ns	0.161	.06
Insulin sum	0.019	ns	0.165	<.04	−0.149	ns
HOMA-IR	0.079	ns	−0.019	ns	−0.182	<.03
ISI	0.117	ns	0.006	ns	0.310	<.001
QUICKI	0.175	<.008	0.124	ns	0.207	<.01
SPISE	0.028	ns	0.026	ns	0.088	ns
INS	0.082	ns	−0.035	ns	0.061	ns
DI HOMA	0.002	ns	−0.001	ns	0.065	ns
DI ISI	0.081	ns	−0.061	ns	0.112	ns

Abbreviations: AUC, area under the curve; BMI, body mass index; DBP, diastolic blood pressure; DI HOMA, disposition index calculated by HOMA-IR; DI ISI, disposition index calculated by Matsuda-ISI; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; INS, Insulinogenic Index; ISI, Matsuda Insulin Sensitivity Index; ns, not significant; QUICKI, Insulin Sensitivity Check Index; SBP, systolic blood pressure; SPISE, Single Point Insulin Sensitivity Estimator.

retrospective nature of our study, we cannot exclude that aldosterone levels are also modulated by a different salt inhibitory effect across pubertal stages [47].

The effects of obesity and metabolic derangement have a great impact on RAAS, likely influencing BP levels as a second step. The effect of obesity seems prevalent on renin. Accordingly, we observed that high renin levels positively correlated with increased glucose and insulin excursions during an OGTT, and inversely correlated with associations for RAAS. These findings parallel those of the observational part of the recently published KORA F4/FF4 study in an adult Western European population [48]. In addition, we observed that aldosterone levels were positively associated with insulin sensitivity and negatively with fasting glucose, age, and puberty, suggesting that the pubertal regulation we observed in our patients with obesity has a major effect on aldosterone. Our data on aldosterone are also in line with those of the KORA F4/FF4 study, where negative odds ratios were observed between aldosterone and incident T2D or insulin resistance, suggesting that the association with glucose alterations is prevalent for renin [48]. Indeed, findings of both the KORA F4/FF4 study and our cohort suggest that the associations of primary hyperaldosteronism with glucose derangement are not applicable to the general population, also in the pediatric age group. Because of the associative nature of these data, it could be speculated that insulin resistance and subtle hyperglycemia per se might induce first renin and after RAAS activation and HTN, as suggested also by the Insulin Resistance Atherosclerosis Study on the risk of incident HTN in adulthood [49]. Furthermore, alterations in the secretion of adipokines could also contribute to the picture, since adiponectin, which is decreased in obesity, inhibits renin secretion [47].

Our study has some limitations since it is retrospective. First, we analyzed only morning BP without data about circadian rhythms. However, most of the studies on children and adolescents had the same design, and as all used the old cut-off for HTN were unable to observe the progression of the associations we identified [4, 26, 34, 45, 46]. Second, data on salt intake or urinary sodium excretion were not available. However, only 1 pediatric study in a small cohort of patients analyzed sodium excretion in children and adolescents, demonstrating a diurnal rhythm independent of puberty and sex [45]. Third, blood samples for measurements of renin and aldosterone were unavailable for all individuals due to the requested volume of serum; however, our cohort had a similar sample size to the most relevant published papers on renin and aldosterone in childhood. Four, since ARR and potassium were normal in all our patients, we did not perform adrenal imaging to detect potential adenomas. Since insulin resistance is linked to adrenal adenomas in adults [50] and well validated ARR cut-offs for pediatric age groups are not available, reducing the assurance of a negative diagnosis with ARR, further studies with ARR and insulin resistance measurements, as well as adrenal imaging in pediatric age groups, should give us interesting findings. Five, a control group of normal weight subjects with normal glucose and BP levels was not available.

In light of the limitations described, multicentric pediatric studies with a larger sample size are needed to overcome our limitations. Prospective studies focused on renin and aldosterone levels in relation to obesity-related insulin resistance that also considers daily salt intake thorough a detailed dietary history, urinary sodium excretion, 24-hour BP monitoring, and potential cardiac and kidney precocious damage should be planned to detect the role of these hormones in the precocious metabolic and organ alteration. Furthermore, performing adrenal imaging in both pediatric subjects with and without obesity could detect adenomas and define pediatric ARR cut-offs for the screening of secondary HTN.

In conclusion, in childhood obesity, we have shown that insulin resistance and/or glucose alterations are strictly and progressively associated with HTN. From a clinical point of view subjects with HTN, glucose alterations, or marked insulin resistance should be followed reciprocally for these parameters. High renin levels and a low AAR have a relationship with altered glucose homeostasis in childhood obesity. Longitudinal studies with RAAS evaluation, adrenal imaging, and screening of organ damage are needed to define the long-term pathologic implications of our observations.

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Disclosures

Authors have nothing to declare.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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