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ORIGINAL ARTICLE

Effect of plasma NO_x values on cardiac function in obese hypertensive and normotensive pediatric patients

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Abstract
Background Hypertension (HT) is a major comorbidity of obesity that is associated with an increased risk of cardiovascular disease and higher mortality. The aim of our study was to evaluate cardiac function in obese hypertensive (OHT) and obese normotensive (ONT) pediatric patients and determine the effects of plasma nitric oxide (NO_x) values on cardiac function, while demonstrating the role of plasma NO_x in HT in obese pediatric patients.
Methods The study population consisted of 62 patients (27 boys, 35 girls), aged 13–18 years and 21 age-matched healthy controls. All subjects enrolled in the study underwent echocardiography (Echo) evaluation and ambulatory blood pressure monitoring for HT. Plasma NO_x and biochemical values were studied in both patient groups separately.
Results Plasma NO_x levels were found to be lower in the OHT group than in the ONT and control groups ($p<0.001$) and to be negatively correlated with left ventricular mass index values ($p<0.05$). Both the OHT and ONT groups had concentric hypertrophy of the heart.
Conclusions Plasma NO_x plays an essential role in obesity-induced HT. Concentric hypertrophy of the left ventricle was found in both the OHT and ONT groups, indicating structural deformation of the heart.

Keywords Plasma NO_x · Hypertension · Obese · Children · Echocardiography

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Introduction

The prevalence of obesity is increasing significantly in pediatric age groups worldwide and is a public health problem of epidemic proportions in many countries [1].

Hypertension (HT) is the most serious comorbidity of obesity and an important risk factor for cardiovascular disease [2]. Two separate studies on obesity-related hypertension and childhood obesity revealed that nearly one-third of the obese children and four-fifths of the obese adolescents were obese in adulthood [2, 3]. Although clinical complications of coronary heart disease mostly occur in middle age or in later life, atherosclerosis has its roots in childhood and progresses over decades [4]. Blood pressure (BP) level values are reported to be one of the most important measurable markers in obese children of potential cardiovascular risk factors later in life [5]. Echocardiography (Echo) is an important diagnostic technique to not only confirm HT but also to assess end-organ damage [6]. Recent studies have demonstrated that obese patients have a deformation in the geometric shape of their hearts even if they are not hypertensive or have left ventricle hypertrophy (LVH) [7, 8].

The mechanism of HT in obesity seems to be multifactorial [9], with hyperinsulinemia, hyperleptinemia, renal structural deformation, inflammation, oxidative stress, activation of the central nervous system and endothelial dysfunction comprising major components of this mechanism [10, 11]. Nitric oxide (NO_x) is a potent regulator of vasomotor tone and an important anti-atherogenic molecule. There is growing evidence that NO_x is critically involved in obesity and its clinical consequences, such as cardiovascular diseases, diabetes and HT [12]. Obesity-related oxidative stress reduces the bioavailability of NO_x [13].

The aims of our study were to evaluate cardiac function in obese hypertensive (OHT) and obese normotensive (ONT) pediatric patients and to determine the effect(s) of plasma NO_x values on cardiac function, as well as to demonstrate the role of plasma NO_x in HT in obese pediatric patients and evaluate the role of inflammation.

Patients and methods

Patients

The study population consisted of 62 patients (27 boys, 35 girls), aged 13–18 years, who were followed-up for obesity for at least 2 years in the Department of Pediatric Endocrinology, Gazi University School of Medicine, Ankara, Turkey. Patients were divided into two groups based on ambulatory blood pressure monitoring (ABPM) measurements, with one group consisting of OHT children (16 boys, 19 girls) and the second group comprising ONT children (11 boys, 16 girls). The children in the study population did not have any acute or chronic kidney, cardiac or neurological disorders and were not taking any medication. The control group consisted of 21 healthy pubertal normotensive children (11 boys, 10 girls), aged 13–18 years, who were not obese, did not have any kidney, cardiac or neurological disorders and were not taking any medication. These healthy children were selected from children admitted to the Child and Adolescent Outpatient Clinic for follow-up and subsequently diagnosed as healthy. Glomerular filtration rate was estimated (eGFR) by the Schwartz Formula [14].

Anthropometric measurements

As part of routine clinical care, height was measured with a stadiometer and weight was measured on a calibrated scale with the child wearing light clothing. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared (kg/m^2). Obesity was defined, according to the BMI percentiles for the Turkish population based on gender and age, as being≥95th percentile [15]. Waist circumference (WC) of all patients was measured in the standing position using a non-flexible tape, from the intersection of the midaxillary line and iliac crests at the end of expiration, and was evaluated according to the Turkish population-based data based on age and gender [16]. The BMI *z*-score was calculated using Cole's lambda-mu-sigma method [17].

Insulin resistance and metabolic syndrome

Insulin resistance (IR) was analyzed using the homeostasis model assessment of IR (HOMA-IR). HOMA-IR was calculated by the following formula: [fasting glucose (mg/dL) × fasting insulin (U/L)]/405. IR was defined according to the HOMA-IR values for the population as the 95th percentile of the HOMA-IR values according to Tanner stage; the cut-off values for IR were determined to be 5.22 in boys and 3.82 in girls [18].

The International Diabetes Federation (IDF) consensus criteria were used to define whether patients had metabolic syndrome (MS) [19].

Office BP measurements

All patients and children in the control group were evaluated for hypertensive status. Office BP measurements were performed using an aneroid sphygmomanometer with the appropriately sized cuff for the child's upper arm. The sphygmomanometer was calibrated before starting the study and once a month thereafter with a mercury sphygmomanometer. Systolic BP (SBP) was defined by the first Korotkoff sound (appearance of sounds), and diastolic BP (DBP) was identified by the fifth Korotkoff sound (disappearance of sounds). Measurements were performed while the children were sitting with their back supported and the cubital fossa supported at the heart level, after a rest of at least 5 min. The mean of three readings was recorded as the office BP. SBP and DBP percentiles were calculated according to the normograms recommended by the National High Blood Pressure in Children and Adolescents Institute [20]. HT was defined in a child or adolescent if the mean SBP or DBP was above the 95th percentile for gender, age and height on three or more occasions. A child was diagnosed as normotensive if both or either of the SBP and DBP percentiles were<90th percentile, and prehypertension was diagnosed if both or either of the SBP and DBP percentiles were≥90th percentile but both were<95th. For the classification of HT, each patient was evaluated by ABPM. The BP index was the ratio of the BP and corresponding 95th BP percentile from the Fourth Report [20]. Office BP *z*-score was calculated based on the 95th percentile for age, sex and height from the Fourth Report [20].

Ambulatory blood pressure monitoring

Ambulatory blood pressure monitoring measurements were obtained for all patients in the study and the subjects in the control group using a Spacelabs monitor (model no: 90207; SpaceLabs Medical, Redmond, WA). Cuff size was determined by measuring the circumference of the mid-arm. The device was set to take measurements every 30 min during the day (08:00 to 23:00 hours) and every 60 min during sleep (23:00 to 08:00 hours). The patients were instructed to avoid heavy physical exercise but to continue their normal daily activities. The patient or their parents were asked to keep a diary to record events on a 24-h basis, including the awake and asleep times; daytime and nighttime periods were determined according to the times recorded in these diaries. At least 40 readings were considered satisfactory for analysis. ABPM data (24-h mean SBP and DBP, daytime SBP and DBP, nighttime SBP and DBP) were registered. Elevated BP load was defined as more than 25 % of recordings of SBP or DBP measurements being≥95th percentile for gender and height, respectively [6, 21]. Office BP percentiles, 24-h mean SBP and 24-h mean DBP percentiles and SBP and DBP load were used to classify HT in patients [22]. Patients were grouped as having white-coat HT, masked HT, ambulatory HT and severe ambulatory HT, respectively, according to the data [22]. White-coat HT was defined as office BP percentiles of>95th percentile, 24-h mean SBP of<95th percentile and SBP load of <25 %; masked HT was defined as office BP of<95th percentile, 24-h mean SBP of>95th percentile and SBP load of>25 %; ambulatory HT was defined as office BP of>95th percentile, 24-h mean SBP of>95th percentile and SBP load of 25–50 %; severe ambulatory HT was defined as office BP of>95th percentile, and 24-h mean SBP of>95th percentile and SBP load of>50 % [22]. Our study did not include prehypertensive patients. The patients were classified as dippers if the mean SBP and/or DBP decreased by ≥10 % during the sleep period, and this was calculated as follows: (mean daytime − mean nighttime/mean daytime) × 100. Subjects with a nighttime drop of SBP or DBP of <10 % of daytime values were considered to be non-dippers.

The OHT group consisted of masked, ambulatory, severe ambulatory hypertensive and obese patients. The ONT group consisted of obese and normotensive or white-coat hypertensive patients. The control group consisted healthy children matched for age and gender who were neither obese nor hypertensive.

All of the patients and control subjects underwent the Echo evaluation.

Serum blood sample measurements

Blood samples were obtained from all study subjects after a 12-h fasting period. Blood glucose, lipids and routine laboratory parameters were measured using enzymatic spectrophotometric methods on an automated clinical chemistry analyzer (Olympus AU2700 plus analyzer; Beckman Coulter Inc., Pasadena, CA). Low-density lipoprotein was calculated using the Friedewald formula. Insulin levels were measured using an electrochemiluminescence immunoassay method (Architect i2000 Analyzer; Abbott Laboratories, Chicago, IL). Lipoprotein A, high-sensitivity C-reactive protein (hsCRP) and urine albumin levels were measured by a nephelometric method using automated equipment (BN ProSpec; Siemens Healthcare GmbH, Erlangen, Germany). Fibrinogen was measured by the coagulometric method (ACL TOP700; Beckman Coulter).

Plasma NO_x measurements

The NO_x level in whole blood was determined by measuring nitrite and nitrate production using the classical colorimetric reaction. Plasma samples for the determination of NO_x concentration were diluted 1:1 (v/v) with 0.3 M NaOH and incubated for 5 min and then protein-precipitated using 10 % $ZnSO_4$. The mixture was then centrifuged for 10 min at 14, 000 rpm. The supernatants were collected and plated briefly, and then equal volumes of sample and Griess reagent (sulfanilamide and naphthalene–ethylene diaminedihydrochloride) were mixed at room temperature. After 5 min, absorbance was measured at 540 nm using a spectrophotometer. The concentration of nitrite was determined by comparison with a standard curve prepared with sodium nitrite [23].

Echocardiographic evaluation

Echocardiography was performed on the same day as the collection of blood samples by the same pediatric cardiologist who was blinded to the groups. All Echo examinations were performed using a Vivid 7 ultrasound system (GE Vingmed Ultrasound AS, Horten Norway). Left ventricular (LV) functions were evaluated according to the American Society of Echocardiography Pediatric Guidelines [24]. LV mass (LVM) was calculated according to the formula of Devereux et al. [25]. The LVM index (LVMI) was obtained by dividing LVM by height to the power of 2.7. The use of height$^{2.7}$ as a denominator to calculate the LVMI minimizes the effect of age, gender, obesity and race [26]. Three different models were used to determine (LVH, as described by Khoury et al. [27]: LVMI>38.6 g/m$^{2.7}$, LVMI>51 g/m$^{2.7}$ and LVMI>95th percentile for age and gender in normal children and adolescents.

LV diastolic function was evaluated by determining the maximal mitral early (E) and late (A) diastolic flow velocity ratio (E/A) and mitral annular velocity by tissue Doppler examination. To evaluate late diastole, the mitral early inflow wave (E) to lateral mitral annulus Doppler wave (E') velocity

ratio was calculated (E/E'). An E/E' ratio of >10 represents abnormal LV diastolic functions, while an E/A ratio of<1 or>3 is considered normal [28].

Relative wall thickness (RWT) was calculated to assess the LV geometric pattern using the following formula: interventricular septum+LV posterior wall/LV end diastolic diameter. Patients with a LVMI of>95 % and RWT of>0.42 were considered to have concentric LVH, while those with a LVMI of>95 % and RWT of<0.42 were considered to have eccentric LVH. Concentric remodeling was defined as normal LVMI and a RWT of >0.42 [29].

Statistical analysis

Data analysis was performed using SPSS ver. 15.0 (IBM Corp., Armonk, NY). Categorical variables were analyzed by the chi-square test and are presented as frequency and percentage. Each continuous independent and dependent variable was analyzed using the Kolmogorov–Smirnov or Shapiro-Wilk tests for normality of distribution. Normally distributed data were presented as the mean and standard deviation. One-way analysis of variance with Scheffe post hoc test was used to compare differences between the three groups. Non-normally distributed data are presented as the median with interquartile range, and data on the three groups were compared using the Kruskal–Wallis H test and the Mann–Whitney *U* test with Bonferroni correction. Bivariate correlation coefficients (*r*) were calculated using the Pearson product moment or Spearman's rank test, depending on whether or not the data were distributed normally. Multiple regression analyses were used to investigate the association between plasma NO_x level and the measured laboratory parameters. All statistical analyses were two-sided, and a *P* value<0.05 was considered to be statistically significant.

Results

Demographic data and the physical characteristics, of the study and control groups are given in Table 1. The OHT and ONT group had significantly higher white blood cell (WBC), neutrophil and platelet counts, and higher hsCRP, fibrinogen and uric acid levels than the control group. The results of selected laboratory tests are given in Table 2.

Plasma NO_x levels were lower in the OHT group than in the ONT and control groups ($P<0.001$) (Table 3).

In the Echo evaluation, LVMI values were higher in the OHT and ONT groups than in the control group ($P=0.001$). LVH, defined as a LVMI ($g/m^{2.7}$) of>95th percentile, and a LVMI ($g/m^{2.7}$) of>38.6 were found at similar percentages in both the OHT and ONT groups. The E/A ratios were lower and the trend in the E/E′ ratios was highest in the OHT group, reflecting diastolic dysfunction, but neither parameter was significantly different from that in the ONT and control groups, respectively ($p>0.05$). (Table 3).

Twenty-three patients in the OHT group (88.5 %) and three patients in the ONT group (11.5 %) were assessed as having MS based on related criteria [19], and this difference was significant ($P<0.001$). Insulin levels in the OHT and ONT groups were 19.7±7.4 and 17.8±7.4 U/mL, respectively; this difference was not significant ($P>0.05$). The mean values of the HOMA index did not differ between the two patient groups ($P>0.05$). Sixteen patients in the OHT group (45.7 %) and seven patients in the ONT group (25.9 %) were diagnosed as having IR; this difference was not significant ($P>0.05$).

The ABPM values of all ONT patients were higher than those of the control group, even if the former were not defined as hypertensive ($P<0.001$). The ONT patients also had higher BP loads than the control group ($P<0.001$). Five patients (8.06 %) in the ONT group had white-coat HT according to the classification by Urbina et al. [21]. In the OHT group, 20 patients (57.1 %) had masked HT, ten patients (28.6 %) had ambulatory HT and five patients had severe ambulatory HT, according to the same classification [21]. A non-dipping pattern was found to be present in 60 % ($n = 21$ patients) of the children in the OHT group (60 %) and in 38.1 % of the children in the control group.

Plasma NO_x levels negatively correlated with office SBP and DBP, daytime and nighttime mean SBP and DBP and daytime and nighttime SBP and DBP loads ($P<0.05$) (Fig. 1). They did not differ between the dipping and non-dipping groups, nor between the different HT groups ($p<0.05$).

eGFR values were found to be higher in the OHT group than in the ONT and control groups, but the difference did not reach significance ($P>0.05$). A positive correlation was found only between BMI, BMI *z*-scores and eGFR ($P<0.05$).

There was a negative correlation between plasma NO_x levels and BMI, BMI *z*-scores, WBC, uric acid, very low-density lipoprotein-cholesterol (VLDL-C) and triglyceride values ($P<0.05$). Patients with IR and MS had lower plasma NO_x levels ($P<0.05$). Plasma NO_x values were negatively correlated with LVMI values ($P<0.05$) (Fig. 2).

ABPM results are shown in Table 4. BMI values and BMI *z*-scores positively correlated with office SBP and DBP values and with all ABPM parameters ($P<0.05$). A positive correlation was observed between WC and office DBP and SBP loads ($P<0.05$).

We found a positive correlation between ABPM values and hsCRP, fibrinogen, uric acid, VLDL-C and triglyceride values ($P<0.05$) and a negative correlation between ABPM values and high-density lipoprotein-cholesterol (HDL-C) values ($P<0.05$).

To identify independent risk factors for plasma NO_x, we entered the parameters significantly associated with plasma

Table 1 Demographic variables of the study population and control group

Demographic variables	OHT (n = 35)	ONT (n = 27)	Control (n= 21)	P
Males (n = 38) Females (n = 45)	16 (42.2) 19 (42.2)	11 (28.9) 16 (35.6)	11 (28.9) 10 (22.2)	0.724
Age (year)	14.9±1.4	14.7±1.5	15.4±1.7	0.279
Weight (kg)	90.8±12.6	87.6±10.4	54.6±10.0	<0.001[a]
Weight z-score	3.1±1.3	3.0±1.0	−0.5±0.7	<0.001[a]
Height (cm)	166.1±7.8	164.0±7.3	163.0±10.0	0.351
Height z-score	0.4±1.1	0.2±1.4	−0.4±0.9	0.087
BMI (kg/m^2)	33.1±4.7	32.6±3.3	20.5±2.6	<0.001[a]
BMI z-score	2.8±1.0	2.7±0.4	−0.4±1.0	<0.001[a]
Waist circumference (cm)	105.3±9.7	102.8±7.2		0.267
WC (cm), males	100.9±9.3	102.1±7.0		0.715
WC (cm), females	108.7±8.7	103.2±7.6		0.055
SBP (mmHg)	126.5±12.5	118.0±11.5	98.6±12.0	<0.001[b]
SBP z-score	1.4±1.2	0.6±1.1	−1.3±1.1	<0.001[b]
SBP index	0.98±0.10	0.93±0.09	0.77±0.09	<0.001[b]
DBP (mmHg)	83.9±8.8	76.9±10.1	63.3±9.7	<0.001[b]
DBP z-score	1.6±0.8	1.1±0.9	−0.2±1.1	<0.001[a]
DBP index	1.01±0.11	0.93±0.12	0.77±0.12	<0.001[a]
eGFR(mL/min/1.73 m^2)	116.7±20.4	110.0±18.2	104.1±78.7	0.052
Birth weight (g)	3467.4±810.0	3441.5±509.9	2766.7±251.7	<0.001[a]

Data are presented as a number with the percentage in parenthesis, i.e. the frequency, or as mean ± standard deviation (SD); see section Statistical analysis

OHT, Obese hypertensive; ONT, obese normotensive; BMI, body mass index; SBP, Systolic blood pressure; DBP, diastolic blood pressure; WC, waist circumference; eGFR, estimated glomerular filtration rate

[a] $P < 0.05$ for controls vs. OHT and ONT groups combined

[b] $P < 0.05$ for controls vs. OHT and ONT groups, separately

NO_x values in the univariate analyses into the logistic regression multivariate analysis. This latter analysis revealed that HDL-C values (β=0.332, P=0.005) and lipoprotein A (β= 0.184, P=0.020) levels were independent predictors of plasma NO_x levels in obese children (Table 5).

Multivariate regression analysis also determined that 45 % of daytime and 46 % of variability in nighttime SBP loads, and 29 % of daytime and 35 % of variability in nighttime DBP loads, respectively, were explained by plasma NO_x and fibrinogen values.

Discussion

Hypertension is a well-known consequence of obesity in both adults and children, and among adolescents, obesity-related HT has become one of the commonest forms of HT [30]. Increased rates of death, premature heart failure, coronary artery disease and vascular stiffness among individuals younger than 55 years of age have been associated with elevated BP levels during childhood and adolescence [31]. It has been proven that structural deformations in the vascular endothelium play a primary role in HT physiopathology, as well as in systemic and vascular inflammation [10]. Endothelial dysfunction is the first step in the development of atherosclerotic disease: it is present in the early course of all known cardiovascular risk factors and is characterized by impaired bioavailability of NO_x [32]. Potential mechanisms for the pathogenic relationship between impaired NO_x bioavailability and HT include defects in the L-arginine/NO pathway, leading to decreased NO_x production. Genetic polymorphisms in endothelial NO-synthase may cause reduced availability of cofactors essential for NO_x formation. Another possible mechanism could be high levels of NO_x inhibitors and also the destruction of NO_x reactive oxygen species [32]. Obesity-related oxidative stress reduces the bioavailability of NO_x [13], which may result in impaired endothelium-dependent vasodilatation and possibly promote atherosclerosis [33]. Gruber et al. [33] showed that the level of NO_x was decreased in obese juveniles compared to normal weight juveniles and was also significantly negatively correlated with body weight. These findings provide additional support for low plasma NO_x levels in obese individuals even when not HT, but the authors only took office BP measurements into account in their evaluation of HT, and ABPM data were not evaluated [33]. In a recent experimental study on male mice, strong evidence emerged which suggests

Table 2 Results from selected laboratory tests carried out on both patient groups and the control group

Variables	OHT	ONT	Control	*P*
WBC ($\times 10^3$ /mm^3)	7.45 (6.83–8.20)	6.63 (5.85–8.12)	5.80 (5.18–6.925)	<0.001[a]
Neutrophil ($\times 10^3$/mm^3)	4.22 (2.70–7.90)	3.66 (2.59–6.87)	2.90 (2.15–4.80)	0.001[a]
Lymphocyte ($\times 10^3$/mm^3)	2.43 (1.25–4.33)	2.27 (1.40–3.29)	2.10 (1.00–3.02)	0.039[b]
Neutrophil/lymphocyte ratio	1.8±0.7	1.8±0.6	1.6±0.6	0.226
PLT ($\times 10^3$ /mm^3)	291.8 (265–330.6)	268.8 (254.8–336.9)	235.6 (212.6–275.65)	<0.001[a]
hsCRP (mg/dL)	0.33 (0.33–0.38)	0.33 (0.33–0.33)	0.03 (0.02–0.33)	<0.001[a]
Fibrinogen (mg/dl)	330.0 (283.0–372.0)	323.0 (295.0–350.0)	260.0 (248.5–288.5)	<0.001[a]
Fasting glucose (mg/dL)	87.0 (82.0–91.0)	86.0 (81.0–92.0)	90.0 (86.0–94.0)	0.035[b]
Insulin (U/mL)	19.7±7.4	17.8±7.4		0.299
HOMA index	4.2±1.6	3.9±1.7		0.373
Uric acid (mg/dL)	5.6±1.2	5.5±1.4	4.4±1.5	0.005[a]
HDL-C (mg/dL)	40.0 (36.0–45.0)	43.0 (38.0–46.0)	50.0 (41.0–59.0)	0.006[b]
LDL-C (mg/dL)	99.0 (86.6–124.6)	88.2 (81.0–102.8)	83.2 (66.9–93.0)	0.005[b]
VLDL-C (mg/dL)	24.4 (20.0–37.0)	20.0 (15.0–27.0)	16.0 (12.5–19.0)	<0.001[a]
Total cholesterol (mg/dL)	172.0±24.2	158.6±26.3	149.5±33.2	0.011[b]
Triglyceride (mg/dL)	122.0 (98.0–176.0)	107.0 (77.0–140.0)	80.0 (62.5–95.0)	<0.001[a]
Lipoprotein A (mg/dL)	9.7 (9.5–19.1)	9. 5 (9.5–31.9)	17.7 (9.5–25.8)	0.762
Urine microalbumin (mg/day)	8.9 (5.5–18.2)	6.9 (4.8–7.9)	4.9 (2.1–12.7)	0.035[b]
Plasma NO_x (nmol/mL)	50.2±9.4	62.2±11.1	64.8±12.6	<0.001[b]

Data are presented as mean±SD or as the median with the interquartile range in parenthesis, as appropriate; see section Statistical analysis

WBC, White blood cell; PLT, platelet count; hsCRP, highly sensitive C-reactive protein; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; VLDL-C, very low-density lipoprotein cholesterol; HOMA (homeostasis model assessment) index = plasma insulin (U/mL) × plasma glucose (mg/dL)/405; OHT, obese hypertensive; ONT, obese normotensive; NO_x, nitric oxide

[a] $P<0.05$ for control vs. OHT and ONT combined

[b] $P<0.05$ for control vs. OHT

a reduction in cellular transport of L-arginine for NO_x biosynthesis, indicating that reduced bioavailability of NO_x may contribute to the development of obesity-induced HT [34].

Our results confirm the clinical importance of plasma NO_x levels in obesity-induced HT. First, we found significantly lower values of plasma NO_x only in the OHT group. Second, plasma NO_x levels were significantly negatively correlated with BP levels and LVMI. Third, BMI, BMI *z*-scores, WBC levels, uric acid levels and lipid levels, such as VLDL and triglycerides, showed significant negative correlations with plasma NO_x levels. Finally, MS and IR levels also demonstrated significant negative correlations with plasma NO_x levels. Taken together, our findings support the hypothesis that plasma NO_x is involved in HT in obese patients.

Table 3 Selected echocardiographic parameters of both patient groups and the control group

Variables	OHT	ONT	Control	*P*
LVMI (g/m$^{2.7}$)	43.60±11.68	43.68±14.52	32.28±6.83	0.001[a]
RWT (mm)	0.46±0.9	0.43±0.7	0.46±0.6	0.202
E/A ratio	1.47±0.17	1.51±0.2	1.74±0.3	0.296
E/E′ ratio	6.23±1.30	5.67±1.22	5.76±1.12	0.177

Data are presented as the mean±SD or as the median with the IQR in parenthesis, as appropriate; see section Statistical analysis

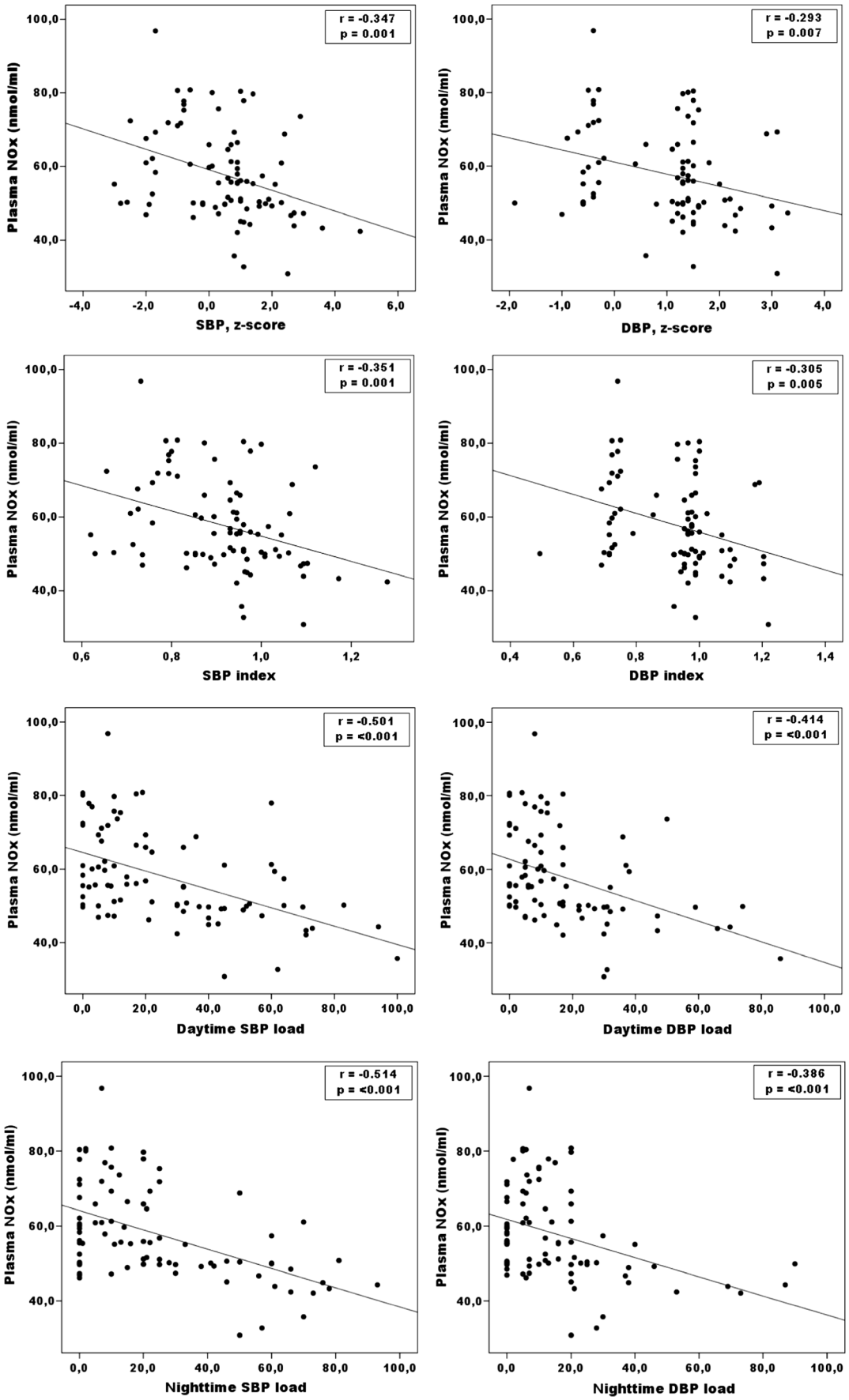
LVMI, left ventricle mass index; RWT, relative wall thickness; E, mitral diastolic flow velocity curve–E wave; E′, mean of the septum and lateral mitral annulus E′ wave; A, mitral diastolic flow velocity curve–A wave

[a] $p<0.05$ for control vs. OHT and ONT combined

[b] $P<0.05$ for control vs. OHT

Obesity has other significant adverse cardiovascular implications in addition to elevated BP, including inflammation and dyslipidemia [4]. Systemic inflammation has been accepted as a cardiovascular risk factor, and increased levels of inflammation-sensitive plasma proteins have been shown to be associated with an increased incidence of HT [4]. Meng et al. reported that in their longitudinal study of urban Han Chinese, higher total leukocyte levels were significantly related to MS in obesity [35]. Demir [36] studied the relationship between neutrophil lymphocyte ratio and non-dipper hypertension and reported that total leukocyte levels and monocyte levels were significantly related to non-dipping HT and that he

Fig. 1 Correlations of plasma nitric oxide (NO_x) levels and blood pressure values. *DBP* Diastolic blood pressure, *SBP* systolic blood pressure


r = -0.347
p = 0.001
Plasma NOx (nmol/ml)
SBP, z-score
r = -0.293
p = 0.007
DBP, z-score
r = -0.351
p = 0.001
SBP index
r = -0.305
p = 0.005
DBP index
r = -0.501
p = <0.001
Daytime SBP load
r = -0.414
p = <0.001
Daytime DBP load
r = -0.514
p = <0.001
Nighttime SBP load
r = -0.386
p = <0.001
Nighttime DBP load

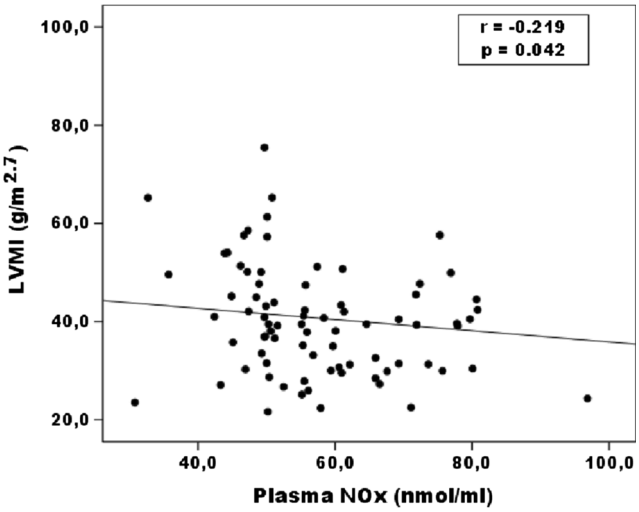


Fig. 2 Correlations of left ventricular mass index (*LVMI*) and plasma nitric oxide (NO*x*) values

observed a significant positive correlation between higher levels of neutrophils and HT [36]. Uric acid is an important factor that causes endothelial dysfunction and supports inflammation by causing an increase in renal renin levels and

Table 4 Ambulatory blood pressure monitoring values of both patient groups and the control group

Variables	OHT	ONT	Control	*P*
Mean 24-h ABPM				
SBP (mmHg)	128.1±10.6	115.8±5.1	109.3±7.2	<0.001[a]
DBP (mmHg)	71.3±9.7	63.7±4.9	65.3±6.3	<0.001[b]
Mean daytime ABPM				
SPB (mmHg)	132.3±10.8	118.0±6.8	112.3±7.0	<0.001[b]
DBP (mmHg)	75.7±8.9	66.3±5.9	67.5±4.6	<0.001[b]
Mean nighttime ABPM				
SBP (mmHg)	118.8±9.8	111.2±5.3	99.9±5.9	<0.001[a]
DBP (mmHg)	64.7±10.8	59.8±7.4	56.7±4.8	<0.001[c]
SBP load (%)				
Daytime	51.8±18.6	11.6±6.5	4.6±5.6	<0.001[a]
Nighttime	44.8±25.7	15.7±8.7	2.3±4.0	<0.001[a]
DBP load (%)				
Daytime	32.0±19.1	9.9±9.8	3.6±3.7	<0.001[a]
Nighttime	27.2±23.5	10.2±8.0	3.3±4.1	<0.001[a]
Nocturnal BP				
Dipping (%)	40	18.5	38.1	0.009[a]
Non-dipping (%)	60	81.5	61.9	0.009[a]

Data are presented as the mean± SD

ABPM, Ambulatory blood pressure monitoring; SBP, systolic blood pressure; DBP, diastolic blood pressure; OHT, obese hypertensive; ONT, obese normotensive

[a] All groups vs. each group

[b] OHT vs. ONT and control combined

[c] Control vs. OHT

Table 5 Multiple regression analyses for plasma nitric oxide values

Variables	β	95 % Confidence interval	*P*
Constant	68.282	44.587–91.977	<0.001
HDL-C	0.332	0.105–0.558	0.005
Lipoprotein A	0.184	0.030–0.338	0.020

Adjusted R^2 : 0.434

HDL-C, high-density Lipoprotein cholesterol

a decrease in plasma NO_x levels, as well as by inhibiting NO_x synthesis [37, 38]. In our study, the OHT and ONT groups had significantly higher WBC, neutrophil and platelet counts and higher hsCRP and fibrinogen levels than the control group, reflecting the systemic inflammation parameters in obese patients. Lymphocyte count was only significantly higher in the OHT group compared to the control group, but the neutrophil/lymphocyte ratio did not differ between the groups.

As lipid profiles were significantly higher in both the OHT and ONT groups in our study, we suggest that both hyperlipidemia and inflammation should be monitored carefully in obese patients. We found a significant positive correlation between ABPM values and hsCRP, fibrinogen, uric acid, VLDL-C and triglyceride levels ($P<0.05$) and a significant negative correlation between ABPM values and HDL-C levels ($P<0.05$). The significant differences in inflammation and lipid parameters between the control group and both the ONT and OHT groups should indicate increased vascular risk factors in obese patients, even if they are not HT. In our study, the OHT and ONT groups had significantly higher levels of uric acid. Patients satisfying the criteria for MS had significantly higher uric acid levels, which showed a significant positive correlation with both the BP and ABPM parameters. These results emphasize the importance of uric acid in the presence of HT with obesity. Thus, obese patients should avoid overconsumption of soft drinks containing high levels of fructose. Uric acid level monitoring could be accepted as an important early biomarker reflecting the high potential risk of HT in obese patients.

Obesity is the major risk factor for HT, and it has been shown that mean BP values are significantly directly correlated with BMI values [39]. Daytime and nighttime BP loads are significantly higher in obese patients than in normal weight individuals [40]. As the severity of the obesity increases, office BP measurements increase significantly, and WC, which is an important marker of probable visceral fat accumulation, shows a positive correlation with DBP values [41]. Patients with non-dipper HT have a threefold higher risk for atherosclerotic events than patients with dipper HT [42]. There is a strong correlation between a non-dipping pattern and LVMI, silent cerebrovascular events, microalbuminuria and also progression in renal disease [43]. Civilibal et al. [44] studied a group of children with MS and found that higher nighttime

SBP was significantly related to early atherosclerosis and endothelial dysfunction. In our study, patients with a non-dipping pattern had significantly higher levels of fibrinogen ($P<0.05$), indicating the significant risk for inflammation in these patients. Also in our study, the ABPM values of all ONT patients were significantly higher than those of the control group, even though according to our criteria the former were classified as normotensive. The ONT patients also had significantly higher BP loads than the control group. In the OHT group, 20 patients (57.1 %) were defined as masked HT, which is very high. BMI values and BMI *z*-scores significantly positively correlated with office SBP and DBP values, as well as with all ABPM parameters ($P<0.05$); there was a significant positive correlation between WC and office DBP and systolic BP loads ($P<0.05$). In our study population, office BP values and systolic night loads were significantly higher in patients with IR ($P<0.05$). These results indicate that obese patients should routinely be evaluated by ABPM as well as by office BP monitoring where the incidence of masked HT is much higher than expected. In routine clinical follow-ups, changes in BMI, BMI *z*-scores and WC should be evaluated carefully in order to define HT risk in obese patients.

Data reported to date suggest that end-organ damage is present at the time of diagnosis in a substantial number of children with HT. Thompson et al. found that LVH was present in up to approximately 40 % of adolescents who had recently received a diagnosis of HT [45]. In children, LVMI is correlated directly and strongly with BMI and HT [46]. BMI is known as an independent predictor of LV diastolic dysfunction [41]. Dhuper et al. reported that the LVMI, RWT and E/E′ ratio in the obese adolescents enrolled in their study were significantly higher than those of the controls, suggesting diastolic dysfunction [8]. Obese and normotensive children show significantly higher E/A ratios than controls and also show diastolic dysfunction [47]. Obese and overweight children have lower E/A ratios, also reflecting diastolic dysfunction [48]. We found that LVH defined by a LVMI ($g/m^{2.7}$) of>95th percentile and by a LVMI ($g/m^{2.7}$) of>38.6, respectively, was equally prevalent in both the OHT and ONT groups compared to the healthy control group. There was no significant difference in RWT, an indicator of remodeling, between the groups, resulting in all of the groups having concentric hypertrophy. In our patients, the E/E′ ratio and E/A ratio were within normal ranges, but the OHT and ONT groups had lower E/A ratios and the OHT group had higher E/E′ ratios than the controls, reflecting impaired diastolic functions, but without statistical significance.

Overweight and obese children are reported to have decreased renal function and to be at risk for developing chronic kidney disease [49]. However, hyperfiltration has also been reported to be associated with cardiometabolic risk factors [50]. Our OHT patients had increased eGFR compared to the other two groups, but the difference was not statistically significant ($P>0.05$), and the eGFR was significantly and positively correlated with the BMI and BMI *z*-scores. BP loads, the ratio of night non-dipping patients and IR and MS did not affect eGFR values. The patients having higher eGFR may be candidates for renal function deterioration with or without diabetes.

It was formerly believed that obese children did not develop cardiovascular problems until they reached adulthood. However, increasing evidence now indicates that obesity in children and adolescents is associated with short- and long-term cardiovascular risks that include both hemodynamic changes and structural and functional changes in the heart and blood vessels [51]. LVMI has been significantly associated with MS, BMI, central obesity, fasting blood glucose, hyperinsulinemia, IR and arterial BP [26, 51]. Our study confirms that cardiac hypertrophy might be developed by both hemodynamic and non-hemodynamic factors, such as HT and BMI. In our study, BMI and BMI *z*-scores showed a significant positive correlation with LVMI. We also found a significant positive correlation between LVMI and both insulin levels and HOMA-IR values. Our patients in the OHT and ONT groups had significantly higher LVMI indexes than the control group subjects, even though the children in the ONT group were not hypertensive. As their RWT values did not differ significantly from each other, both ONT and OHT groups showed concentric hypertrophy of the left ventricle. It is known that in the absence of volume and pressure load, fat distribution may influence LV remodeling by adipose depots which may be visualized by cardiac magnetic resonance imaging [52].

In the multiple regression analyses, the parameters that significantly affected ABPM values were fibrinogen and plasma NO_x levels. Plasma NO_x levels were significantly lower in the OHT group, thus supporting the role of NO_x in obesity-induced HT. In routine clinical practice, WBC, hsCRP and fibrinogen levels should be used to indicate the risk of obese children for HT. Plasma NO_x levels are significantly affected by HDL-C and lipoprotein A, such that lipoprotein A should be used as a biomarker to follow-up obese children.

In conclusion, based on our results, we suggest that for the follow-up of obese patients, WC, fibrinogen levels, lipid profiles and lipoprotein A levels are the most important biomarkers for the risk of HT and cardiovascular diseases. Concentric hypertrophy of the left ventricle was found in both of our obese patient groups, indicating structural deformation of the heart.

Compliance with ethical standards

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

Ethical statement The study adhered to the principles of the Declaration of Helsinki and was approved by the local Ethics Committee (Date: 27 April 2011; Protocol Number: 105). Written

informed consent was obtained from all patients enrolled in the study and also from all control subjects and their parents.

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