

Original Article

Plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (α -MSH) levels in patients with or without hypertension and/or obesity: a pilot study

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Abstract: Aim: Obesity frequently co-exists with hypertension (HTN). Hypothalamus neuropeptides such as neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (α -MSH) interact with leptin, an anorexigenic peptide produced mainly by adipose tissue and are involved in the regulation of appetite, energy balance and sympathetic nervous system (SNS) activity, possibly contributing to blood pressure (BP) elevation. We compared plasma NPY and α -MSH levels between patients with or without hypertension and/or obesity and the differences in these neuropeptides between patients with or without pathological heart echo findings, aiming to investigate the possible role of these peptides in obesity induced HTN. Patients and Methods: 160 non-diabetic, treatment-naïve individuals were randomly recruited from our outpatient clinics. Study population was divided into 6 groups, according to body mass index-BMI (OB=obese, OW=overweight, NW=normal weight) and blood pressure. Waist circumference (WC) and heart rate (HR) were also recorded. A heart echo was performed and plasma NPY and α -MSH levels were measured for all participants. Results: Plasma NPY levels and HR were higher in OW and OB hypertensives compared with NW hypertensives. OW and OB hypertensives had also higher NPY concentrations compared with OW and OB normotensives, respectively. However, in NW patients, plasma NPY concentrations did not differ between hypertensives and normotensives. Patients with central obesity (COB) had also higher NPY levels compared with patients without COB, a difference also observed in hypertensives but not in normotensive patients. Furthermore, plasma NPY concentrations were significantly correlated with BMI, WC, HR, systolic and diastolic BP. Patients with left ventricle hypertrophy had higher plasma NPY levels compared with those with normal findings, but this was not seen in hypertensives. The majority of these differences were also observed in male and female patient populations. In contrast, plasma α -MSH levels were similar in all study groups. Conclusions: These results suggest that NPY may be involved in obesity-related HTN, possibly via increased SNS activity. Further investigation is needed to elucidate the role of both NPY and α -MSH in obesity-related HTN.

Keywords: Neuropeptide Y, alpha-melanocyte stimulating hormone, obesity, hypertension

Introduction

Obesity is a rapidly growing public health problem with epidemic proportions, which increases the risk for type 2 diabetes mellitus (T2DM) and coronary heart disease (CHD) [1]. Furthermore, obesity frequently co-exists with hypertension (HTN), an important risk factor for cardiovascular disease (CVD) [2]. Therefore, in order to prevent CVD, there is a need to elucidate the mechanisms controlling food intake and body weight and to clarify whether they also influence

blood pressure (BP) regulation, leading to HTN.

The brain and mainly the hypothalamus, is the organ responsible for maintaining the balance between food intake and energy expenditure by receiving peripheral signals from adipose tissue (adipose signals) and responding to them [3]. Several neuropeptides are involved in this process, divided in 2 major groups: (a) orexigenic, such as neuropeptide Y (NPY), which increase food consumption and decrease energy expenditure, and (b) anorexigenic, such as alpha-

melanocyte stimulating hormone (α -MSH), which suppress appetite and increase energy expenditure [1]. NPY, acting through specific receptors (Y1, Y2, Y4, Y5, and Y6) [4-8], plays an important role in several physiological functions, including cardiovascular homeostasis, regulation of the sympathetic nervous system (SNS) activity [9-11] and control of BP [10]. Similarly, α -MSH is involved in several physiological processes, mainly skin pigmentation and regulation of energy homeostasis, acting through 5 melanocortin receptor (MCR) subtypes, with MC3R and MC4R primarily involved in the feeding procedure [12,13].

Leptin, a potent anorexic peptide produced mainly by adipose tissue [14,15], acts on the hypothalamus to regulate food intake and energy balance by interacting with neurons expressing either α -MSH [16,17] or NPY [18]. Leptin promotes weight loss not only by reducing appetite but also by increasing energy expenditure through SNS activation [3,19]. It has been suggested that leptin also influences SNS activity through the regulation of hypothalamic neuropeptides, such as NPY and α -MSH [3,10,20].

Stimulation of hypothalamic nuclei leads to sympathetic responses and therefore hypothalamus controls the activity of the autonomic nervous system, coordinating it with the function of neuroendocrine systems. As HTN is frequently associated with increased SNS activity, it is reasonable to assume that hypothalamus may play a role in BP regulation [21].

NPY and/or α -MSH, apart from being involved in food intake and body weight regulation, seem to contribute in increased SNS activity, increased BP, vasoconstriction vascular smooth muscle cell proliferation and abnormal myocardial growth according to many studies [22-32], but their role in the increased prevalence of HTN seen in obese subjects has not been fully elucidated [33,34].

The aim of the present study was to compare plasma NPY and α -MSH levels between patients with or without hypertension and/or obesity (defined by body mass index-BMI and waist circumference-WC), thereby indicating a possible role of these neuropeptides in obesity-related HTN. We also considered any differences in these neuropeptides plasma concentrations between patients with and without pathological

heart echo findings.

Methods

Patients and study design

A total of 160 participants were randomly recruited from our outpatient hypertension and obesity clinics. Inclusion criteria were: a) aged between 30- 65 years, b) either grade 1 and grade 2 HTN [35] or normal BP and c) newly diagnosis of HTN, therefore without a need to neither discontinue pharmaceutical treatment nor to immediately initiate drug therapy, according to European Society of HTN (ESH) and European Society of Cardiology (ESC) guidelines [35] and their recent (2009) reappraisal [36].

Exclusion criteria were: a) all situations that either affect plasma NPY and α -MSH levels or do not permit drug discontinuation, such as secondary HTN or HTN with target organ damage, CVD, cerebrovascular disease, Diabetes Mellitus (DM), renal failure, endocrinopathies related to obesity, pregnancy, alcohol abuse, malignancies, retinopathy and depression (defined by the use of Beck Depression Inventory-BDI questionnaire) [37-39], and b) medical conditions influencing HR, such as fever, anemia and postural hypotension.

Study population was divided into the following 6 groups, according to BMI (normal weight-NW= BMI <25 kg/m², overweight-OW= BMI 25.1 – 29.9 kg/m²) and obese-OB= BMI \geq 30 kg/m²) and BP [normotensives (H-) and hypertensives (H+), according to ESH/ESC guidelines (44)]: Group 1: H+ NW 26 patients, 13 Males (M) and 13 Females (F); Group 2: H+ OW 28 patients, 14 Males and 14 Females; Group 3: H+ OB 28 patients, 14 Males and 14 Females; Group 4: H- NW 22 patients, 11 Males and 11 Females; Group 5: H- OW 28 patients, 14 Males and 14 Females and Group 6: H- OB 28 patients, 14 Males and 14 Females.

Furthermore, participants were categorized into those with central obesity (COB+) and those without central obesity (COB-), according to Waist Circumference (WC) measurements (COB+ defined by WC>102cm in Males and >88cm in Females).

BP (both systolic-SBP and diastolic-DBP) and HR were defined as the mean of three different measurements in three different office visits

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Table 1. Patients' characteristics in the total study population

	Group 1 (H+ NW)	Group 2 (H+ OW)	Group 3 (H+ OB)	Group 4 (H- NW)	Group 5 (H- OW)	Group 6 (H- OB)
Age (years)	47.4±1.9	47.2±12.4	46.5±10.1	46.2±13.5	45.6±10.8	47.3±9.2
Gender (M)	13/26	14/28	14/28	11/22	14/28	14/28
BMI (kg/m ²)	23.5±0.9	27.9±1.2	35.3±3.6	23.4±1.1	27±1.3	34.8±3.2
WC (cm)	84.5±7.5	97.8±7.9	112.1±6.6	83.9±7.1	95.5±5.1	112.5±9.2
SBP (mmHg)	153.5±7.3	154.1±7	156.8±8.2	118.9±6.9	121.8±9.4	124.5±9.8
DBP (mmHg)	94.8±5.8	94.4±5.1	98.8±5.6	72.3±6.9	74.3±5.9	76.1±7.7
HR (beats/min)	73.1±7.3	78.7±5.8 ¹	80.7±6.5 ²	73.5±4.5	75.9±4.9	77.2±6.1 ³
NPY (ng/ml)	0.32±0.1	0.55±0.11 ⁴	0.71±0.13 ⁵	0.41±0.28	0.37±0.12 ⁶	0.42±0.09 ⁷
a-MSH (ng/ml)	0.38±0.11	0.41±0.12	0.42±0.09	0.44±0.1	0.45±0.12	0.43±0.15

H+: hypertensives; H-: normotensives; NW: normal weight; OW: overweight; OB: obese; M: male; BMI: body mass index; WC: waist circumference; SBP: systolic blood pressure; DBP: diastolic blood pressure; HR: heart rate; NPY: neuropeptide Y; a-MSH: alpha-melanocyte stimulating hormone; ¹ p = 0.005 vs Group 1; ² p = 0.001 vs Group 1; ³ p = 0.021 vs Group 4; ⁴ p < 0.001 vs Group 1; ⁵ p < 0.001 vs Group 1; ⁶ p < 0.001 vs Group 2; ⁷ p < 0.001 vs Group 3.

within one week, according to ESH/ESC guidelines [35]. All participants had steady nutrition and physical stress for at least three weeks before entering the study in order to avoid possible effects of nutrition and stress status on plasma NPY levels [40,41].

Plasma NPY (Phoenix Pharmaceuticals Inc.) and a-MSH levels (BioSupply UK Ltd.) were measured by an enzyme-linked immunosorbent assay (ELISA) with an intra-assay error <5% and an inter-assay error <14%.

Statistical analysis

Statistical analyses were performed using the SPSS version 15 software package (SPSS Inc., Chicago, USA). Kolmogorov-Smirnov test was used to assess normal distribution. Values are expressed as mean \pm SD. Differences between 2 groups were analyzed by performing Student's t-test or Mann-Whitney (for non-normally distributed parameters), whereas continuous variables between ≥ 3 groups were compared by one-way ANOVA or the non-parametric alternative Kruskal-Wallis test. Correlations between various parameters were determined by Pearson r and Spearman Rho correlation coefficients for parametric and non-parametric variables, respectively. A 2-tailed p < 0.05 was considered significant.

Results

Comparisons between study groups

Patients' characteristics are shown in **Table 1**.

Age and gender distribution were similar in all groups. SBP and DBP did not differ significantly between hypertensive patients (groups 1, 2 and 3) or between normotensives (groups 4, 5 and 6). Mean BMI and WC were similar between hypertensives and normotensives in NW (groups 1 and 4), OW (groups 2 and 5) and OB (groups 3 and 6).

The patient groups did not differ significantly with regard to smoking and alcohol consumption. In terms of lipids, only LDL-C differed significantly between group 4 and the other groups (group 4 = 104 vs group 1 = 127, group 2 = 137, group 3 = 138, group 5 = 134 and group 6 = 130 mg/dl; p < 0.05 for all comparisons). No differences in LDL-C levels were found between the other groups.

Plasma NPY concentrations were higher in OW (group 2) and OB hypertensives (group 3) compared with NW hypertensives (group 1) (0.55 \pm 0.11 and 0.71 \pm 0.13 vs. 0.32 \pm 0.1 ng/ml, respectively; p < 0.001 for all comparisons). OW (group 2) and OB hypertensives (group 3) had also higher NPY levels compared with OW (group 5) and OB normotensives (group 6), respectively (p < 0.001 for all comparisons). However, in NW patients, plasma NPY concentrations did not differ significantly between hypertensives (group 1) and normotensives (group 4). OB hypertensives patients (group 3) had the highest plasma NPY levels compared with all the other study groups (p < 0.001 for all comparisons).

No significant differences were observed in

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Table 2. Heart rate (HR), plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (a-MSH) levels in male (M) patients

Male population	Group 1 (H+ NW)	Group 2 (H+ OW)	Group 3 (H+ OB)	Group 4 (H- NW)	Group 5 (H- OW)	Group 6 (H- OB)
HR (beats/min)	73.7 \pm 5.7	80.9 \pm 6.7 ¹	83 \pm 6.4 ²	73.8 \pm 4.9	75.3 \pm 6.4 ³	74.9 \pm 5.4 ⁴
NPY (ng/ml)	0.28 \pm 0.09	0.58 \pm 0.13 ⁵	0.70 \pm 0.11 ⁶	0.34 \pm 0.08	0.35 \pm 0.08 ⁷	0.42 \pm 0.06 ⁸
a-MSH (ng/ml)	0.36 \pm 0.09	0.41 \pm 0.14	0.37 \pm 0.07	0.43 \pm 0.06	0.43 \pm 0.12	0.42 \pm 0.12

H+: hypertensives; H-: normotensives; NW: normal weight; OW: overweight; OB: obese; ¹ p = 0.006 vs. Group 1; ² p = 0.001 vs. Group 1; ³ p = 0.032 vs. Group 2; ⁴ p = 0.001 vs. Group 3; ⁵ p < 0.001 vs. Group 1; ⁶ p = 0.001 vs. Group 1; ⁷ p < 0.001 vs. Group 2; ⁸ p = 0.001 vs. Group 3.

Table 3. Heart rate (HR), plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (a-MSH) levels in female (F) patients

Female population	Group 1 (H+ NW)	Group 2 (H+ OW)	Group 3 (H+ OB)	Group 4 (H- NW)	Group 5 (H- OW)	Group 6 (H- OB)
HR (beats/min)	72.5 \pm 8.8	76.4 \pm 3.7 ¹	78.4 \pm 5.9 ²	73.1 \pm 4.2	76.6 \pm 2.9	79.6 \pm 6.1 ³
NPY (ng/ml)	0.35 \pm 0.11	0.53 \pm 0.09 ⁴	0.72 \pm 0.16 ⁵	0.48 \pm 0.39	0.39 \pm 0.15 ⁶	0.41 \pm 0.12 ⁷
a-MSH (ng/ml)	0.41 \pm 0.12	0.42 \pm 0.09	0.46 \pm 0.09	0.45 \pm 0.14	0.48 \pm 0.11	0.45 \pm 0.18

H+: hypertensives; H-: normotensives; NW: normal weight; OW: overweight; OB: obese; ¹ p < 0.001 vs. Group 1; ² p < 0.001 vs. Group 1; ³ p = 0.005 vs Group 4; ⁴ p < 0.001 vs. Group 1; ⁵ p < 0.001 vs. Group 1; ⁶ p = 0.01 vs. Group 2; ⁷ p < 0.001 vs. Group 3.

plasma a-MSH concentrations between study groups.

Similarly to plasma NPY levels, HR was higher in OW (group 2) and OB hypertensives (group 3) compared with NW hypertensives (group 1) (78.7 \pm 5.8 and 80.7 \pm 6.5 vs. 73.1 \pm 7.3 beats/min; p = 0.005 and 0.001, respectively). In normotensive study groups, OW (group 5) and OB patients (group 6) had also higher HR compared with NW ones (group 4), but this difference was significant only between OB and NW normotensives (p = 0.021). No significant differences in HR were found between hypertensives and normotensives in NW (groups 1 and 4), OW (groups 2 and 5) and OB patients (groups 3 and 6).

Comparisons between male and female patients

When study population was analyzed according to gender (**Tables 2 and 3**), similar significant differences were observed in plasma NPY levels between groups in both Male and Female patients, as in the total patient population. In both genders, OB hypertensives patients (group 3) had the highest plasma NPY concentrations

compared with all the other study groups (p = 0.008 to < 0.001 for M; p = 0.007 to < 0.001 for Females). Plasma a-MSH levels did not differ significantly between groups in both Male and Female populations.

In Male patients, HR was higher in OW and OB hypertensives compared with NW hypertensives (80.9 \pm 6.7 and 83 \pm 6.4 vs. 73.7 \pm 5.7 beats/min; p = 0.006 and 0.001, respectively). In normotensives, HR did not differ between NW, OW and OB patients. In contrast, HR was significantly higher in hypertensives compared with normotensives, in OW (80.9 \pm 6.7 vs. 75.3 \pm 6.4 beats/min; p = 0.032) and OB groups (83 \pm 6.4 vs. 74.9 \pm 5.4 beats/min; p = 0.001).

In Female patients, HR was also higher in OW and OB hypertensives compared with NW hypertensives (76.4 \pm 3.7 and 78.4 \pm 5.9 vs. 72.5 \pm 8.8 beats/min; p < 0.001 for all comparisons). In normotensive study groups, OW and OB patients had also higher HR compared with NW ones, but this difference was significant only between OB and NW normotensives (p = 0.005). No significant differences in HR were found between hypertensives and normotensives in NW (groups 1 and 4), OW (groups 2 and

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Table 4. Heart rate (HR), plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (a-MSH) levels in patients with and without central obesity, in the total study population, hypertensives, normotensives, males (M) and females (F) separately.

	Patients with central obesity	Patients without central obesity	p
In the total study population			
HR (beats/min)	78.2±5.9	74.8±6.7	0.001
NPY (ng/ml)	0.53±0.18	0.39±0.19	< 0.001
a-MSH (ng/ml)	0.43±0.12	0.42±0.12	ns
In M patients			
HR (beats/min)	78.8±7.0	75.7±6.5	ns
NPY (ng/ml)	0.54±0.16	0.38±0.15	< 0.001
a-MSH (ng/ml)	0.36±0.09	0.41±0.14	ns
In F patients			
HR (beats/min)	77.8±4.9	73.2±6.8	0.002
NPY (ng/ml)	0.51±0.19	0.42±0.26	0.004
a-MSH (ng/ml)	0.45±0.12	0.44±0.12	ns
In hypertensives			
HR (beats/min)	79.3±6.1	75.2±8.1	0.026
NPY (ng/ml)	0.63±0.15	0.39±0.17	< 0.001
a-MSH (ng/ml)	0.41±0.09	0.39±0.12	ns
In normotensives			
HR (beats/min)	77.1±5.2	74±5.3	0.008
NPY (ng/ml)	0.40±0.11	0.39±0.23	ns
a-MSH (ng/ml)	0.44±0.14	0.44±0.11	ns

5) and OB patients (groups 3 and 6).

Comparisons between patients with and without central obesity (COB+ and COB-, respectively)

COB+ patients had higher plasma NPY levels compared with COB- individuals, a difference observed not only in the total study population, but also in Male and Female patients separately ($p = 0.004$ to < 0.001) (**Table 4**). Furthermore, COB+ hypertensives had higher plasma NPY concentrations compared with COB- hypertensives ($p < 0.001$), a finding that was not observed in normotensive patients. HR was significantly higher in COB+ patients compared with COB- individuals for all study populations ($p = 0.026$ to 0.001), except M patients.

Plasma a-MSH levels did not differ significantly between COB+ and COB- patients, in the total population, hypertensives, normotensives, Male and Female patients separately.

Comparisons between patients with normal and abnormal findings in heart Echo

In the total study population, patients with LVH had higher plasma NPY levels compared with those with diastolic dysfunction and those with

normal findings (**Table 5**). However, this difference was significant only between patients with LVH and normal echo findings ($p = 0.024$). Similar results were observed in the Male patient population ($p = 0.008$), whereas in Female patients and in hypertensives no significant differences in plasma NPY concentrations were observed between patients with normal and abnormal echo findings.

Plasma a-MSH levels and HR did not differ significantly between patients with different echo findings in the total study population, hypertensives, Male and Female patients separately.

Correlations

In the total study population, plasma NPY levels significantly correlated with BMI ($\rho = 0.480$; $p < 0.001$), WC ($\rho = 0.398$; $p < 0.001$), SBP ($\rho = 0.377$; $p < 0.001$), DBP ($\rho = 0.414$; $p < 0.001$) and HR ($\rho = 0.226$; $p = 0.004$). Similar correlations between plasma NPY concentrations and BMI ($\rho = 0.571$; $p \leq 0.001$), WC ($\rho = 0.514$; $p \leq 0.001$), SBP ($\rho = 0.365$; $p \leq 0.001$), DBP ($\rho = 0.417$; $p \leq 0.001$) and HR ($\rho = 0.393$; $p \leq 0.001$) were observed in Male patients. In Female patients, plasma NPY levels correlated with BMI ($\rho = 0.405$; $p < 0.001$),

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Table 5. Heart rate (HR), plasma neuropeptide Y (NPY) and alpha-melanocyte stimulating hormone (a-MSH) levels in patients with normal and abnormal heart echo findings, in the total study population, hypertensives, males (M) and females (F) separately.

	Patients with normal findings	Patients with dilated dysfunction	Patients with LVH
In total patient population			
HR (beats/min)	76.1±6.1	78.2±7.1	78.8±7.7
NPY (ng/ml)	0.44±0.19	0.52±0.23	0.55±0.17*
a-MSH (ng/ml)	0.42±0.12	0.45±0.09	0.4±0.12
In M patients			
HR (beats/min)	75.8±5.9	79.1±7.5	81±8.3
NPY (ng/ml)	0.41±0.14	0.49±0.25	0.56±0.19**
a-MSH (ng/ml)	0.41±0.11	0.42±0.07	0.37±0.13
In F patients			
HR (beats/min)	76.3±6.1	77.1±6.8	74.3±3.9
NPY (ng/ml)	0.47±0.22	0.54±0.21	0.51±0.14
a-MSH (ng/ml)	0.43±0.13	0.49±0.09	0.46±0.07
In hypertensives			
HR (beats/min)	76.7±7.1	78.2±7.1	78.8±7.3
NPY (ng/ml)	0.53±0.2	0.52±0.22	0.55±0.17
a-MSH (ng/ml)	0.39±0.10	0.45±0.09	0.40±0.12

LVH: Left ventricular hypertrophy; *p = 0.024 vs. patients with normal findings; **p = 0.008 vs. patients with normal findings

WC ($\rho = 0.388$; $p < 0.001$), SBP ($\rho = 0.365$; $p = 0.001$) and DBP ($\rho = 0.410$; $p < 0.001$), but not with HR.

In normotensives, no significant correlations were observed between plasma NPY concentrations and other parameters. In contrast, in patients with hypertension, plasma NPY levels correlated with BMI ($\rho = 0.766$; $p < 0.001$), WC ($r = 0.657$; $p < 0.001$), DBP ($\rho = 0.217$; $p = 0.05$) and HR ($\rho = 0.331$; $p = 0.002$). No correlations were found between plasma a-MSH concentrations and other variables in the total study population, hypertensives, normotensives, Male and Female patients separately.

Discussion

Obesity is a rapidly growing public health problem with epidemic proportions. It is a chronic multifactorial disease, with both genetical and environmental factors implicated in its pathogenesis. Obesity predisposes several pathological situations including T2DM, CHD and cancer [1]. It also frequently co-exists with HTN, a major risk factor for CVD [42,43]. Increased SNS activity is considered as one of the most important

mechanisms contributing to obesity-induced HTN [49].

Leptin, an adipose-derived peptide that regulates appetite and energy homeostasis, decreases food intake and increases SNS activity in cases of calorie excess. Elevated leptin levels activate anorexigenic and inhibit orexigenic pathways, while in nutritional deficiency circulating leptin concentrations are decreased, therefore enhancing food intake [45,46]. Leptin interacts with several neuropeptides produced in the central nervous system (CNS) and mainly in the hypothalamus, including the orexigenic NPY and the anorexigenic a-MSH. Obesity is characterized by leptin resistance in which elevated circulating leptin levels cannot suppress food intake. In contrast, leptin effects on SNS activity are preserved [19,47,48], representing a potential mechanism that contributes to obesity-induced HTN [49,50]. Up-to-date scientific data support a role of NPY and a-MSH as possible mediators of leptin actions not only in appetite control, but also in SNS activity regulation [3,51-53]. Chronic overfeeding in rats was shown to change NPY and a-MSH levels in the hypothalamus, leading also to increased BP following SNS

activation [20]. However, the precise mechanisms of leptin-induced increased SNS activity have not been fully elucidated yet. Furthermore, whether an intervention in those mechanisms may contribute to the prevention and/or treatment of obesity-induced HTN remains to be proved.

NPY exerts various effects on the cardiovascular system such as vasoconstriction, induction of vascular SMCs growth and angiogenesis [26,53]. Possible mechanisms involved in NPY-related elevation of BP observed in both experimental and human studies include activation of Central Nervous System (CNS) nuclei, especially in the hypothalamus, and increased vascular tone following the contraction of peripheral and renal artery SMCs [54-59]. Interestingly, in animal studies intracerebrovascular (ICV) NPY administration was associated with both increased [60] and decreased BP [61]. Furthermore, NPY effects on sympathetic nerve activity to interscapular brown adipose tissue were either stimulatory or suppressive, according to the CNS area in which NPY was administered [62].

In several human studies, increased plasma NPY levels were reported in patients with primary HTN or some forms of secondary HTN such as pheochromocytoma, preeclampsia and end stage renal disease (ESRD) [9,26,53,63,64]. Of note, these studies did not report body weight. Interestingly, hypertensive patients with absence of the physiological nocturnal decrease in BP during ambulatory BP measurement (non-dippers) and those with target organ damage had also higher circulating NPY levels compared with dippers and those without target organ damage, respectively [22]. Furthermore, the T1128C polymorphism of the NPY gene has been associated with increased prevalence of hypertension [3,65,66]. Several mechanisms are implicated in the hypertensive effects of NPY, including catecholamine production in the hypothalamus, co-localization with norepinephrine in hypothalamic neurons, facilitation of sympathetic transmitter vasoconstriction, direct vasoconstriction [23-26] and stimulation of vascular smooth muscle cell (SMC) proliferation [26-28].

In the present study, plasma NPY levels were higher in OW and OB hypertensives compared with NW hypertensives. OW and OB hypertensives had also higher NPY concentrations com-

pared with OW and OB normotensives, respectively. However, in NW patients, plasma NPY levels did not differ significantly between hypertensives and normotensives. Based on these results, it seems reasonable to suggest that NPY may contribute to obesity-induced HTN. These findings are consistent with other studies supporting a role for NPY in obesity-related HTN [67-69]. In contrast, other investigators reported no significant differences in plasma NPY concentrations between obese hypertensives, obese normotensives and control subjects [70].

It should be noted that smoking and alcohol consumption, factors that may contribute to hypertension development [71,72], did not differ significantly between the study groups. Furthermore, among lipid parameters, only low density lipoprotein C (LDL-C) differed significantly between group 4 and the other patient groups. This observation cannot interfere with our results since there is a significant difference only between normotensive-normal weight patients (group 4) and the rest of the groups, whereas no significant differences in LDL-C were found between those patient groups where NPY levels were shown to be associated with obesity-related HTN.

Differences in plasma NPY levels observed in both Male and Female patients were similar with those observed in the total population. These findings suggest that Male and Female cardiovascular system may respond similarly in NPY actions. In this context, no gender differences in NPY immunoreactivity were observed in obese and lean Zucker diabetic fatty rats [73]. However, in another animal study NPY responses in stress were different between Male and Female, with up-regulation of NPY and adrenergic receptors only in Male animals [74]. The authors suggested that elevated circulating catecholamine levels may be involved in the down-regulation of vascular adrenergic receptors observed in the Female animals [74].

In our study, COB+ patients had higher plasma NPY levels compared with COB- individuals, a difference observed not only in the total study population, but also in M and F patients separately ($p = 0.004$ to < 0.001). Furthermore, COB+ hypertensives had higher plasma NPY concentrations compared with COB- hypertensives ($p < 0.001$), a finding that was not observed in normotensive patients. Interestingly,

experimental studies have shown that NPY is also produced by visceral adipose tissue (VAT) and that it enhances adipocyte proliferation rate, acting through Y1 receptor [75]. Of note, NPY mRNA expression in VAT was up-regulated 6-fold in a rat model of increased visceral adiposity and >2-fold in obese Zucker rats [75]. In another animal study, chronic ICV NPY administration increased adiposity, without however raising BP [61]. These findings suggest that high circulating NPY concentrations may be related to abdominal obesity.

In the present study, plasma NPY levels significantly correlated with BMI, WC, SBP, DBP and HR. Similar correlations were observed in Male patients, whereas in Female patients plasma NPY levels correlated with BMI, WC, SBP and DBP, but not with HR. These results may be partly explained by a possible interaction between NPY and female hormones, as suggested by animal studies reporting a decrease in luteinizing hormone releasing hormone (LHRH) following IV NPY administration [76]. Of note, in hypertensives, plasma NPY concentrations correlated with BMI, WC, DBP and HR, whereas in normotensives no correlations were found between plasma NPY concentrations and other variables.

Elevated plasma NPY levels were also observed in patients with HTN, left ventricular hypertrophy (LVH) and congestive heart failure, implying that overstimulation of NPY receptors in cardiomyocytes may lead to abnormal myocardial growth [29]. In our study, patients with LVH had higher plasma NPY levels compared with those with diastolic dysfunction and those with normal findings, but this difference was significant only between patients with LVH and normal echo findings. Similar results were observed in M patient population, whereas in F patients and hypertensives no significant differences were found in plasma NPY concentrations between patients with and without echo findings. NPY has been reported to stimulate the hypertrophy of rat ventricular cardiomyocytes [29], thus possibly contributing to the LVH frequently seen in HTN [41,77]. Furthermore, elevated plasma NPY levels were independently associated with LVH in patients with pheochromocytoma [78] and ESRD [79]. The absence of a significant difference in plasma NPY levels between hypertensive patients with and without LVH in the present study may be attributed to the fact that

all hypertensives were newly diagnosed or had grade 1 or grade 2 HTN, according to our inclusion criteria, and therefore less likely to have target organ damage such as LVH.

On the other hand, no significant differences were observed in plasma α -MSH levels between study groups in the total population, Male and Female patient groups. Furthermore, plasma α -MSH concentrations did not differ between patients with different echo findings in the total study population, hypertensives, Male and Female patients separately. No correlations were found between plasma α -MSH levels and other variables. In contrast, in animal studies acute ICV administration of α -MSH resulted in increased mean arterial pressure and HR through central stimulation of SNS, an effect mediated by the MC4R [30-32,80,81]. Interestingly, in another animal study ICV injection of α -MSH increased BP and renal sympathetic nerve activity in conscious rabbits, whereas intravenous (IV) administration of α -MSH had no effect on either BP or SNS activity [82]. Of note, both chronic ICV administration of α -MSH and injection to selected sites such as the nucleus of the solitary tract or the dorsovagal complex of the medulla, have been reported to lower BP and HR [31,80,83]. Therefore, the effects of α -MSH on BP and HR remain to be established.

In the present study and similarly to plasma NPY levels, HR was higher in OW and OB hypertensives compared with NW hypertensives. In normotensive study groups, OW and OB patients had also higher HR compared with NW ones, but this difference was significant only between OB and NW normotensives. No significant differences in HR were found between hypertensives and normotensives in NW (groups 1 and 4), OW (groups 2 and 5) and OB patients (groups 3 and 6). These findings are in agreement with previous studies reporting that elevated circulating NPY levels contribute to HR rise through increased SNS activity [3,59,60]. In contrast, α -MSH does not influence HR, probably due to the fact that its actions are mediated by different receptors in the CNS.

Conclusions

In the present study, plasma NPY levels were higher in OW and OB hypertensives compared with NW hypertensives. OW and OB hypertensives had also higher NPY concentrations com-

pared with OW and OB normotensives, respectively. However, in NW patients, plasma NPY concentrations did not differ significantly between hypertensives and normotensives. COB+ patients had higher plasma NPY levels compared with COB- ones, a difference also observed in hypertensives but not in normotensive patients. Furthermore, plasma NPY concentrations significantly correlated with BMI, WC, SBP, DBP and HR. Apart for the total study population, the majority of these differences were also observed in M and F patients separately. These results may suggest a role for NPY in obesity-induced HTN. HR was also higher in the same patient populations, possibly as a result of NPY-induced increase in SNS activity.

In contrast, plasma α -MSH levels were similar in all groups. This finding can be explained as the action of α -MSH takes place mainly through its receptors in CNS while NPY has well known actions through receptors in peripheral organs involved in BP control as the vessels, kidney and myocardium. On the other hand, this discrepancy may also represent the different central effects of α -MSH and NPY, as these neuropeptides act through different receptors in the CNS.

Patients with LVH had higher plasma NPY levels compared with those with dilated dysfunction and those with normal findings. No significant differences in plasma NPY concentrations were found between hypertensive patients with normal and abnormal echo findings. This result may be possibly attributed to the fact that all hypertensives were newly diagnosed or had grade 1 or 2 HTN. Plasma α -MSH levels did not differ significantly between patients with normal and abnormal echo findings in the total population, M, F and hypertensive groups.

Further investigation is needed to elucidate the role of NPY and α -MSH in obesity-related HTN. Moreover, whether agonists and/or antagonists of these neuropeptides will prove to be beneficial therapeutic targets in both prevention and treatment of this form of HTN remains to be established.

Conflict of interest: There is no conflict of interest.

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