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Differential sympathetic activation in muscle and skin neural districts in the metabolic syndrome

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Abstract

The present study was designed to determine whether and to what extent the activation of the sympathetic nervous system reported in the metabolic syndrome is generalized to the whole cardiovascular system or if it is rather confined to selected vascular districts. In 16 untreated patients with metabolic syndrome, 12 essential hypertensive subjects, 12 obese subjects, and 14 lean healthy normotensive controls, we measured blood pressure (Finapres, Englewood, CO), heart rate (electrocardiogram), venous plasma norepinephrine (high-performance liquid chromatography), and postganglionic sympathetic nerve traffic in the skeletal muscle and in the skin districts (microneurography). The muscle and skin nerve traffic measurements were obtained in a randomized sequence. Measurements also included skin sympathetic nerve responses to an arousal (acoustic stimulus). The 4 groups of subjects had superimposable ages. Muscle sympathetic nerve traffic values were significantly higher in subjects with hypertension and in those with obesity than in controls (51.2 ± 2.8 and 52.0 ± 3.0 vs 37.2 ± 3.3 bursts per 100 heart beats, respectively; $P < .01$ for all). A further significant increase in muscle sympathetic nerve traffic was detected in subjects with the metabolic syndrome (61.0 ± 3.2 bursts per 100 heart beats, $P < .05$). In contrast, skin sympathetic nerve traffic was not significantly different in the 4 groups of individuals (13.0 ± 0.7, 14.3 ± 1.3, 12.5 ± 0.8 vs 15.4 ± 1.0 bursts per minute, respectively; P = not significant). The skin sympathetic responses to an acoustic stimulus were also similar in the different groups. The present data provide the first direct evidence that in the metabolic syndrome the sympathetic activation is not uniformly distributed over the cardiovascular system. This may depend on the fact that muscle and skin sympathetic nerve activities are regulated by mechanisms that are affected in a different fashion by the various components of the disease.

1. Introduction

Metabolic syndrome is characterized by a marked sympathetic overactivity, as documented by the increase in urinary excretion of epinephrine and norepinephrine, in sympathetic nerve firing rate to the skeletal muscle district, in total norepinephrine spillover, and in the low-to-high frequency ratio of the power spectral analysis of the heart rate signal [1-6]. Evidence has been also provided that in the metabolic syndrome the magnitude of the adrenergic overdrive is potentiated as compared with the one already characterizing each condition clustering in the metabolic syndrome [2,4,7]. In contrast, no information is available as to whether the hyperadrenergic state of the metabolic syndrome has a regional or rather a more generalized distribution. The issue has important pathophysiologic and clinical implications, given the evidence that in the metabolic syndrome the adrenergic overdrive (1) is one of the major driving forces of the metabolic disarray [7-9]) and (2) represents the target of nonpharmacologic as well as pharmacologic interventions aimed at correcting the dysmetabolic state and thus at reversing the elevated cardiovascular risk profile of the patient [7,8].

The present study was designed to determine whether the sympathetic activation of the metabolic syndrome is generalized to the whole cardiovascular system or if it is rather confined to selected vascular beds. Because in the metabolic syndrome the adrenergic overdrive seen in the muscle neural district is greater in magnitude than that seen in its major single components, that is, obesity and hypertension

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[2,4,7-8], the study was also designed to assess whether a similar behavior is detectable in the skin sympathetic neural drive. To this aim, we systematically performed microneurographic recording of muscle as well as of skin sympathetic nerve traffic in patients with metabolic syndrome and compared the results with those obtained in healthy subjects and in patients with either obesity or hypertension alone.

2. Methods

2.1. Study population

Our study was carried out in 54 subjects of both sexes (42 men and 12 women; age range, 30-54 years). Twelve subjects had (1) mild untreated essential hypertension, that is, a diastolic blood pressure (BP) between 90 and 109 mm Hg at sphygmomanometric measurements performed in the outpatient clinic, and (2) no history and/or clinical evidence of hypertension-related complications or major end-organ damage. Twelve subjects had a normal BP, that is, a diastolic value less than 90 mm Hg and a systolic value less than 140 mm Hg, but displayed a marked increase in body mass index (>30 kg/m^2) and waist circumference (men, >102 cm; women, >88 cm). Sixteen subjects had a metabolic syndrome based on the presence of at least 3 of the 5 diagnostic criteria proposed by the National Cholesterol Educational Program Adult Treatment Panel in the 2005 revised version [10]. These included abdominal obesity (waist circumference >102 cm in men and >88 cm in women), fasting hypertriglyceridemia (plasma triglycerides >150 mg/dL), low high-density lipoprotein cholesterol (plasma high-density lipoprotein cholesterol <40 mg/dL in men and <50 mg/dL in women), high BP (BP values ≥130/85 mm Hg or treatment with antihypertensive drugs), and fasting hyperglycemia (plasma glucose >100 mg/dL). Antihypertensive drug treatment, if present, was withdrawn at least 4 days before the study. To exclude the concomitant presence of left ventricular dysfunction (see below), all subjects underwent an echocardiographic examination that showed that left ventricular ejection fraction was always greater than 55%. Subjects were excluded from the study if they had atrial fibrillation and/or major cardiac arrhythmias, history of myocardial infarction, clinical or laboratory evidence of valvular heart disease, history of smoking and/or excessive alcohol consumption, presence of diabetes, congestive heart failure, obstructive sleep apnea or other conditions known to affect autonomic function, history of regular exercise habit, or involvement in physical training programs. The study protocol was approved by the Ethics Committee of our hospital. All of the subjects agreed to participate after being informed of the study's nature and purpose.

2.2. Measurements

At the beginning of the experimental session, BP was measured by a mercury sphygmomanometer, taking the first and fifth Korotkoff sounds to identify systolic and diastolic values, respectively. During the study proper (see below), BP was measured by a finger photoplethysmographic device (Ohmeda 2300; Finapres, Englewood, CO) capable of providing accurate and reproducible beat-to-beat systolic and diastolic values [3-4,11-12]. This procedure to assess BP values has been shown not to affect muscle sympathetic nerve traffic at variance from sphygmomanometric BP assessment [13]. Heart rate was continuously monitored by a cardiotachometer triggered by the R wave of an electrocardiographic lead. Respiration rate was monitored by a strain gauge pneumograph positioned at midchest level. Multiunit recordings of efferent postganglionic sympathetic nerve activity to skeletal muscle (muscle sympathetic nerve activity [MSNA]) or skin (skin sympathetic nerve activity [SSNA]) areas were obtained through a tungsten microelectrode inserted into the right or left peroneal nerve, as previously described [3-4,11]. The nerve signal was amplified ×70 000, fed through a band-pass filter (700-2000 Hz), and integrated with a custom nerve traffic analyzer (Bioengineering Department, University of Iowa). Integrated nerve activity was monitored by a loudspeaker; displayed on a storage oscilloscope (model 511A; Tektronix, Beaverton, OR); and recorded together with BP, heart rate, and respiration rate on thermic paper by an ink polygraph (Gould 3800; Gould Instruments, Cleveland, OH). The muscle or skin nature of sympathetic nerve activity was assessed by the criteria detailed in previous studies [3-4,11-16]. For MSNA, these criteria were that (1) a weak electrical stimulation through the microelectrode induced an involuntary muscle contraction but not paresthesias, (2) tapping or passive stretching of the muscle supplied by the nerve caused afferent mechanoreceptive impulses, and (3) the recording consisted of spontaneous pulse-synchronous bursts that increased during held expiration [3-4,11,14]. For SSNA, the criteria were that (1) electrical stimulation through the microelectrode induced skin paresthesias without concomitant muscle contraction, (2) light skin touching evoked afferent nerve impulses, and (3) tapping or passive stretching of the muscle supplied by the nerve did not elicit afferent mechanoreceptive impulses [15-16]. Neurograms were accepted only if they did not show simultaneous SSNA and MSNA activity and if the signal-to-noise ratio was greater than 3. Muscle sympathetic nerve activity was quantified over each 30-minute period either as bursts per minute or as bursts per 100 heart beats, whereas SSNA was quantified as bursts per minute. The SSNA response to an acoustic stimulus (see below) was quantified as the absolute change in the amplitude of the burst after the stimulus as compared with the mean amplitude of the spontaneous bursts occurring over the 3 minutes preceding the stimulus itself. Body mass index was obtained by dividing body weight in kilograms by the square of the height in meters. Plasma norepinephrine was measured by high-performance liquid chromatography [17] from a

Table 1
Demographic, anthropometric, hemodynamic, and neuroadrenergic variables in the 4 groups of subjects enrolled in the study

Variable	Controls (n = 14)	Essential hypertensive subjects (n = 12)	Obese subjects (n = 12)	Subjects with metabolic syndrome (n = 16)
Age (y)	46.1 ± 2.2	48.2 ± 2.3	46.3 ± 2.6	49.4 ± 2.8
Sex (male/female)	10/4	10/2	9/3	13/3
BMI (kg/m^2)	24.3 ± 0.8	25.1 ± 0.7	38.7 ± 1.2 †,§	32.4 ± 1.0*,‡
Waist circumference (cm)	93.8 ± 1.4	94.2 ± 1.5	109.1 ± 1.7 †,§	104.3 ± 1.5*,‡
Sphygmomanometric BP, S/D (mm Hg)	125.4 ± 2.5/78.6 ± 1.8	154.4 ± 2.2†,‖/96.1 ± 2.4†,‖	129.6 ± 2.8/81.1 ± 2.2	147.3 ± 3.1†,‖,§/85.1 ± 2.3*,§
Finger BP, S/D (mm Hg)	123.6 ± 2.6/76.8 ± 2.0	152.6 ± 2.3†,‖/94.5 ± 2.5†,‖	128.0 ± 3.0/79.7 ± 2.4	145.1 ± 2.8†,‖,§/83.3 ± 2.5§
Heart rate (beats/min)	72.3 ± 2.3	71.7 ± 2.4	75.8 ± 2.6	78.3 ± 2.9*,‡
Respiration rate (breaths/min)	18.4 ± 1.1	18.8 ± 1.3	19.1 ± 1.4	19.0 ± 1.4
LVEF (%)	64.2 ± 0.9	63.7 ± 1.0	64.0 ± 0.9	63.5 ± 0.8
Plasma NE (pg/mL)	222.9 ± 35	266.6 ± 29	297.8 ± 41	342.2 ± 38*
MSNA (bursts/min)	26.2 ± 2.2	37.3 ± 2.2*	41.6 ± 2.2†	49.0 ± 2.6†,‡,‖
MSNA (bursts/100 heart beats)	37.2 ± 3.3	51.2 ± 2.8*	52.0 ± 3.0†	61.0 ± 3.2†,‡,‖
SSNA (bursts/min)	15.4 ± 1.0	13.0 ± 0.7	14.3 ± 1.3	12.5 ± 0.8

Data are shown as means ± SEM. Symbols refer to the statistical significance between groups. BMI indicates body mass index; S, systolic; D, diastolic; LVEF, left ventricular ejection fraction; NE, norepinephrine.

* $P < .05$ vs controls.
† $P < .01$ vs controls.
‡ $P < .05$ vs essential hypertensive subjects.
§ $P < .01$ vs essential hypertensive subjects.
‖ $P < .05$ vs obese subjects.

venous blood sample. The same blood sample was also used to assess the various metabolic variables useful for the diagnosis of metabolic syndrome (glycemia, triglycerides, total and high-density lipoprotein cholesterol) as well as routine hematologic and blood chemistry data (hemoglobin, blood urea, electrolytes, blood creatinine, etc).

2.3. Protocol and data analysis

All subjects were examined in the morning after a light breakfast and an overnight abstinence from alcohol, smoking, and coffee consumption. They were placed supine and fitted with the intravenous cannula and the devices to measure sphygmomanometric BP, finger BP, heart rate, and respiration rate. After a 30-minute interval, a blood sample for the assay of plasma norepinephrine and other metabolic variables was then drawn from the cannula. Thereafter, BP was measured 3 times by a mercury sphygmomanometer (see above); and then the microelectrode was inserted into a peroneal nerve to obtain MSNA or SSNA, which was recorded together with finger BP, heart rate, and respiration rate during a 30-minute period. The

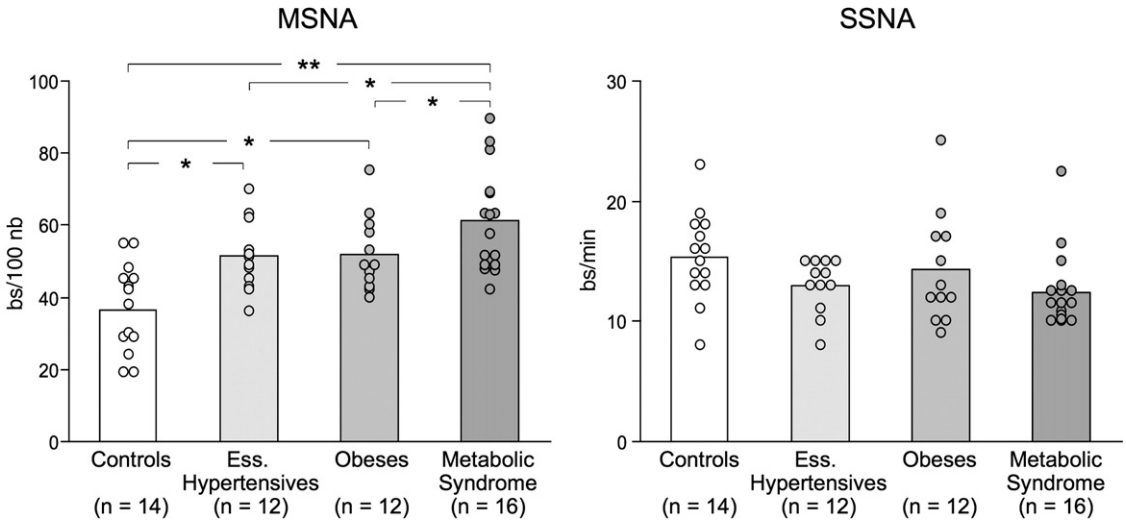

Fig. 1. Individual and average values of muscle sympathetic nerve traffic (MSNA, expressed as bursts corrected for heart rate [bursts per 100 heart beats]) and skin sympathetic nerve traffic (SSNA, expressed as bursts per minute) in control subjects and in patients with essential hypertension, obesity, or metabolic syndrome. Asterisks (*$P < .05$, **$P < .01$) refer to the statistical significance between groups. Bs indicates bursts; hb, heart beats.

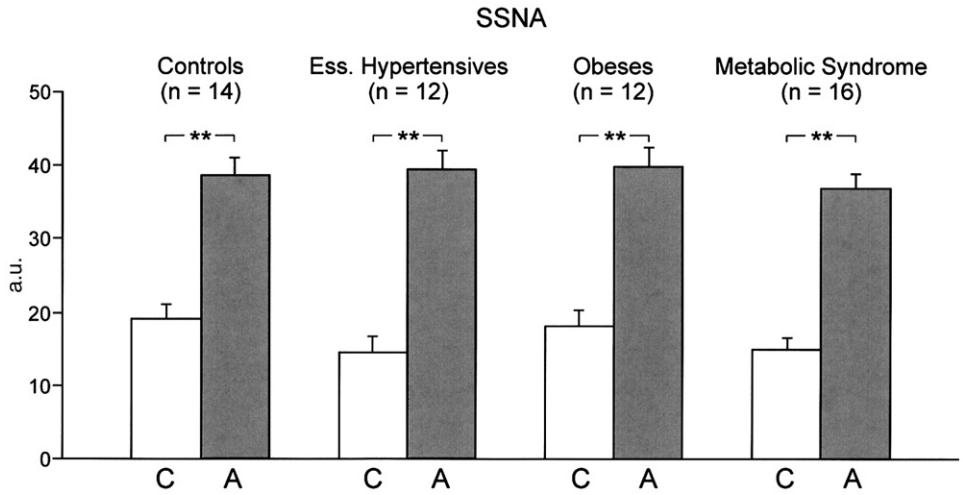

Fig. 2. Skin sympathetic nerve traffic (SSNA) burst area responses (in arbitrary units) to an acoustic stimulus in healthy control subjects and in patients with essential hypertension, obesity, or metabolic syndrome. In each group, control value was calculated as the average area of the bursts over the 3 minutes preceding the stimulus, whereas arousal response was calculated as the area of the single burst immediately after the stimulus itself. Data are shown as means ± SEM. $^{**}P <$ .01, arousal response vs control value. C indicates control value; A, arousal response; au, arbitrary units.

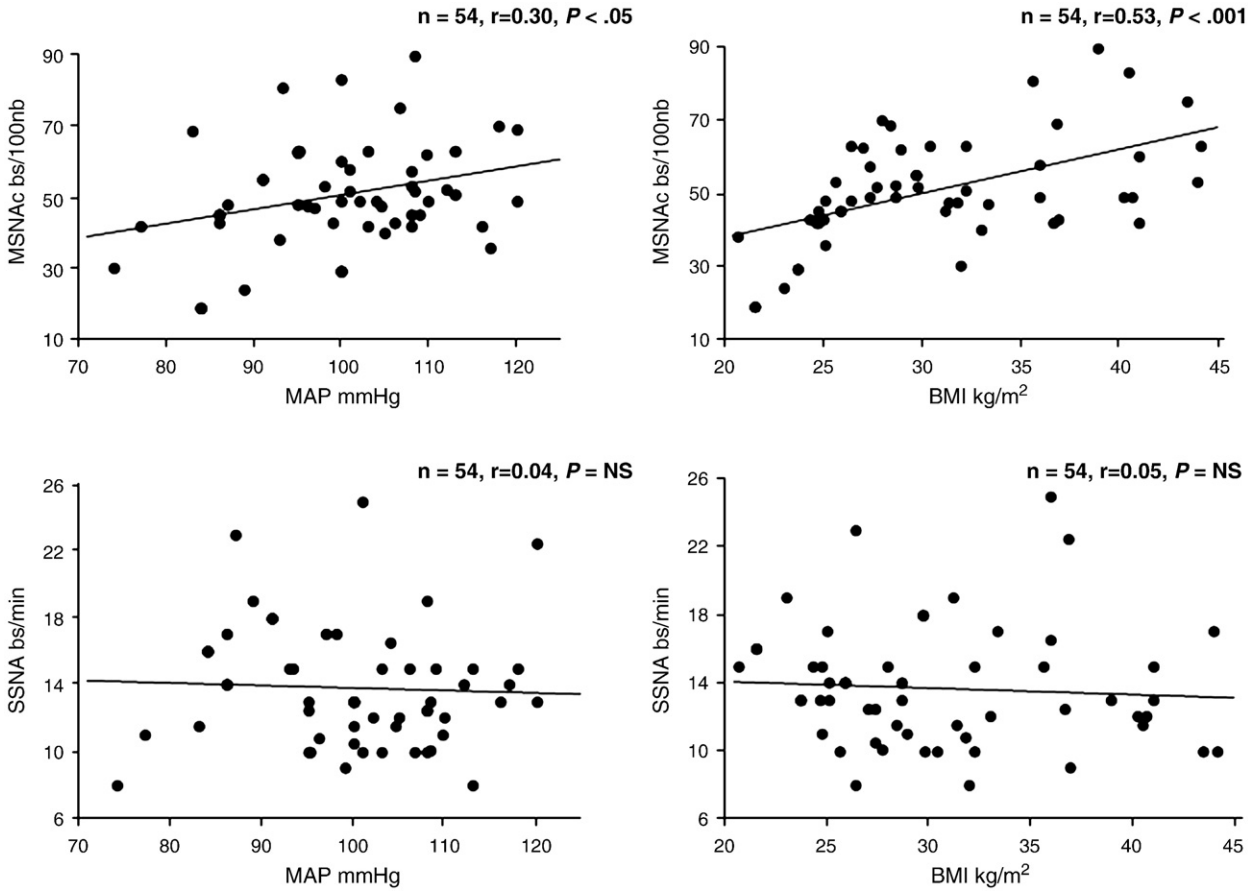

Fig. 3. Relationships between muscle sympathetic nerve traffic (MSNA, upper panels), skin sympathetic nerve traffic (SSNA, lower panels), and mean arterial pressure or body mass index in the whole study population. Correlation coefficients (r) and P values are shown. MAP indicates mean arterial pressure; BMI, body mass index; NS, not significant.

microelectrode was then repositioned in the peroneal nerve fascicles to obtain the sympathetic nerve activity (MSNA or SSNA) that was not obtained in the previous recording period. Also in this instance, finger BP, heart rate, and respiration rate were all recorded during a 30-minute period. Muscle sympathetic nerve activity was evaluated before SSNA in 26 subjects and after SSNA in 28 subjects. At the end of the SSNA recording period, a 5-second acoustic signal provided by an alarm clock was delivered to check the SSNA ability to increase. The delivery of the stimulus was not anticipated by the subjects. Each protocol step was separated from the following one by a 20- to 30-minute interval. Data were collected in a semidark and quiet room at a constant temperature of 20°C to 21°C. Calculations of finger BP, heart rate, respiration rate, as well as MSNA and SSNA were made by a single independent observer. Values from individual subjects were averaged for each group and expressed as means ± SEM. Comparisons between groups were made by 2-way analysis of variance using the Bonferroni correction for multiple comparisons. The Pearson correlation coefficient was used to determine the relationships between resting MSNA, resting SSNA, body mass index, mean arterial finger BP (diastolic BP plus one third of pulse pressure), and plasma norepinephrine. $P < .05$ was taken as the minimal level of statistical significance.

3. Results

As shown in Table 1, the 4 groups of subjects had similar age. Body mass index and waist circumference were increased in obese subjects and in those with metabolic syndrome, who also displayed greater BP values. Greater BP values were also seen, as expected, in the hypertensive subjects. Heart rate was not significantly different in control, obese, and hypertensive subjects but slightly, although significantly, increased in individuals with the metabolic syndrome. Respiration rate was superimposable in all the 4 groups.

As shown in the average data of Table 1 and in the individual values of Fig. 1, compared with that in controls, MSNA was significantly greater in subjects with hypertension and in those with obesity. A further significant MSNA increase was detected in subjects with the metabolic syndrome. In contrast, SSNA was not significantly different in the 4 groups of individuals, who also displayed superimposable increases in SSNA in response to the acoustic stimulus (Fig. 2). No significant difference in MSNA and SSNA values was found in the male and female subjects belonging to the different groups. In the population as a whole, resting MSNA, but not SSNA, was significantly and directly related to mean finger arterial pressure and body mass index (Fig. 3). Plasma norepinephrine was also directly and significantly related to MSNA ($r = 0.37$, $P < .02$), but not to SSNA ($r = 0.07$, P = not significant).

4. Discussion

Confirming previous findings based on indirect or direct assessment of adrenergic neural drive [1-5], the present study shows that sympathetic cardiovascular influences are potentiated in a high-risk condition such as the metabolic syndrome, resulting in a sympathetic activation greater in magnitude than that seen in its major individual components. It adds to this information, however, the evidence that this sympathetic potentiation does not appear to be uniformly distributed over the whole cardiovascular system. This is because, in our patients with metabolic syndrome, the increase in MSNA was not paralleled by a concomitant increase in SSNA, which on the other hand showed values within the reference range. This allows us to conclude that in the metabolic syndrome the sympathetic overactivity is not uniformly distributed over the whole cardiovascular system.

There are at least 2 potential mechanisms that may account for the MSNA/SSNA dichotomy. It is indeed possible that baroreflex mechanisms are involved, given the evidence that (1) in several conditions and diseases, MSNA but not SSNA is under baroreflex influences [3-4,18] and (2) an impairment in baroreflex MSNA modulation has been reported in the metabolic syndrome [3]. It is on the other hand possible to speculate that metabolic and/or humoral substances whose release is augmented in this clinical condition could be responsible for this finding by exerting nonhomogeneous effects on MSNA and SSNA. This may be particularly the case for insulin and leptin because both these substances, whose circulating levels are increased in the metabolic syndrome [7,9], trigger a marked sympathetic activation at the level of the muscle circulation, unaffecting the cutaneous one [19-20]. Several mechanisms can be advanced for explaining the insulin-induced sympathetic activation. For example, it has been suggested that the sympathoexcitatory effects of insulin can be regarded as the compensatory response of the adrenergic nervous system to the vasodilatation induced by insulin [21]. It has also been suggested, however, that insulin may exert adrenergic activation throughout its direct sympathoexcitatory effects on specific regions of the central nervous system [22].

Several other results of our study deserve to be discussed. First, in our patients with metabolic syndrome, heart rate values were significantly increased as compared with those recorded in healthy controls and in patients with obesity or hypertension alone. Because this hemodynamic variable depends not only on vagal but also on sympathetic influences on the sinus node [23], the increased heart rate values seen in patients with metabolic syndrome may indicate that at cardiac level sympathetic activity is enhanced, thereby paralleling the behavior of sympathetic nerve traffic at the level of the muscle vascular district. This hypothesis, however, should be balanced against the finding that the heart rate differences between subjects with and without metabolic syndrome, although significant, were small in magnitude. This prevents any definite conclusion on this issue to be drawn. Second,

plasma norepinephrine values were also significantly increased in patients with metabolic syndrome but not in those with obesity or hypertension alone, despite the concomitant marked increase in MSNA seen in all these clinical conditions. This may be regarded as a further example of the inability of this humoral marker to fully reflect a stable increase in sympathetic drive reported in previous studies by our group and others [24-26]. Finally, our study does not allow to clarify whether sympathetic activity in other districts behaves similarly to SSNA or to MSNA. As mentioned above, however, the behavior of heart rate in patients with metabolic syndrome suggests that, at least at the cardiac level, an increase in sympathetic drive does occur. Whether this is the case also for cerebral, splanchnic, or other regional circulations deserves to be investigated by future studies based on the only available approach allowing precise quantification of regional sympathetic drive, that is, the norepinephrine spillover technique [26,27].

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