Time delay of baroreflex control and oscillatory pattern of sympathetic activity in patients with metabolic syndrome and obstructive sleep apnea

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SYMPATHETIC NERVE ACTIVITY is increased in patients with metabolic syndrome (MetS) (11, 22), which enhances the risk of cardiovascular disease and likely cardiac death (5, 20, 21). Obstructive sleep apnea (OSA), a frequent comorbidity in patients with MetS (8), is characterized by recurrent episodes of partial or complete upper airway obstruction during sleep, leading to intermittent hypoxemia and frequent arousals from sleep (40). Additionally, OSA exacerbates the muscle sympathetic nerve activity (MSNA) in patients with MetS (12, 37) and impairs arterial baroreflex control (ABR) of heart rate (HR) (12, 37). Grassi et al. (12) demonstrated that ABR of HR was further decreased in patients with MetS and OSA. We confirmed that OSA exacerbates the dysfunction of the ABR of HR in patients with MetS (37).

The ABR exerts a major inhibitory influence on sympathetic outflow (15). This autonomic reflex control determines the sympathetic nerve activity cardiovascular response (12, 37) and the oscillatory pattern of sympathetic nerve activity (31). The effectiveness of the ABR depends on their magnitude and the time delay of the effectors’ response (33). However, the intrinsic characteristics of baroreflex function of MSNA and HR in patients with MetS and OSA are unknown.

Yet, the influence of sympathetic drive on cardiovascular control is dependent of its tonus (quantification of its frequency and incidence, expressed in bursts/min and bursts/100 heart beats, respectively) and its modulation (intensity, rhythm, and oscillation of discharge). The oscillatory pattern of MSNA depends on the relationship between low-frequency (LF) and high-frequency (HF) components of oscillation, i.e., the ratio of LF and HF of MSNA. The efficiency of effectors’ sympathetic response is then influenced not only by their tonus but also by their oscillatory pattern. It is of clinical implication since the absence of the LF component of MSNA modulation during baseline and, in consequence, a decreased oscillatory pattern of MSNA have been associated with an increased mortality in patients with heart failure (39). The oscillatory pattern of MSNA in patients with MetS and OSA remains unknown.

We investigated the magnitude and the time delay of the ABR of MSNA and HR, and the oscillatory pattern of MSNA in patients without and with OSA. Our hypotheses were that 1) MetS would decrease the magnitude and increase the time delay of the ABR of MSNA and HR 2) MetS would impair the oscillatory pattern of MSNA; and 3) OSA would exacerbate these autonomic dysfunctions.

MATERIALS AND METHODS

Study population. Forty-three consecutive outpatients (18 women), 35 to 65 yr of age, diagnosed with MetS by the presence of least three of the five criteria according the Adult Treatment Panel III Report (13); i.e., waist circumference > 102 cm in men and > 88 cm in women, fasting glucose level ≥ 6.1 mmol/l, fasting triglyceride level > 1.69 mmol/l, HDL cholesterol < 1.03 mmol/l in men and < 1.29 mmol/l in women, and blood pressure > 130/85 mmHg were included in the study.
Patients were eligible for participation in the study if they were taking no medications, were sedentary, were nonsmokers, had no history of excessive alcohol consumption and had no evidence of overt cardiovascular disease at the time of the study. A week after clinical examination, all participants were submitted to a nocturnal polysomnography to detect the presence of OSA. The MetS patients were then divided into two groups: 1) with OSA (MetS + OSA; n = 21) and 2) without OSA (MetS − OSA; n = 22). In addition, twelve healthy controls without OSA (C), 35 to 57 yr of age, were enrolled in the study. This protocol was approved by Scientific Committee of the Clinical Hospital, University of São Paulo in the study. This protocol was approved by Scientific Committee of healthy controls without OSA (C), 35 to 57 yr of age, were enrolled in the study. This protocol was approved by Scientific Committee of the Clinical Hospital, University of São Paulo Medical School, and written, informed consent was given by the participants.

Experimental design. The study was performed at ~8:00 am, with the subjects supine in a quiet air-conditioned room (22 to 24°C). After obtaining an adequate microneurographic nerve recording site in the leg, all participants rested for 10 min. Baseline recordings of MSNA, arterial pressure, HR, and respiratory rate were taken for 10-min. Polysomnography. The sleep pattern was recorded during a nocturnal polysomnography (EMBLA digital system, Flaga hf. Medical Devices, Reykjavik, Iceland) (14). Apnea was defined as complete cessation of airflow for at least 10 s, associated with oxygen desaturation of 3%. Hypopnea was defined as a reduction (>50%) in respiratory signals for at least 10 s associated with oxygen desaturation of 3%. The apnea-hypopnea index (AHI) was calculated as the total number of respiratory events (apneas plus hypopneas) per hour of sleep. OSA was defined by an AHI of at least 15 events per hour of sleep (37).

Muscle sympathetic nerve activity. MSNA was recorded from the peroneal nerve using the microneurography technique (35, 38). In brief, multunit postganglionic muscle sympathetic nerve recordings were made using a tungsten microelectrode (tip diameter, 5 to 15 μm). The signals were amplified by a factor of 50 000 to 100 000 and band-pass filtered (700 to 2,000 Hz). For recordings and analysis, nerve activity was rectified and integrated (time constant, 0.1 s) to obtain a mean voltage display. Muscle sympathetic bursts were identified by inspection and were expressed as burst frequency (in bursts/min) and burst incidence (in burst per 100 heart beats).

Arterial pressure, HR, and respiratory rate. Arterial pressure was monitored noninvasively by a finger photoplethysmography device (Finapres 2300, Ohmeda, Englewod, CO) on a beat-to-beat basis. Simultaneously, HR was monitored through lead II of ECG and respiratory rate was monitored with a piezoeletric thoracic belt (Pneumotrace II, model 1132) placed around the upper abdomen.

Cardiac autonomic evaluation. An automated computer program (Winadq/Pro, Datqa Instruments, Akron, OH) with sampling frequency of 500 Hz and with a resolution of 16 bits was used to process the ECG, pulsatile arterial pressure, and integrated MSNA signals extracting, respectively, the beat-to-beat time series of HR (considering the RR interval), systolic arterial pressure (SAP), and MSNA. Regarding to MSNA analysis, sympathetic bursts were identified by careful inspection of the mean voltage neurogram. For each individual sympathetic burst, the computer program provided the time of occurrence and its amplitude (time × voltage area) (4, 23). In addition, time series of MSNA were provided through integration of the continuous MSNA signal: MSNA(t) = ∫0 t MSNA(τ)dτ, where each integral was performed over the time window between two consecutive RRi delimiting the i-th cardiac cycle of period t(i) (4, 23). The MSNA time series were normalized by mean values of integrated area of bursts per cardiac cycle and expressed in arbitrary units.

Cardiovascular fluctuations of the RRi, SAP, and MSNA time series were assessed in the frequency domain by means of autoregressive spectral analysis (25, 30). Briefly, on stationary segments of the time series, autoregressive variables were estimated via the Levinson-Durbin recursion, and the order of the model was chosen according to Akaike’s criterion (30). An autoregressive spectral decomposition was then performed. This procedure permitted the automatic quantification of the center frequency and the power of each relevant component in absolute as well as in normalized units. The spectral components in LF of RRi, SAP, and MSNA variabilities were considered in range between 0.04 and 0.15 Hz, and the components with the range between 0.15 and 0.40 Hz, synchronized with the breath, were considered as HF. The power spectra of RRi, SAP, and MSNA variabilities were expressed in squared milliseconds, squared millimeters of mercury, and squared arbitrary units, respectively, and power spectral densities were also calculated in normalized units. The LF component of RRi variability reflects both cardiac sympathetic and parasympathetic modulation and the HF component synchronized with the breath reflects cardiac parasympathetic modulation (34). The LF component of SAP variability reflects vasomotor sympathetic modulation as well as the buffering ability of endothelial nitric oxide (NO) (32), whereas the HF component expresses the mechanical effect of respiration on the heart and vessels and did not represent an autonomic index (2). The LF component of MSNA reflects the profile of oscillation of sympathetic modulation associated to 0.1–Hz rhythm because of central medullary sympathetic premotor oscillatory circuits and/or baroreflex resonance, whereas HF component reflects the marked influence of the central respiratory drive on medullary sympathetic premotor neurons (24).

The normalization procedure was performed by dividing the power of the LF or HF component by the total spectral power from which the power of the very low frequency (range, 0 to 0.04 Hz) component had been subtracted and by multiplying the result by 100 (25, 30). Furthermore, the ratio of LF and HF of MSNA and RR intervals were calculated for estimation of the oscillatory pattern of MSNA (LFMSNA/HFMSNA) and cardiac sympathovagal balance (LFRR/HFRR), respectively.

Arterial baroreflex control. The magnitude (gain) and the latency (time delay) of the ABR of MSNA and HR were obtained by means of the transfer function analysis, using the bivariate autoregressive identification procedure (1). This procedure permits the quantification of coherence (K2), phase shift (φ), and transfer function magnitude (gain) between the time series of MSNA or RRi (output signal) and SAP (input signal). The coherence function measures the degree of linear correlation between the oscillations at the same frequency in both variability signals and was accepted when K2 > 0.5 (1). The phase shift measures the time lag or lead between the signals and was considered when negative (φ < 0 radians, SAP changes precede MSNA or RRi changes) (1). The transfer function magnitude (gain) quantifies the intensity of response of output signal (MSNA or RRi) per unit of spontaneous change of input signal (SAP), being expressed in arbitrary units per millimeters of mercury or milliseconds per millimeters of mercury, respectively. Their values represent estimations the spontaneous gain of ABR on MSNA or HR (1, 3).

All these transfer function analysis variables can be calculated at the LF or HF ranges. We calculated the coherence and the phase shift and gain of the transfer function in the central frequency corresponding to the maximum coherence within the LF range. In consideration that the phase shift is the product between the time delay (tφ) and the angular velocity (ω), the time delay of ABR of MSNA or RRi in the LF range was quantified according to the following equation: tφ LF = ωLF/φLF, in which φLF is equal to the product between central frequency of LF band and 2π. The time delay was expressed in seconds (1, 3).

Statistical analysis. The data are presented as means ± SE. The Kolmogorov-Smirnov test was used to assess the normality of distribution of each variable studied. A χ2-test was used to assess categorical data differences. Demographic data and baseline physical characteristics and hemodynamic and autonomic data were compared using one-way ANOVA followed by Scheffé’s post hoc multiple
 MetS for HR (Fig. 1) observed between the MetS differences in the characteristics of the sleep pattern were increased HFMSNA band (Fig. 1) and oscillatory pattern (LFMSNA/HFMSNA, Fig. 1) compared with patients in the MetS OSA and C groups. Besides, the MetS OSA had lower ABR of MSNA and HR when compared with the MetS OSA and C groups (Fig. 2, B and D, respectively). MetS OSA patients had similar time delay of the ABR of MSNA and HR to C subjects. Examples of individual raw data of arterial pressure, sympathetic neurogram, and cross-spectral analysis (gain of ABR, coherence and shift phase) between MSNA and SAP variabilities of a C subject, a patient with MetS OSA, and a patient with MetS + OSA are shown in Fig. 3.

DISCUSSION

We see three new and important findings in the present study: 1) the modified oscillatory pattern of MSNA in patients with MetS, since they have a reduced LFMSNA and increased HFMSNA components; 2) OSA exacerbates these autonomic dysfunctions in MetS patients, and 3) patients with MetS associated to OSA had increased latency of the baroreflex response of HR and MSNA. Increased MSNA levels in MetS patients and exacerbation of this autonomic control in the presence of OSA have been reported (12, 37). MetS and OSA also provoke alteration in the modulation of MSNA. These findings have potential clinical implications. The LF component of the MSNA is reduced in our patients, which means that MSNA is predominantly modulated in a HFMSNA band. Similar response has been observed in congestive heart failure patients (39). Some investigators have suggested that in patients with heart failure, the absence of the LF oscillatory component of MSNA is associated not only with augmented MSNA levels but also with decreased ejection fraction and increased mortality rate (39).

Explanations for the changes in oscillatory pattern of MSNA variability associated with sympathetic overactivity in clinical conditions such as OSAS or chronic heart failure are not available. However, the involvement of the arterial baroreceptors may be contributed to LF oscillations of sympathetic activity via resonance loop (3, 31). Thus a depressed ABR may explain, at least in part, the changes in oscillatory pattern of MSNA.

Our study confirms the reduction in the baroreflex sensitivity of HR in MetS patients and that the presence of OSA exacerbates...
bates this autonomic dysfunction (12, 37). The present study shows that the baroreflex sensitivity of MSNA is impaired in MetS and the presence of OSA exacerbates the alteration in the baroreflex sensitivity of MSNA (Fig. 2A). There is no clear explanation for contradictory results (12). We cannot rule out that methodological strategy explains such findings. Our paradigm was based on spontaneous ABR, whereas others used drug administration to activate and deactivate arterial baroreceptors (12).

Not only the magnitude (sensitivity) but also the latency (time delay) of the effector baroreflex response can determine the efficiency of ABR (3). The latency of the baroreflex response of MSNA and HR is preserved in patients with MetS but profoundly altered in patients with MetS and OSA. When OSA was associated to MetS, the time delay of baroreflex response of MSNA and HR was augmented. These findings reinforce the idea that the magnitude of baroreflex and the time delay contribute to the decrease the ABR of MSNA and HR in patients with MetS associated to OSA. Thus we demonstrated the intrinsic characteristic of baroreflex control, gain, coherence, and time delay not only of HR but also of the MSNA in a closed-loop context in patients with MetS with or without OSA.

Parasympathetic nerve activity is important for the time delay of the ABR of HR (9, 17). Fisher et al. (9) demonstrated that the parasympathetic nervous activity blockade of the muscarinic receptors with glycopyrrolate increased the latency of the HR response of carotid baroreflex control in healthy individuals. Since our data show a marked reduction in HF component of RRi, the increased time delay observed in patients with MetS + OSA (Table 3) may be associated to reduced cardiac parasympathetic modulation. Further analysis of our data suggests an inverse correlation between cardiac parasympathetic modulation (expressed as absolute HF power density) and time delay of RRi response (r = −0.38, P < 0.01) and an association between cardiac parasympathetic modulation and AHI (r = −0.41, P = 0.002).

However, we cannot rule out that decreased ABR of MSNA and HR may be associated with arterial stiffness, since there is a correlation between the severity of OSA and the arterial stiffness (7). The carotid intima-media thickness increase and the reduction of arterial compliance observed in patients with OSA (6) may impair the ability of baroreceptors to translate spontaneous oscillations of pressure arterial (19). In this condition, the mechanical stimulus to activate the loop of the regulation of ABR depends on large oscillations in arterial pressure, which results in increases in arterial pressure variability (28). Patients with MetS + OSA have increased vascular sympathetic modulation (LF_{SAP}) and SAP variability compared with C subjects (Table 3). However, this increased LF oscillation of SAP was followed by a conflicting decrease in LF oscillation in MSNA. We speculate that contradictory results could be explained by a change in endogenous endothelial NO. Some investigators have documented a marked buffering capacity of NO continuously released by endothelium, reducing the LF oscillations of arterial pressure (32).

**Table 3. Cardiac and peripheral autonomic control**

<table>
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<th>C</th>
<th>MetS − OSA</th>
<th>MetS + OSA</th>
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<tr>
<td><strong>Cardiac autonomic control</strong></td>
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<tr>
<td>RRi</td>
<td></td>
<td></td>
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<tr>
<td>Variance, ms²</td>
<td>2.897 ± 679</td>
<td>2.083 ± 350</td>
<td>1.201 ± 142*</td>
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<tr>
<td>LF, ms²</td>
<td>1,937 ± 903</td>
<td>731 ± 122</td>
<td>640 ± 126*</td>
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<tr>
<td>LF, nu</td>
<td>43 ± 3</td>
<td>65 ± 3*</td>
<td>73 ± 2**</td>
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<tr>
<td>HF, ms²</td>
<td>919 ± 266</td>
<td>432 ± 132*</td>
<td>153 ± 28*</td>
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<tr>
<td>HF, nu</td>
<td>52 ± 3</td>
<td>35 ± 3*</td>
<td>26 ± 2*</td>
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<tr>
<td>LF/HF</td>
<td>0.9 ± 0.1</td>
<td>2.5 ± 0.4*</td>
<td>3.3 ± 0.3*</td>
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<td><strong>Peripheral autonomic control</strong></td>
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<tr>
<td>SAP</td>
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<tr>
<td>Variance, mmHg²</td>
<td>17.1 ± 3.8</td>
<td>30.0 ± 4.0</td>
<td>35.0 ± 3.1*</td>
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<tr>
<td>LF, mmHg²</td>
<td>12.6 ± 3.7</td>
<td>20.7 ± 3.6</td>
<td>21.7 ± 2.8</td>
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<tr>
<td>LF, mmHg²</td>
<td>2.6 ± 0.3</td>
<td>5.7 ± 0.7*</td>
<td>8.1 ± 0.9†</td>
</tr>
<tr>
<td>HF, mmHg²</td>
<td>1.7 ± 0.3</td>
<td>2.5 ± 0.4</td>
<td>3.8 ± 0.6*</td>
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Values are means ± SE. RRi, R-R interval; VLF, very low frequency (LF); HF, high frequency; LF/HF, sympathovagal balance; nu, normalized units. *P < 0.05 vs. C; †P < 0.05 vs. MetS – OSA.
Since endothelial dysfunction in MetS patients worsened by the association of OSA despite a reduction in LF oscillation of MSNA, the lack of buffering capacity of NO could be translated in a higher LF oscillation in SAP.

Another explanation for the changes in oscillatory pattern of MSNA variability in patients with MetS and OSA is the hypersensitization of peripheral chemoreceptors. The shift of the oscillatory pattern of MSNA toward HF range is observed in the MetS + OSA group compared with the MetS − OSA and C groups. Time delay SAP-MSNA and SAP-RRI were greater in the MetS + OSA group compared with the MetS − OSA and C groups.

Fig. 2. Arterial baroreflex control of MSNA [gain systolic arterial pressure (SAP)-MSNA, A] and heart rate (gain SAP-RRI, C) and latency of arterial baroreflex control of MSNA (time delay SAP-MSNA, B) and heart rate (time delay SAP-RRI, D) in patients in the C, MetS − OSA, and MetS + OSA groups. Gain SAP-MSNA and gain SAP-RRI were lower in the MetS − OSA group compared with the C group. MetS + OSA group had further decreases in gain SAP-MSNA and SAP-RRI compared with MetS − OSA and C groups. Time delay SAP-MSNA and SAP-RRI were greater in the MetS + OSA group compared with the MetS − OSA and C groups.

Yet, the levels of MSNA are increased in MetS − OSA in relation to C subject and expressively increased in MetS + OSA subjects. The cross-spectral analysis shows that the MetS − OSA patient had decreased gain of arterial baroreflex control of MSNA compared with C subject, whereas the MetS + OSA patient shows reduced gain of arterial baroreflex control of MSNA compared with the MetS − OSA patient and the C subject.

Fig. 3. Examples of blood pressure, sympathetic neurogram, and cross-spectral analysis [gain of arterial baroreflex control, solid black line; coherence (K^2), dotted black line; shift phase (ϕ), solid gray line; and central frequency of the peak coherence (*) with the respective phase shift and gain of arterial baroreflex control] between MSNA and SAP variability of a C subject (A), a patient with MetS − OSA (B) and a patient with MetS + OSA (C). AHI, apnea-hypopnea index; au, arbitrary units. The variance of blood pressure is expressively increased in the MetS + OSA patient in relation to C subject but not in the MetS − OSA patient.
with reduction of LF oscillations may be dependent on an overexcitation of central respiratory nuclei associated with hyperactivation of chemoreceptors. Since there are direct connections between ventrolateral respiratory neurons and pre-sympathetic neurons in the rostral ventral lateral medulla (26), an increased respiratory drive may cause a leak to these pre-sympathetic neurons, not only increasing the sympathetic outflow (26) but also changing their oscillatory profile. Effects of stimulation of chemoreceptors on sympathetic nerve activity reinforce our hypothesis (27). We cannot discard a possible influence of peripheral stretching pulmonary mechanoreceptors, which via vagal afferent neurons can affect the pre-sympathetic neurons in the medulla (18).

In conclusion, MetS decreases the oscillatory pattern of MSNA and the magnitude of the ABR control of MSNA and HR. In addition, OSA exacerbates these autonomic dysfunctions and further increases the time delay of the baroreflex response of MSNA and HR in these patients.

Perspective. The changes in oscillatory profile of MSNA may represent new markers of cardiovascular risk in patients suffering of MetS and OSA. Similarly to what have been reported in patients with chronic heart failure (39), patients with MetS and OSA have impaired modulation of MSNA.

REFERENCES


AUTHOR CONTRIBUTIONS


DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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