Original Article
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Obesity

Diet and Exercise Improve Chemoreflex Sensitivity in Patients with Metabolic Syndrome and Obstructive Sleep Apnea

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Objective: Chemoreflex hypersensitity was caused by obstructive sleep apnea (OSA) in patients with metabolic syndrome (MetS). This study tested the hypothesis that hypocaloric diet and exercise training (D+ET) would improve peripheral and central chemoreflex sensitivity in patients with MetS and OSA.
Methods: Patients were assigned to: (1) D+ET ($n = 16$) and (2) no intervention control (C, $n = 8$). Minute ventilation (VE, pre-calibrated pneumotachograph) and muscle sympathetic nerve activity (MSNA, microneurography) were evaluated during peripheral chemoreflex sensitivity by inhalation of 10% O_2 and 90% N_2 with CO_2 titrated and central chemoreflex by 7% CO_2 and 93% O_2 for 3 min at study entry and after 4 months.
Results: Peak VO_2 was increased by D+ET; body weight, waist circumference, glucose levels, systolic/diastolic blood pressure, and apnea–hypopnea index (AHI) (34 ± 5.1 vs. 18 ± 3.2 events/h, $P = 0.04$) were reduced by D+ET. MSNA was reduced by D+ET at rest and in response to hypoxia (8.6 ± 1.2 vs. 5.4 ± 0.6 bursts/min, $P = 0.02$), and VE in response to hypercapnia (14.8 ± 3.9 vs. 9.1 ± 1.2 l/min, $P = 0.02$). No changes were found in the C group. A positive correlation was found between AHI and MSNA absolute changes ($R = 0.51$, $P = 0.01$) and body weight and AHI absolute changes ($R = 0.69$, $P < 0.001$).
Conclusions: Sympathetic peripheral and ventilatory central chemoreflex sensitivity was improved by D+ET in MetS+OSA patients, which may be associated with improvement in sleep pattern.

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Introduction

Obstructive sleep apnea (OSA) is a common condition characterized by recurrent episodes of partial (hypopnea) or complete (apnea) obstruction of the upper airway, intermittent hypoxia, and frequent arousal from sleep (1,2). Consistent evidence pointed to a positive correlation between OSA and cardiovascular events (3-5).

Metabolic syndrome (MetS) is associated with increased cardiovascular risk (6). Growing evidence suggests that OSA is common and more than an epiphenomena in patients with MetS (7-13). As both conditions are tightly linked to obesity, it is possible that MetS and OSA share the same pathophysiological mechanisms involved in cardiovascular diseases.

In a recent study, we found that OSA causes chemoreflex hypersensitivity in patients with MetS (14). During hypoxia, patients with MetS and OSA have increased sympathetic nerve activity response compared with patients with MetS without OSA. Similarly, patients with MetS and OSA have increased sympathetic nerve activity and ventilation in response to hypercapnia, compared with healthy control subjects. These alterations in peripheral and central chemoreflex sensitivity seem to contribute to the sympathetic hyperactivation in patients with MetS and OSA.

The previous studies have consistently shown that changes in lifestyle by means of a hypocaloric diet (D) and exercise training (ET) reduce obesity, high blood pressure (BP), and diabetes (15-18). This nonpharmacological treatment also improves OSA severity (19). Interventional studies demonstrated that a 10% reduction in body weight is associated with a 30% reduction in apnea–hypopnea index (AHI) (20,21). However, the mechanisms explaining the effects of D+ET in patients with MetS and comorbid OSA are unknown.

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We tested the hypothesis that weight loss and increase in physical capacity by means of D and ET would reduce sympathetic peripheral chemoreflex hypersensitivity and ventilatory central chemoreflex hypersensitivity in patients with MetS+OSA. In addition, D and ET would improve OSA severity.

Methods

Study population

Never-treated outpatients with a recent diagnosis of MetS, according to the ATP-III (22), were selected for this study. Patients with body mass index (BMI) of >35 kg/m^2, orthopedic limitation, and a history of regular alcohol consumption were excluded. In addition, smokers, pregnant women, patients under chronic use of any medication, and those participating in regular exercise or a diet program were not included in the study. During the clinical examination, each subject underwent three standard BP measurements (the mean BP was used in the analysis) as well as the assessment of body weight and height (BMI calculation) and the measurements of waist circumference. Total serum cholesterol, triglycerides, and high-density lipoprotein cholesterol (HDL-C) (enzymatic method) and plasma glucose (standard glucose oxidase method) concentrations were assayed from a venous blood sample that was taken when the patients were in the fasting state. Patients who met the inclusion criteria underwent standard polysomnography. Patients with MetS who also had OSA (AHI >15 events/h) were assigned to one of the two groups: (1) D and ET as the experimental group (D+ET) and (2) control group (C), receiving no intervention. All participants performed the evaluations before and 4 months after the intervention or control period. It is worth noting that part of the patients included in this study participated in a previous study from our Laboratory (14).

This study was approved by the Scientific Committee of the Heart Institute (#2728/05/148) and Human Subject Protection Committee of the Heart Institute (InCor) and the Ethics Committee of Clinical Hospital (#1222/05), University of São Paulo Medical School, and all participants gave written informed consent.

Measurements and procedures

MetS diagnosis. MetS was diagnosed according to the National Cholesterol Education Program, Adult Treatment Panel III (22) when the patient had at least three out of five risk factor criteria: Waist circumference (≥102 cm in men and ≥88 cm in women); triglycerides (≥150 mg/dl); HDL (<40 mg/dl in men and <50 mg/dl in women); arterial BP (≥130 or 85 mmHg for systolic, and diastolic BP, respectively); fasting glucose ≥100 mg/dl.

Sleep study. All participants underwent a standard overnight polysomnography, (EMBLA—Flaga hf. Medical Devices, Reykjavik, Iceland), which is the "gold standard" for OSA diagnosis as described previously (9,13,14). Apnea was defined as complete cessation of airflow for at least 10 s associated with oxygen desaturation of 3%, and hypopnea was defined as a reduction in respiratory signals for at least 10 s associated with oxygen desaturation of 3% (23). The AHI was calculated as the total number of respiratory events (apneas plus hypopneas) per hour of sleep. OSA was defined by an AHI of ≥15 events/h of sleep because of a high expected prevalence of OSA among patients with MetS (8,9).

Functional capacity measurement. Functional capacity was measured by using a cardiopulmonary maximal exercise test on an electromagnetically braked cycle ergometer (Medifit 400L, Medical Fitness Equipment, Maarn, The Netherlands), using a ramp protocol with workload increments of 10 or 15 W every minute at 60 rpm up to exhaustion. Oxygen uptake (VO_2) and carbon dioxide production were determined by means of gas exchange on a breath-by-breath basis in a computerized system (SensorMedics, Vmax 229 model, BuenaVista, CA). Peak VO_2 was defined as the maximum attained VO_2 at the end of the exercise period in which the subject could no longer maintain the cycle ergometer velocity at 60 rpm. This method is considered the "gold standard" for assessing patients' exercise capacity. Anaerobic threshold was determined to occur at the breakpoint between the increase in the carbon dioxide production and VO_2 (V-slope) or the point at which the ventilator equivalent for oxygen and end-tidal oxygen partial pressure curves reached their respective minimum values and began to rise (24). Respiratory compensation point was determined at the point which the ventilatory equivalent for carbon dioxide was lowest before a systematic increase and when end-tidal carbon dioxide partial pressure reached a maximum and began to decrease (25). Heart rate (EKG) was continuously monitored during exercise testing and for 6 min during the recovery period. BP (auscultatory method) levels were measured every 2 min during the exercise test and at the first, second, fourth, and sixth minute of the recovery period.

Muscle sympathetic nerve activity. During peripheral (hypoxic stimulus) and central (hypercapnia) evaluations, muscle sympathetic nerve activity (MSNA) was recorded directly through a multiunit recording of the post-ganglionic efferent nerve from the muscle fascicle, on the posterior side of the peroneal nerve, immediately inferior to the fibular head, using the microneurography technique (26). This technique has been validated and employed in numerous laboratory studies in humans. Nerve recordings were made using a tungsten microelectrode and grounded by a reference microelectrode placed 1-2 cm away. The electrodes were connected to a pre-amplifier, and the nerve signal was fed through a band-pass filter and led to an amplitude discriminator to be stored in an oscilloscope. Signals were amplified by a factor of 100,000 and band-pass filtered (700-2,000 Hz). For recording and analysis, the neurogram was fed through a resistance–capacitance integrator, and nerve activity was rectified and integrated (time constant, 0.1 s) with acquisition sampling frequency of 500 Hz to obtain a mean voltage display of sympathetic nerve activity that was recorded through a software program (WinDaq Software, Transonic Systems, Akron, OH). The nerve signal was evaluated by measuring bursts per minute.

Other measurements. During peripheral and central evaluations, BP was continuously and noninvasively monitored from an automatic BP cuff, on a per-minute basis (Dixtal 2010, Manaus, Brazil). Heart rate (HR) was monitored continuously through lead II of the EKG. The oxygen saturation (O_2 saturation) was monitored through a pulse oximeter (DX 2405, OXYPLETH, Super Bright, Manaus, Brazil), and the exhaled carbon dioxide (end-tidal CO_2) was monitored with a capnograph (Dixtal, DX 1265 ETCO2 CAPNOGARD, Manaus, Brazil). The minute ventilation (VE) was monitored by pneumotacograph (Hans Rudolph, Kansas City, MO) and a differential pressure transducer linked to a signal integrator. The respiratory

rate (RR) was monitored with a piezoelectric thoracic belt (1132 Pneumotrace II; UFI) placed around the upper abdomen.

Experimental protocol

At pre- and post-4 months of intervention, the patients underwent the chemoreflex evaluation protocols, which occurred between 07:00 and 09:00 with the patients in a supine position in a quiet air-conditioned room (22 °C). Patients were instructed to sleep at least 7 h, to not participate in physical exercise for 24 h prior to the study, and to consume a light meal without caffeine before participating in the protocol. Electrodes and an automatic BP cuff were then placed. After the patients were positioned and the instruments were placed, the patient had his or her leg positioned for microneurography, and a microelectrode was placed in the peroneal nerve and an adequate nerve-recording site was obtained. After a rest period of 15 min, the experimental sequence was 3 min of baseline records followed by 3 min of exposure to isocapnic hypoxia to assess peripheral chemoreflex control. After a new recovery period of at least 15 min to allow patients' biological signals returned to baseline values, we recorded 3 min of baseline followed by hyperoxic hypercapnia to assess central chemoreflex control.

Peripheral chemoreflex evaluation

The purpose of this protocol was to study the hemodynamic, neurovascular, and ventilatory responses to peripheral chemoreflex stimulation in MetS patients with OSA. At least 2 h after a light meal without caffeine, each individual had his or her leg positioned for microneurography, and a microelectrode was placed in the peroneal nerve. Electrodes were then placed to monitor the electrocardiogram, cuffs were placed to monitor BP, and mouthpiece and nasal clip were placed to allow inhaling of gases. MSNA, HR, mean BP, VE, O_2 saturation, end-tidal CO_2, and RR were recorded for 3 min at rest, followed by 3 min when the peripheral chemoreflex control was evaluated by inhalation of a hypoxic gas mixture (10% O_2 and 90% N_2) as described previously (14,27,28).

Central chemoreflex evaluation

The purpose of this protocol was to study the hemodynamic, neurovascular, and ventilatory responses to central chemoreflex stimulation in MetS patients with OSA. MSNA, HR, mean BP, VE, O_2 saturation, end-tidal CO_2, and RR were recorded for 3 min at rest, followed by 3 min when the central chemoreflex control was evaluated by inhalation of the hypercapnic gas mixture (7% CO_2 and 93% O_2) as described previously (14,27,28).

Hypocaloric diet

The patients consumed a diet under the supervision of a nutritionist with a decrease of 500 kcal/day for 4 months. The expected weight loss was about 10% of the initial weight. The caloric intake was determined according to the basal energy requirements estimated using the World Health Organization equation (29), which takes into consideration the sex and age of the individuals. Because of the fact that under normal circumstances physical activity accounts for 15-30% of a person's total daily energy expenditure, the basal metabolic rate must be multiplied by 1.15 or 1.3 to calculate the daily energy expenditure (30). On alternate weeks, every patient visited the clinical nutritionist for a regular checkup. On each visit, the subjects were weighed and encouraged to record their intake to ensure adherence to the dietary protocol.

Exercise training

ET consisted of three 60-min exercise sessions three times per week for 4 months. Each exercise session consisted of 5 min of warm-up/stretching exercises, 40 min of aerobic cycling exercise, and 15 min of strengthening exercises. The aerobic exercise intensity was established by HR levels that corresponded to the anaerobic threshold up to the respiratory compensation point, obtained in a progressive cardiopulmonary exercise test (25,31,32).

Statistical analysis

The data are presented as mean ± SEM. Physical characteristics, MetS diagnostic criteria, and polysomnography data comparisons within-group and between MetS+OSA groups (D+ET and C, pre- vs. post-intervention periods) were the subjects of two-way analysis of variance with repeated measures (two-way ANOVA). A χ^2 (chi-square) test was used to assess gender distribution differences. Two-way ANOVA with repeated measures was also performed to test possible differences within-groups and between-groups and phases (pre- and post-period) and responses (absolute changes) to the assessment of peripheral (isocapnic hypoxia) and central (hyperoxic hypercapnia) chemoreflex responses. When significance was found, *post hoc Scheffé* comparisons were performed. Probability values of $P \leq 0.05$ were considered statistically significant.

Results

Baseline measures

Physical characteristics, MetS diagnostic criteria, BP levels, and polysomnography data in both D+ET and C groups are summarized in Table 1. There were no differences between groups in gender, weight, BMI, and peak VO_2. The patients with D+ET were older compared to C-group patients. Waist circumference, blood measures, BP levels, and polysomnography data were not different between groups. In addition, MSNA was similar between groups (Table 2).

Hemodynamic and ventilatory responses during peripheral and central chemoreceptors stimulation were similar between patients selected to D+ET and C groups (Table 2). Similarly, MSNA responses were not different between groups.

Effects of exercise training and hypocaloric diet

D+ET significantly decreased body weight, BMI, waist circumference, and fasting glucose levels. In addition, D+ET decreased systolic and diastolic BP and significantly increased peak VO_2 (Table 1). D+ET did not change triglycerides and HDL–C levels (Table 1). No significant changes were found in the C group. In contrast to C condition, D+ET reduced MSNA (Table 2). Interestingly, the number of patients with the diagnosis of MetS significantly declined in the D+ET group (Figure 1). No changes were found in the C group. In regard to OSA severity, D+ET significantly reduced AHI (Table 1) and tended to increase minimal O_2 saturation ($P = 0.07$, Table 1). D+ET caused no changes in total sleep time, sleep efficiency, or

TABLE 1 Physical characteristics, metabolic syndrome measures, and polysomnography data pre- and post-hypocaloric diet and exercise training or control period in metabolic syndrome patients with obstructive sleep apnea

	D+ET ($n = 16$)		C ($n = 8$)	
	Pre	Post	Pre	Post
Physical characteristics				
Age (years)	53 ± 1.7[a]	–	42 ± 2.6	–
Gender (M/F)	11/5	–	6/2	–
Weight (kg)	87 ± 2.0	82 ± 1.8[b]	90 ± 5.3	91 ± 6.0
BMI (kg/m^2)	32 ± 0.7	30 ± 0.6[b]	32 ± 1.3	32 ± 1.6
Peak VO_2 (ml/kg/min)	23.5 ± 1.9	28.2 ± 1.5[b]	24.2 ± 3.0	25.9 ± 1.9
Metabolic syndrome – absolute values and percentages of each positive criterion				
Waist circumference (cm)	106 ± 1.7	100 ± 1.3[b]	106 ± 3.5	108 ± 2.9
(%)	94	50	94	94
Triglycerides (mg/dl)	165 ± 18.2	136 ± 12.7	148 ± 22.0	155 ± 26.1
(%)	56	44	50	50
HDL-C (mg/dl)	44 ± 2.3	46 ± 2.7	42 ± 3.5	42 ± 4.5
(%)	44	38	50	63
Glucose (mg/dl)	109 ± 3.0	101 ± 2.8[b]	100 ± 5.4	102 ± 4.5
(%)	81	56	38	38
Systolic BP (mmHg)	136 ± 4.7	125 ± 3.3[b]	129 ± 4.7	129 ± 5.3
(%)	75	44	63	63
Diastolic BP (mmHg)	93 ± 2.9	84 ± 2.3[b]	93 ± 2.3	88 ± 5.0
(%)	75	56	100	63
Polysomnography data				
TST (min)	403 ± 12.8	411 ± 10.7	424 ± 21.3	411 ± 17.9
Sleep efficiency (%)	87 ± 1.6	89 ± 1.5	93 ± 2.3	82 ± 4.1
S2 (%)	62 ± 1.1	60 ± 3.3	63 ± 4.9	59 ± 7.4
S3 (%)	11 ± 1.6	14 ± 2.2	12 ± 3.3	15 ± 3.6
REM (%)	20 ± 1.7	20 ± 1.6	18 ± 3.0	17 ± 2.3
AHI (events/h)	34 ± 5.1	18 ± 3.2[b]	36 ± 10.1	47 ± 12.4
Minimum O_2 saturation (%)	80 ± 2.0	84 ± 1.7	82 ± 2.2	79 ± 2.6
Arousal index (events/h)	21 ± 2.8	17 ± 3.2	12 ± 2.3	34 ± 10.3

Values are mean ± SE.
Abbreviations: AHI, apnea-hypopnea index; BMI, body mass index; BP, blood pressure; C, control; D+ET, hypocaloric diet and exercise training; HDL-C, high-density lipoprotein cholesterol; peak VO_2, maximal oxygen consumption; REM, rapid eye movements; *S2* and *S3*, stages 2 and 3 of sleep; TST, total sleep time.
[a]$P<0.05$ vs. C.
[b]$P<0.05$ vs. pre-intervention.

proportional sleep stage levels (Table 1). No significant changes in sleep pattern were found in the C group.

D+ET significantly reduced sympathetic peripheral chemoreflex response (MSNA absolute change, interaction, $P = 0.02$) during isocapnic hypoxia (Table 2 and Figure 2A). No changes were found in the C patients. Ventilatory peripheral chemoreflex responses (minute ventilation absolute change) during isocapnic hypoxia were unchanged in D+ET and C groups (Figure 2C). Similarly, HR and mean BP, RR, and oxygen desaturation and end-tidal CO_2 during isocapnic hypoxia stimulation did not change in D+ET and C groups (Table 2).

D+ET caused no changes in sympathetic central chemoreflex responses (Figure 2B). D+ET significantly reduced ventilatory central chemoreflex response ($P = 0.02$, Figure 2D). No changes were found in the C group. HR, mean BP, RR, O_2 saturation, and end-tidal CO_2 responses during hyperoxic hypercapnia stimulation were unchanged in D+ET and C groups (Table 2).

Further analysis showed a significant association between AHI absolute change and MSNA absolute change ($R = 0.51$, $P = 0.01$, Figure 3A). Additionally, body weight absolute change was associated with AHI absolute change ($R = 0.69$, $P < 0.001$, Figure 3B).

Discussion

In a previous study (14), we found that the exposure to isocapnic hypoxia, which preferentially stimulates the peripheral chemoreceptors, provoked a greater increase in MSNA in patients with MetS and OSA than in patients with MetS without OSA. On the other hand, the MSNA responses during exposure to hyperoxic hypercapnia, which

TABLE 2 Peripheral and central chemoreflex responses pre- and post-hypocaloric diet and exercise training or control period in metabolic syndrome patients with obstructive sleep apnea

			Isocapnic hypoxia		Hyperoxic hypercapnia	
			Baseline	Peak	Baseline	Peak
HR (beats/min)	D+ET	Pre	70 ± 2.5	81 ± 3.1[a]	68 ± 2.7	74 ± 3.0[a]
		Post	69 ± 2.7	82 ± 3.5[a]	66 ± 2.7	70 ± 2.4[a]
	C	Pre	71 ± 3.6	82 ± 3.5[a]	70 ± 3.1	74 ± 3.8[a]
		Post	69 ± 2.5	85 ± 2.1[a]	67 ± 2.3	70 ± 3.1[a]
MBP (mmHg)	D+ET	Pre	108 ± 3.0	119 ± 3.3[a]	107 ± 3.1	120 ± 2.9[a]
		Post	107 ± 2.7	118 ± 4.4[a]	106 ± 3.0	116 ± 3.1[a]
	C	Pre	115 ± 5.2	124 ± 6.2[a]	112 ± 3.5	119 ± 4.1[a]
		Post	113 ± 4.6	120 ± 3.9[a]	116 ± 3.7	132 ± 5.0[a]
MSNA (bursts/min)	D+ET	Pre	32 ± 1.4	41 ± 2.0[a]	33 ± 1.7	39 ± 1.9[a]
		Post	25 ± 1.2[a,b]	31 ± 1.2[a,b]	25 ± 1.3[b]	31 ± 1.1[a,b]
	C	Pre	33 ± 1.7	43 ± 2.9[a]	33 ± 2.6	39 ± 3.1[a]
		Post	34 ± 1.2	45 ± 2.2[a]	31 ± 2.5	37 ± 2.9[a]
VE (l/min)	D+ET	Pre	9 ± 0.5	14 ± 0.9[a]	9 ± 0.5	21 ± 1.9[a]
		Post	8 ± 0.3	13 ± 0.7[a]	8 ± 0.3	17 ± 1.3[a]
	C	Pre	9 ± 0.3	14 ± 1.4[a]	9 ± 0.5	19 ± 2.1[a]
		Post	10 ± 1.3	15 ± 1.8[a]	9 ± 1.1	24 ± 4.2[a]
RR (breaths/min)	D+ET	Pre	15 ± 1.0	17 ± 1.2[a]	14 ± 1.0	18 ± 1.1[a]
		Post	14 ± 1.0	16 ± 1.1[a]	15 ± 1.0	18 ± 0.8[a]
	C	Pre	13 ± 1.8	14 ± 2.1[a]	12 ± 1.7	13 ± 1.7[a]
		Post	14 ± 1.6	15 ± 1.6[a]	13 ± 1.2	16 ± 1.6[a]
O_2 saturation (%)	D+ET	Pre	97 ± 0.3	88 ± 1.5[a]	97 ± 0.2	99 ± 0.2[a]
		Post	97 ± 0.2	84 ± 1.1[a]	97 ± 0.3	99 ± 0.1[a]
	C	Pre	97 ± 0.3	87 ± 2.0[a]	98 ± 0.2	100 ± 0.3[a]
		Post	97 ± 0.3	83 ± 1.5[a]	97 ± 0.3	100 ± 0.2[a]
End-tidal CO_2 (mmHg)	D+ET	Pre	37 ± 0.9	37 ± 0.9	37 ± 0.9	50 ± 0.7[a]
		Post	38 ± 0.9	38 ± 1.0	36 ± 1.0	50 ± 0.8[a]
	C	Pre	40 ± 1.3	40 ± 1.6	40 ± 1.6	52 ± 1.2[a]
		Post	39 ± 2.0	39 ± 1.8	39 ± 1.5	54 ± 1.7[a]

Values are mean ± SEM
Abbreviations: C, control; D+ET, hypocaloric diet and exercise training; HR, heart rate; MBP, mean blood pressure; MSNA, muscle sympathetic nerve activity; RR, respiratory rate; VE, minute ventilation.
[a] $P < 0.05$ vs. baseline.
[b] $P < 0.05$ vs. pre-intervention.

preferentially stimulate the central chemoreceptors, were not different between patients with MetS+OSA and patients with MetS alone. We also found that patients with MetS associated with OSA had increased ventilatory central chemoreflex response, but preserved ventilatory peripheral chemoreflex response. These findings lead us to raise the question that D and ET would improve sympathetic peripheral chemoreflex sensitivity and ventilatory central chemoreflex sensitivity in patients with MetS and OSA. In fact, we found that: (1) D+ET improved sympathetic peripheral chemoreflex sensitivity and ventilatory central chemoreflex hypersensitivity in patients with MetS+OSA and (2) D+ET improved OSA severity in patients with MetS+OSA.

Our study provides no information regarding the mechanisms by which D and ET improves sympathetic peripheral chemoreflex control in patients with MetS+OSA. However, someone may speculate that the improvement in this reflex control is associated with amelioration in sleep disorder. The decrease in AHI and the increase in minimal oxygen desaturation attenuate the compensatory mechanism stimulated by hypoxemia, which in turns reduces the chronic sympathetic peripheral chemoreflex hypersensitization. We cannot rule out the possibility that an improvement in arterial baroreflex control contributes to the improvement in sympathetic chemoreflex control as an interaction between baroreceptor and chemoreceptor reflexes has been described in animals (33,34).

Why there was no change in sympathetic central chemoreflex sensitivity after D and ET? There is no definitive answer for this question. However, it is possible that a longer period of intervention is necessary to reduce sympathetic central chemoreflex hypersensitivity. Despite the significant reduction in body weight and BMI, our patients remained in the overweight range. Similarly, despite the substantial

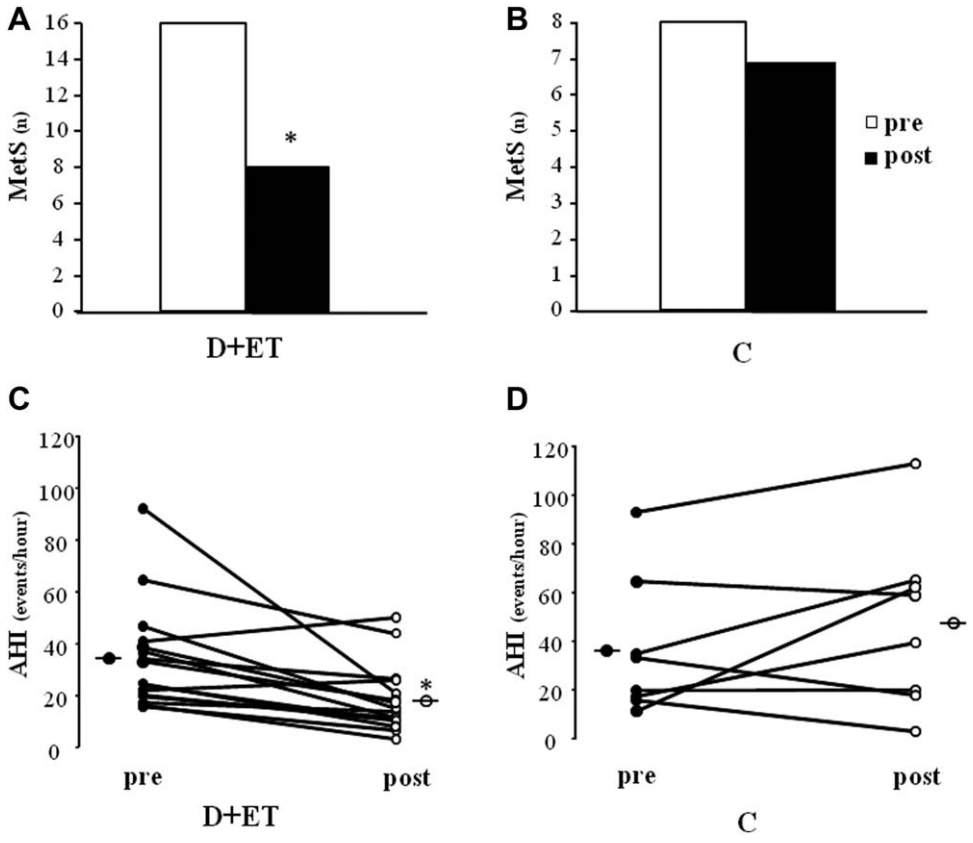

Figure 1 Number of patients with a diagnosis of metabolic syndrome (MetS) pre- and post-hypocaloric diet and exercise training (D+ET, **Panel A**) and control period (C, **Panel B**). Individual apnea/hypopnea index at pre- and post-D+ET (**Panel C**) and **C** (**Panel D**) in patients with MetS and obstructive sleep apnea. *P<0.05 vs. pre.

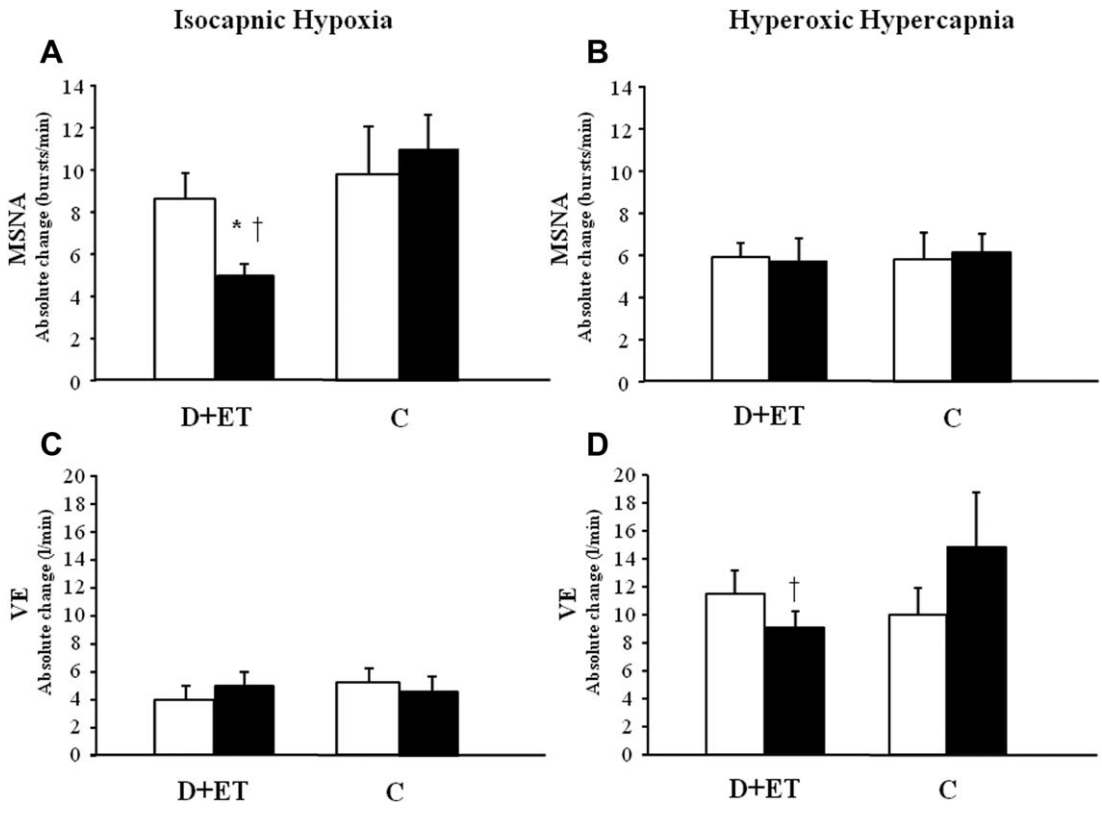

Figure 2 Muscle sympathetic nerve activity (MSNA) response and minute ventilation (VE) during isocapnic hypoxia (**Panels A**,**C**) and during hyperoxic hypercapnia (**Panels B**,**D**) in metabolic syndrome patients with obstructive sleep apnea at pre- and post-diet and exercise training (D+ET) or control (C) period. *$P \leq$ 0.05 vs. pre; † P<0.05 vs. C.

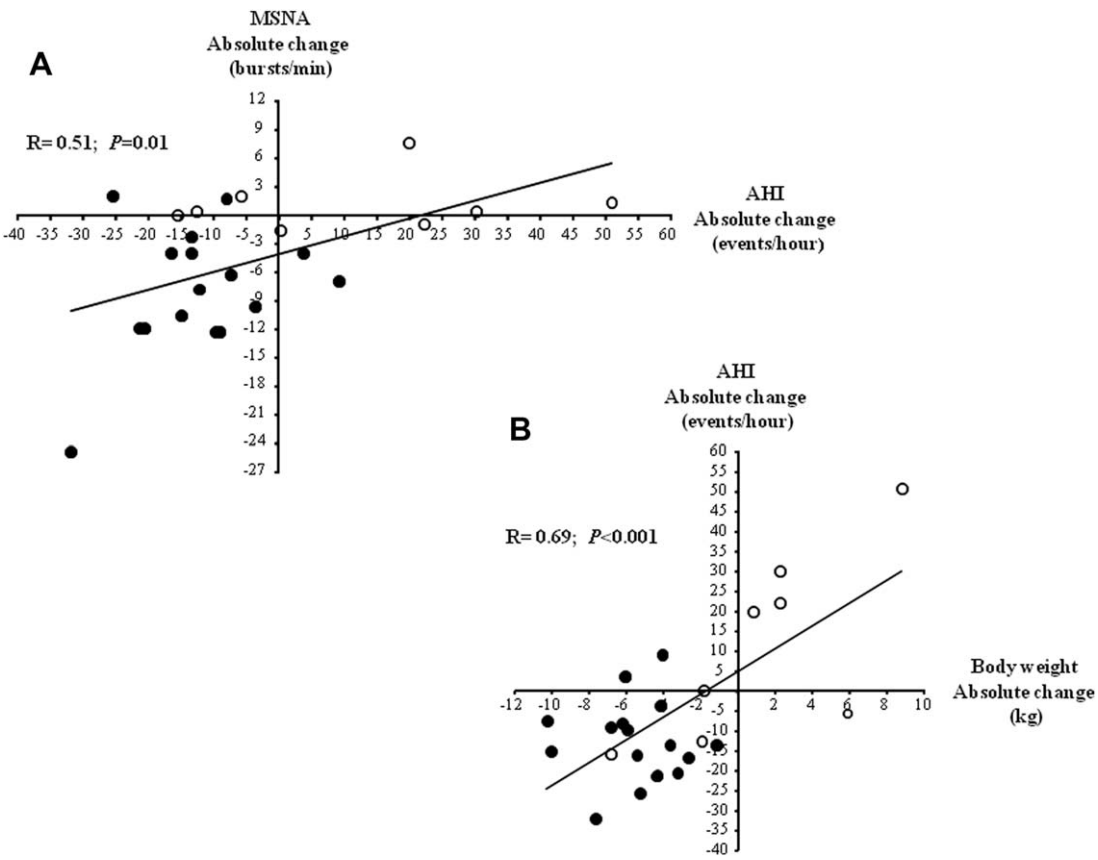

Figure 3 Correlation coefficient between apnea/hypopnea index (AHI) absolute change (post- minus pre-hypocaloric diet and exercise training and control period) and MSNA absolute change (**Panel A**). Correlation coefficient between body weight absolute change per AHI absolute change **(Panel B)**.

amelioration in sleep pattern, our patients remained in the moderate OSA range. Finally, an improvement in sympathetic central chemoreflex sensitivity should not be expected as the previous study (14) demonstrated no differences in this reflex control sympathetic central chemoreflex sensitivity between the patients with MetS+OSA and MetS−OSA, whereas MetS−OSA was similar to that in healthy controls. The same idea should be applied to ventilatory peripheral chemoreflex sensitivity. As no differences in ventilatory peripheral chemoreflex sensitivity among MetS+OSA, MetS−OSA, and healthy controls were found, there is no reason to expect improvement in this autonomic reflex control after D and ET.

Augmented sympathetic nerve activity in patients with MetS has been demonstrated previously. Grassi et al. (35) elegantly showed that patients with MetS had MSNA greater than that in healthy individuals. Recently, other investigators, taking into consideration the presence of occult sleep disorders, reported that OSA causes a further neural burden in patients with MetS (12,13). Indeed, we recently found that a greater sympathetic hyperactivation caused by the overlap of OSA over MetS could explain, at least partially, the impaired postexercise sympatho-vagal balance in patients with both conditions (36). Thus, the reduction in MSNA not only at rest, but also during exposure to isocapnic hypoxia in patients with MetS+OSA provides a clear clinical meaning to our study.

The previous findings (14) suggest a linkage between sympathetic chemoreflex control and sympathetic nerve activity. After D and ET, MSNA was significantly decreased in patients with MetS+OSA in whom sympathetic chemoreflex control was substantially improved. Of course, the modulation of sympathetic nerve activity is a complex issue and, hence, its reduction cannot be attributed only to the improvement in chemoreflex control. In this regard, our study provides another interesting finding. The improvement in sleep disordered breathing after D and ET might have contributed to the reduction in sympathetic outflow in our patients. There is strong evidence that the heightened sympathetic drive, as a consequence of recurrent hypoxia and sleep fragmentation, persists during the awake period (37). Taken together, these findings suggest that the reduction in sympathetic nerve activity in patients with MetS+OSA depends on the improvement in OSA severity and the reduction in chemoreflex hypersensitivity. In fact, D+ET may decrease MSNA in MetS besides improvements on OSA. Indeed, we cannot rule out the possibility that the improvement of insulin sensitivity and decreased waist circumference are involved in the decrease in MSNA. However, in a previous study we found that only patients with MetS+OSA presented chemoreflex-mediated sympathetic outflow (14). In addition, the association between AHI and MSNA changes in the present study reinforced the notion that chemoreflex is an important mechanism to explain the increase in sympathetic nerve activity in MetS+OSA patients.

In addition to the reduction in MSNA, the short period of lifestyle modification increased functional capacity, decreased body weight, waist circumference, glucose levels and BP, and improved sleep disorders in patients with MetS+OSA. These findings have clinical implications. Obesity and high BP are leading risk factors for cardiovascular disease, and OSA seems to increase this risk (7,38).

Our study has some strengths and limitations to be addressed. The strengths include the use of full polysomnography to diagnose OSA and the careful selection of MetS patients who were not receiving any medication. Several limitations should be acknowledged. First, this is not a randomized study. However, sleep studies were performed in a blinded fashion. Second, we found differences in age between patients who underwent D+ET and patients in the C group. Third, despite the substantial improvement in OSA, D and ET did not normalize OSA severity. Thus, we cannot rule out the possibility that a longer period of D and ET might provide even greater benefits in patients with MetS+OSA. Fourth, the present results may not be extrapolated to patients with severe obesity. Finally, we did not evaluate D+ET combined to continuous positive airway pressure (CPAP). A recent study of patients with obesity and moderate to severe OSA showed that the combination of weight loss and CPAP provided an incremental reduction in insulin resistance, serum triglyceride levels, and BP as compared with either intervention alone (39).

Conclusion

In conclusion, the combination of D and ET promotes weight loss and reduction in OSA severity in patients with MetS+OSA. These changes occurred in parallel to a significant reduction in sympathetic peripheral chemoreflex hypersensitivity and ventilatory central chemoreflex hypersensitivity. Therefore, D and ET should be encouraged for these patients as standard OSA treatment with CPAP is very efficient to abolish respiratory events but may promote weight gain (40). O

References

1. Bradley TD, Floras JS. Sleep apnea and heart failure part I: obstructive sleep apnea. *Circulation* 2003;107:1671-1678.
2. Sleep-related breathing disorders in adults: recommendations for syndrome definition and measurement techniques in clinical research. *Sleep* 1999;22:667-689. The Report of an American Academy of Sleep Medicine Task Force.
3. Marin JM, Carrizo SJ, Vicente E, Agusti AG. Long-term cardiovascular outcomes in men with obstructive sleep apnoea-hypopnoea with or without treatment with continuous positive airway pressure: an observational study. *Lancet* 2005;365:1046-1053.
4. Young T, Finn L, Peppard PE, Szklo-Coxe M, Austin D, Nieto FJ, et al. Sleep disordered breathing and mortality: eighteen-year follow-up of the Wisconsin sleep cohort. *Sleep* 2008;31:1071–1078.
5. Campos-Rodriguez F, Martinez-Garcia MA, de la Cruz-Moron I, Almeida-Gonzalez C, Catalan-Serra P, Montserrat JM. Cardiovascular mortality in women with obstructive sleep apnea with or without continuous positive airway pressure treatment: a cohort study. *Ann Intern Med* 2012;156:115-122.
6. Gami AS, Witt BJ, Howard DE, Erwin PJ, Gami LA, Somers VK, et al. Metabolic syndrome and risk of incident cardiovascular events and death: a systematic review and meta-analysis of longitudinal studies. *J Am Coll Cardiol* 2007;49:403-414.
7. Drager LF, Togeiro SM, Polotsky VY, Lorenzi-Filho G. Obstructive sleep apnea: a cardiometabolic risk in obesity and the metabolic syndrome. *J Am Coll Cardiol* 2013;62:569-576.
8. Drager LF, Bortolotto LA, Maki-Nunes C, Trombetta IC, Alves MJ, Fraga RF, et al. The incremental role of obstructive sleep apnoea on markers of atherosclerosis in patients with metabolic syndrome. *Atherosclerosis* 2010;208:490-495.
9. Drager LF, Lopes HF, Maki-Nunes C, Trombetta IC, Toschi-Dias E, Alves MJ, et al. The impact of obstructive sleep apnea on metabolic and inflammatory markers in consecutive patients with metabolic syndrome. *PLoS One* 2010;5:e12065.
10. Venkateswaran S, Shankar P. The prevalence of syndrome Z (the interaction of obstructive sleep apnoea with the metabolic syndrome) in a teaching hospital in Singapore. *Postgrad Med J* 2007;83:329-331.
11. Nock NL, Li L, Larkin EK, Patel SR, Redline S. Empirical evidence for "syndrome Z": a hierarchical 5-factor model of the metabolic syndrome incorporating sleep disturbance measures. *Sleep* 2009;32:615-622.
12. Grassi G, Seravalle G, Quarti-Trevano F, Mineo C, Lonati L, Facchetti R, et al. Reinforcement of the adrenergic overdrive in the metabolic syndrome complicated by obstructive sleep apnea. *J Hypertens* 2010;28:1313-1320.
13. Trombetta IC, Somers VK, Maki-Nunes C, Drager LF, Toschi-Dias E, Alves MJ, et al. Consequences of comorbid sleep apnea in the metabolic syndrome—implications for cardiovascular risk. *Sleep* 2010;33:1193–1199.
14. Trombetta IC, Maki-Nunes C, Toschi-Dias E, Alves MJ, Rondon MU, Cepeda FX, et al. Obstructive sleep apnea is associated with increased chemoreflex sensitivity in patients with metabolic syndrome. *Sleep* 2013;36:41-49.
15. Trombetta IC, Batalha LT, Rondon MU, Laterza MC, Kuniyoshi FH, Gowdak MM, et al. Weight loss improves neurovascular and muscle metaboreflex control in obesity, *Am J Physiol Heart Circ Physiol* 2003;285:H974-H982.
16. Laterza MC, de Matos LD, Trombetta IC, Braga AM, Roveda F, Alves MJ, et al. Exercise training restores baroreflex sensitivity in never-treated hypertensive patients. *Hypertension* 2007;49:1298-1306.
17. Straznicky NE, Lambert EA, Nestel PJ, McGrane MT, Dawood T, Schlaich MP, et al. Sympathetic neural adaptation to hypocaloric diet with or without exercise training in obese metabolic syndrome subjects. *Diabetes* 2010;59:71-79.
18. Lambert E, Straznicky NE, Dawood T, Ika-Sari C, Grima M, Esler MD, et al. Change in sympathetic nerve firing pattern associated with dietary weight loss in the metabolic syndrome. *Front Physiol* 2011;2:52.
19. Ueno LM, Drager LF, Rodrigues AC, Rondon MU, Braga AM, Mathias W Jr, et al. Effects of exercise training in patients with chronic heart failure and sleep apnea. *Sleep* 2009;32:637-647.
20. Newman AB, Foster G, Givelber R, Nieto FJ, Redline S, Young T. Progression and regression of sleep-disordered breathing with changes in weight: the Sleep Heart Health Study. *Arch Intern Med* 2005;165:2408-2413.
21. Peppard PE, Young T, Palta M, Dempsey J, Skatrud J. Longitudinal study of moderate weight change and sleep-disordered breathing. *JAMA* 2000;284:3015-3021.
22. Grundy SM, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation* 2005;112:e297.
23. American Academy of Sleep Medicine. The AASM Manual for the Scoring of Sleep and Associated Events. Westchester, IL: American Academy of Sleep Medicine; 2007.
24. Beaver WL, Wasserman K, Whipp BJ. A new method for detecting anaerobic threshold by gas exchange. *J Appl Physiol* 1986;60:2020-2027.
25. Wasserman K, Hansen JE, Sue DY, Stringer WW, Whipp BJ. Principles of Exercise Testing and Interpretation, 4th ed. Philadelphia: Williams & Wilkins; 2005.
26. Fagius J, Wallin BG. Long-term variability and reproducibility of resting human muscle nerve sympathetic activity at rest, as reassessed after a decade. *Clin Auton Res* 1993;3:201-205.
27. Somers VK, Mark AL, Zavala DC, Abboud FM. Contrasting effects of hypoxia and hypercapnia on ventilation and sympathetic activity in humans. *J Appl Physiol* 1989;67:2101-2106.
28. Somers VK, Mark AL, Zavala DC, Abboud FM. Influence of ventilation and hypocapnia on sympathetic nerve responses to hypoxia in normal humans. *J Appl Physiol* 1989;67:2095-2100.
29. Report of a WHO Expert Committee. *World Health Organ Tech Rep Ser* 1995;854:1-452. Physical status: the use and interpretation of anthropometry.
30. Energy and Protein Requirements. Report of a joint FAO/WHO/UNU Expert Consultation. *World Health Organ Tech Rep Ser* 1985;724:1-206.
31. Skinner JS, McLellan TM. The transition from aerobic to anaerobic metabolism. *Res Q Exerc Sport* 1980;51:234-248.
32. American College of Sports Medicine. Guidelines for Exercise Testing and Prescription, 6th ed. Philadelphia: Lippincott Willians & Wilkins; 2000.
33. Heistad DD, Abboud FM, Mark AL, Schmid PG. Interaction of baroreceptor and chemoreceptor reflexes. Modulation of the chemoreceptor reflex by changes in baroreceptor activity. *J Clin Invest* 1974;53:1226-1236.

34. Brunner MJ, Wallace A, MacAnespie CL. Interaction of carotid chemoreceptor and baroreceptor reflexes in anesthetized dogs. *Am J Physiol* 1988;254:R1-R10.

35. Grassi G, Dell'Oro R, Quarti-Trevano F, Scopelliti F, Seravalle G, Paleari F, et al. Neuroadrenergic and reflex abnormalities in patients with metabolic syndrome. *Diabetologia* 2005;48:1359-1365.

36. Cepeda FX, Toschi-Dias E, Maki-Nunes C, Rondon MUPB, Alves MJNN, Braga AMFW, et al. Obstructive sleep apnea impairs postexercise sympatho-vagal balance in patients with metabolic syndrome. *Sleep* 2014 Dec 20. pii: sp-00376-14. [Epub ahead of print]

37. Somers VK, Dyken ME, Clary MP, Abboud FM. Sympathetic neural mechanisms in obstructive sleep apnea. *J Clin Invest* 1995;96:1897-1904.

38. Wolk R, Shamsuzzaman AS, Somers VK. Obesity, sleep apnea, and hypertension. *Hypertension* 2003;42:1067-1074.

39. Chirinos JA, Gurubhagavatula I, Teff K, Rader DJ, Wadden TA, Townsend R, et al. CPAP, weight loss, or both for obstructive sleep apnea. *N Engl J Med* 2014;370:2265-2275.

40. Drager LF, Brunoni AR, Jenner R, Lorenzi-Filho G, Benseñor IM, Lotufo PA. Effects of CPAP on body weight in patients with obstructive sleep apnoea: a meta-analysis of randomised trials. *Thorax* 2015;70:258-264.