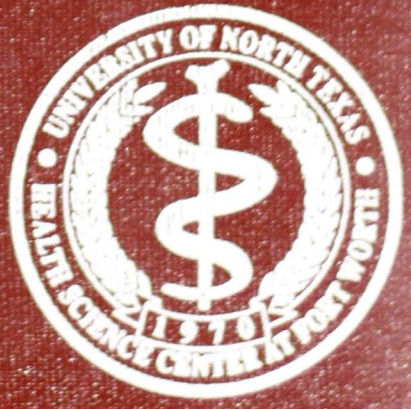
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Hardisty, Janelle M., Mechanisms of Chemoreflex Control of Muscle Sympathetic Nerve Activity and Blood Pressure in Humans. Doctor of Philosophy (Integrative Physiology), May 2004.

The mechanisms linking obstructive sleep apnea (OSA) and cardiovascular disease are not fully understood; however, studies report patients with OSA exhibit chronic elevations in muscle sympathetic nerve activity (MSNA). This appears to be due to altered chemoreflex control of MSNA, mediated primarily by hypoxia. Yet, a correlation between degree of hypoxia and chemoreflex control of MSNA is unknown. Therefore, it was evaluated whether degree of hypoxia occurring during apnea determines the sympathoexcitatory and blood pressure responses, and whether these responses are augmented in OSA patients. Additionally, it was studied whether altered chemoreflex function in OSA patients is predictive of blood pressure response to apnea. In a clinical setting, the blood pressure response to voluntary apnea was determined to evaluate whether this could be used as a non-invasive measure of chemoreflex gain in OSA. Finally, the effect of hyperoxia on MSNA was studied to determine whether 15 min of hyperoxia, following intermittent hypoxic apnea, reverses the elevation of MSNA and altered chemoreflex control of MSNA.

Consistent with the hypotheses, a relationship between MSNA response, blood pressure response and level of hypoxia were determined. MSNA and peak systolic pressure responses were augmented in OSA subjects ($p \leq 0.05$ and $p \leq 0.05$, respectively),

as well as, chemoreflex gain ($p \leq 0.05$). Clinically, peak systolic pressure responses to apnea were augmented in OSA patients ($p < 0.001$). Finally, basal MSNA and chemoreflex control of MSNA, following hyperoxia, was not different from baseline through 180 min of recovery ($p = 0.940$ and $p = 0.278$, respectively).

These data support the hypotheses that chemoreflex gain is predictive of the blood pressure response; and furthermore, the MSNA and blood pressure responses to hypoxic apnea are augmented in OSA. Additionally, peak systolic pressure responses to voluntary apnea are augmented in OSA patients and could possibly be used as a marker of chemoreflex gain. Moreover, these data support the hypothesis that hyperoxia can reverse basal sympathoexcitation and augmented chemoreflex control of MSNA, associated with hypoxic apnea, supporting the hypothesis that elevations in MSNA are hypoxia mediated.

MECHANISMS OF CHEMOREFLEX CONTROL OF

MUSCLE SYMPATHETIC NERVE ACTIVITY

AND BLOOD PRESSURE IN HUMANS

Janelle M. Hardisty, B.S.

APPROVED:

Michael L. Smith

Major Professor

Committee Member

John B Buck MD

Committee Member

Committee Member

University Member

Michael L. Smith

Chair, Department of Integrative Physiology

Thomas Yoo

Dean, Graduate School of Biomedical Sciences

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DISSERTATION

Presented to the Graduate Council of the

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University of North Texas
Health Science Center at Fort Worth

in Partial Fulfillment of the Requirements

For the Degree of

DOCTOR OF PHILOSOPHY

By

Janelle Moseley Hardisty

Fort Worth, Texas

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Original Manuscripts: Published

Ogoh S, PJ Fadel, JM Hardisty, WL Wasmund, DM Keller, PB Raven, ML Smith. Does pulsatile and sustained neck pressure or neck suction produce differential cardiovascular and sympathetic responses in humans? *Exper Physiol* 88:595-601, 2003.

Original Manuscripts: Submitted/in preparation

Hardisty JM, Cutler MJ, Brothers RM, Burk JR, Smith ML. Hyperoxia reverses sympathoexcitation and chemoreflex response following intermittent hypoxic apnea. *J Appl Physiol.* Submitted 2004.

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CHAPTER 1

INTRODUCTION

The mechanisms which link obstructive sleep apnea (OSA) and cardiovascular disease are not fully understood; however, several studies have shown that patients with OSA have sustained daytime elevation of muscle sympathetic nerve activity (MSNA), increased circulating catecholamines, a high incidence of hypertension, and an increased cardiovascular morbidity (12, 22, 23, 26). During sleep, repetitive episodes of hypoxia, hypercapnia and obstructive apnea act through chemoreceptor reflexes and other mechanisms to increase sympathetic drive (34, 58). Studies by Morgan et al. and Cutler et al. have suggested that hypoxia is the primary stimulus for sustained sympathoexcitation following intermittent hypercapnic hypoxia and intermittent hypoxic apnea, respectively (7, 41). Several studies by Smith et al., demonstrated an altered sensitivity in chemoreflex gain in response to hypoxemia, and that acute treatment of OSA patients with hyperoxia significantly reduces MSNA (57, 58). If altered chemoreflex function following hypoxic apnea is accompanied by a sustained elevation of MSNA, then hyperoxia after a period of apneas could be expected to return MSNA towards baseline. Yet, it is unknown whether hyperoxia can produce an effect on chemoreflex function converse to the effect of apneas (i.e. reduce the sensitivity). Thus, Specific Aims #1a and #1b will address the potential use of hyperoxia to reverse the post-

hypoxic apnea elevation of MSNA and the potential effect of hyperoxia on chemoreflex sensitivity.

Previous studies have shown that chemoreflex control of MSNA is more sensitive in OSA patients (42). This has been shown by 1) a greater increased MSNA response to a given degree of hypoxemia and 2) a greater decrease in MSNA in response to hyperoxia. Smith *et al.* demonstrated that the change in sensitivity to hypoxemia is a function of a shift of the threshold for activation to a less severe hypoxemia; thus, even mild hypoxemia can produce sympathoexcitation (57). However, during apnea, it is proposed but unknown whether the MSNA response can be directly related to chemoreflex gain. Furthermore, it is unknown whether MSNA response during apnea determines the blood pressure response to apnea and, whether these responses are altered in OSA. Specific Aims #2a, #2b and #2c will address these questions. A practical question follows: Could a non-invasive method of assessing sensitivity of chemoreflex control of MSNA be developed that could be obtained easily in the clinical setting? Specific Aim #3 will address this question.

Therefore, the proposed studies will address the following Specific Aims. **Specific Aim #1a:** To determine whether hyperoxia, following intermittent hypoxic apnea, will reverse the elevation of basal MSNA. **Hypothesis #1a:** It was hypothesized that hyperoxia will reverse the elevation of basal MSNA, following 20 min of intermittent hypoxic apnea, to baseline levels throughout the 180 min recovery period.

Specific Aim #1b: To determine whether hyperoxia, following intermittent hypoxic apnea, will reverse augmentation of chemoreflex control of MSNA to apnea.

Hypothesis #1b: It was hypothesized that hyperoxia will reverse the augmentation of chemoreflex control of MSNA to apnea compared to post intermittent hypoxic apnea.

Specific Aim #2a: To determine whether the MSNA response during apnea is directly related to degree of hypoxia; and thus, chemoreflex gain. **Hypothesis #2a:** It was hypothesized that progressive degrees of hypoxic end-expiratory apnea will demonstrate elevations of MSNA which can be directly related to nadir SaO_2; and thus, chemoreflex gain.

Specific Aim #2b: To determine whether the blood pressure response during hypoxic apnea is directly related to MSNA; and thus, chemoreflex gain. **Hypothesis #2b:** It was hypothesized that the hemodynamic response to apnea is a function of elevation of MSNA, and thus, can be used as an index of chemoreflex gain.

Specific Aim # 2c: To determine whether the MSNA and blood pressure responses during hypoxic apnea are augmented in untreated OSA subjects. **Hypothesis #2c:** It was hypothesized that the sympathoexcitatory response and hemodynamic responses to hypoxic apnea will be augmented in untreated OSA subjects as compared to controls.

Specific Aim #3: To assess the blood pressure response to apnea as a marker of MSNA and chemoreflex gain in normal and untreated OSA patients. **Hypothesis #3:** It was hypothesized that peak systolic blood pressure response to a voluntary end-expiratory apnea will be greater in untreated OSA patients than normal controls.

CHAPTER 2

REVIEW OF LITERATURE

Over the past several years, the medical community has become increasingly aware that obstructive sleep apnea (OSA) is a common disorder, which adversely affects both the respiratory and cardiovascular systems. OSA has been reported to be associated with an increased incidence of dysrhythmias, myocardial infarction, stroke and sudden death (1, 11, 22, 56). Furthermore, OSA patients are at an increased risk for hypertension (5, 36, 60), with reports of up to 40-60% of OSA patients having been diagnosed with arterial hypertension (18), while only 20-30% of hypertensive patients have OSA (11). While the long term cardiovascular effects of chronic hypoxia and obstructive apnea are difficult to study in humans, animal models have been a significant source of information. Both rat and canine models have demonstrated that chronic intermittent hypoxia leads to a 10-15 mmHg elevation in resting mean arterial pressure (MAP) (4, 16). Therefore, the combined elevation of daytime sympathetic nerve activity with associated elevation in mean arterial pressure, has lead some investigators to postulate the elevation in MSNA may contribute to the increased incidence of hypertension (5, 36, 60).

Many recent studies have suggested that early recognition and treatment of OSA may improve cardiovascular function, reduce the risk of hypertension, and decrease

elevations in sympathetic nerve activity (18, 72, 75). Since increased MSNA also accompanies most cardiovascular diseases, it is probable that the chronic sympathoexcitation found in OSA contributes to the increased incidence and/or risk of cardiovascular disease found in these patients. Therefore, it is logical that interventions that can produce sustained reductions in MSNA will be beneficial to these patients.

Chemoreflex control of MSNA. The chemoreflexes are important modulators of sympathetic activation. The peripheral chemoreceptors located in the carotid bodies have been shown to respond primarily to hypoxia (20, 62). Whereas, the central chemoreceptors located in the brainstem respond to hypercapnia (41). Peripheral chemoreceptors elicit increases in MSNA, with consequent increases in blood pressure (59, 60), as well as invoking an increase in respiratory rate (19, 27). Increased blood pressure and increased minute ventilation both inhibit the sympathetic response to chemoreflex activation (59, 60). Yet, during apnea, when the inhibitory influence of lung stretch is eliminated, there is a potentiation of the sympathetic response to both hypoxia and hypercapnia. Furthermore, the inhibitory influence of the pulmonary afferents has been shown to be more dramatic on the sympathetic response to peripheral, compared with central, chemoreceptor activation (32). Therefore, the chemoreflexes are an important mechanism for regulation of both ventilation and autonomic cardiovascular function. Abnormalities in chemoreflex mechanisms, acting on MSNA have been implicated in increased incidence and/or risk of cardiovascular disease found in patients with OSA (42, 43).

Acute and Chronic Sympathoexcitation in OSA. Patients with obstructive sleep apnea experience repetitive apneic events during sleep, with resulting hypoxia and hypercapnia (46). It has been demonstrated that hypoxia and hypercapnia, acting via the chemoreflexes, elicit increases in sympathetic nerve activity, with consequent increases in blood pressure (46, 61, 68). The increased sympathetic activity during sleep has been shown to carry over into daytime wakefulness with OSA patients demonstrating higher daytime levels of circulating catecholamines (9, 28, 63) and elevated MSNA when compared to healthy control subjects (5). Studies by Morgan *et al.* and Cutler *et al.* have demonstrated sustained sympathoexcitation following intermittent hypercapnic hypoxia and intermittent hypoxic apnea, respectively, for up to 180 min post-perturbation (7, 41). Thus, there appears to be an extended period of sympathoexcitation beyond the primary stimulus of apnea. Furthermore, several studies have suggested that chemoreflex control of MSNA is exaggerated in OSA patients (8, 42, 45, 57). Nevertheless, a question remains: Why is MSNA chronically elevated in OSA patients? Altered chemoreflex sensitivity is now recognized as the primary stimulus for the *acute* sympathoexcitation *during* apnea; therefore, it has been postulated to contribute to the sustained elevation in OSA patients (42).

Altered Chemoreflex Control of MSNA in OSA. As the potential cardiovascular effects of OSA have been established, much work has been undertaken to determine a causal relationship between OSA and chronic elevation of MSNA. While not completely delineated, chemoreflex control of MSNA has been a prime target of investigation for the observed sympathoexcitation and data from several studies suggest

that chemoreflex control of MSNA is altered in patients with OSA (7, 64, 65). Furthermore, several recent studies have demonstrated that hypoxia, acting primarily at the level of the peripheral chemoreceptors, may be the primary mediator of the augmentation in MSNA (5, 41, 42, 65).

A human model of OSA was recently used by Cutler *et al.* to evaluate the MSNA response during recovery from either 20 min of intermittent hypoxic apnea, hypercapnic hypoxia, or isocapnic hypoxia (7). They determined no difference between the various groups ($p=0.51$) and concluded that hypoxia was the primary mediator for the sympathoexcitation (7). Additionally, Xie et al. compared MSNA during recovery from 20 min of sustained isocapnic hypoxia and normoxic hypercapnia, which demonstrated sustained elevation of MSNA in the hypoxia group only (73). Furthermore, Narckiwicz *et al.* demonstrated that *during* hyperoxia exposure (100% O_2), as compared to room air exposure, untreated OSA patients showed reduced basal MSNA while no change in MSNA was found in healthy controls ($p=0.008$ and $p=0.4$, respectively) (45). These authors postulate that the daytime elevation in MSNA in OSA patients may be explained in part by tonic activation of excitatory chemoreflex afferents (42). While altered chemoreflex sensitivity has been demonstrated in sleep apnea patients, it is unknown whether hyperoxia can reverse the effects chemoreflex control on MSNA past the initial exposure.

Role of Hypoxia in MSNA and MAP response to apnea. Hypoxia has been demonstrated to increase muscle sympathetic nerve activity and numerous studies have reported significant increases in MSNA with exposure to hypoxic gas. (57, 55, 59).

Rowel *et al.* (55) investigated the sympathetic effects of exposure to 8%, 10% and 12% oxygen for approximately 20 min. A significant increase in MSNA occurred during all conditions yet the response was reported sooner during the 8% and 10% oxygen. Additionally, Smith *et al.* (57) investigated the effects of acute hypoxemia, in OSA subjects and controls, whereby subjects breathed 1-4 breaths of hypoxic gas (0-5% oxygen). The degree of hypoxemia was titrated in repeat trials to produce a range of arterial oxygen saturation from 70-95% Sa_{O2}. They reported a 'threshold' for sympathoexcitation which was elevated to higher Sa_{O2} levels in OSA subjects. While these studies examined the effect of hypoxia during spontaneous and controlled breathing (respectively), the sympathoexcitatory effect of apnea superimposed on hypoxia has been shown to be more dramatic.

End-expiratory apnea, similar to what occurs repetitively during sleep in OSA, removes the sympathoinhibitory influence from the lung inflation receptors and may contribute to a greater net sympathoexcitation, yet does hypoxia play a role? Hedner *et al.* (27) reported larger desaturations during apnea produced larger increases in MSNA and postulated that hypoxemia superimposed on apnea was mediating the effect. In addition, Hardy *et al.* and Leuenberger *et al.* (24, 34) reported augmented MSNA and MAP responses with hypoxic apnea, as compared to normoxic apnea. Specifically, Hardy *et al.* investigated MSNA and MAP responses to spontaneous breathing of room air, hypoxic gas (10.5% oxygen) and hyperoxic gas (100% oxygen) (24). In healthy subjects breathing spontaneously, MSNA rose during hypoxemia and decreased with hyperoxia while MAP remained unchanged. Compared to spontaneous breathing,

voluntary apnea was found to show both increased MSNA and MAP. Furthermore, the MSNA and MAP responses to apnea were augmented during hypoxic apnea and attenuated following hyperoxia apnea, as compared to normoxic apnea. These data support a role for apnea, superimposed on hypoxia, mediating augmented sympathoexcitatory and pressor responses. Yet, it is unknown if the sympathoexcitation and arterial pressure during hypoxic apnea can be directly related to degree of hypoxia.

Hypoxia and Hemodynamic Alterations in OSA. Investigators have postulated the daytime elevation in MSNA, and elevation of mean arterial pressure in OSA, may be mediated by several different mechanisms, including: humoral factors (13), reduction in sensory input from baroreceptors (5, 44), apnea-related hypoxia and arousals from sleep (35, 59), and altered arterial chemoreflex activation (8, 45). The complex hemodynamic response to hypoxia involves both direct and reflex effects on the vasculature; whereas hypoxia directly induces vasodilation, peripheral chemoreceptor activation leads to changes in heart rate and elevations in MSNA. (24, 54,) The exact mechanisms for altered blood pressure responses of OSA patients to hypoxic apnea have not been determined, yet several areas of investigation are underway.

Both obstructive apnea in sleep apnea patients and voluntary apnea in healthy controls have been shown to produce transient increases in arterial pressure with associated elevation of MSNA. (14, 15) While, the peak MSNA response to apnea is greatest at end apnea, arterial pressure has been shown to be greatest several seconds after the resumption of breathing (13, 24). It has been postulated that the peripheral vascular response to hypoxia in OSA patients is abnormal, with an augmented blood

pressure response demonstrated in several studies. Hedner *et al.* found that OSA subjects have a greater pressor response to hypoxia than controls, yet did not differ in their HR response (26). Additionally, Remsburg *et al.* reported that exposure to isocapnic hypoxia (Sa_{O2} = 80%) produced a vasodilation (as measured by forearm vascular resistance) in controls, yet was not apparent in OSA subjects (54). These authors postulate an altered vascular response to hypoxia in OSA. Yet, these studies did not directly measure MSNA and could not determine if these altered vascular responses are due to direct or reflex mechanisms. While much work has been done to evaluate altered vascular responses to hypoxia, the relative contribution of end-organ response compared to the contribution of chemoreflex control of MSNA and blood pressure response must still be delineated.

Experimental studies in dogs (4) and rats (14, 17) support the hypothesis that intermittent hypoxia causes persistent hypertension. Using experimental protocols that simulate OSA, these studies reported that hypoxia alone can cause an increase in daytime blood pressure (4, 14, 47). This sustained increase in blood pressure has been postulated to be due to enhanced sympathetic activity (2) and has been shown to be prevented by sympathetic denervation using 6-OH dopamine (14).

Furthermore, some have postulated that the increase in negative intrathorasic pressure during apnea may play a role in raising arterial pressure via redistribution of blood volume. Yet, recent reports by O'Donnell *et al.* (48) and Katragadda *et al.* (33) have demonstrated that the acute pressor response to obstructive apnea is primarily sympathetically mediated and is abolished by autonomic blockade. Additionally, Morgan *et al.* reported that chemoreflex stimulation and resulting sympathetic outflow to skeletal

muscle is more likely to mediate arterial pressure elevations than negative intrathorasic pressure during obstructive apnea (40). These data support the hypothesis that augmented MSNA is partially responsible for mediating the cardiovascular effects of sleep apnea and the subsequent altered blood pressure response to apnea.

Increased sympathetic outflow is directly responsible, at least in part, for the acute blood pressure changes seen in OSA (75), yet the precise mechanism has not been determined. While it has been demonstrated that OSA patients have both altered chemoreflex control of MSNA and altered pressor response to apnea-induced hypoxia, it has not been determined if the pressor response during apnea be correlated to chemoreflex gain?

Altered Chemoreflex Gain in OSA? Chemoreflex gain, as defined by $\Delta MSNA/\Delta Sa_{O2}$, has been found to be augmented in untreated OSA patients. Recently, Smith *et al.* reported the Sa_{O2} threshold for sympathoexcitation is elevated in OSA patients compared to controls (57). Their findings demonstrate that the threshold for sympathetic activation during hypoxemia occurred at higher oxygen saturations in sleep apnea patients (84+1%) than control subjects (76+2%; $p<0.01$). In addition, the gain of sympathetic responses, was greater in the OSA patients ($p<0.05$). These data demonstrate that the sensitivity of chemoreflex control of MSNA is increased in OSA patients, particularly for hypoxic stimuli. This increase in sensitivity is due to both a shift in the threshold to a higher SaO_2 and an increase in the gain. The significance of the shift in threshold is such that sympathoexcitation will occur during mild degrees of oxygen desaturation in OSA patients as compared to controls. The shift in gain has been

postulated to be due to augmented chemoreflex control of MSNA following hypoxic apnea. Additionally, a recent human model of OSA, described by Cutler *et al.*, demonstrated that following 20 min of intermittent hypoxic apnea, chemoreflex control of MSNA during apnea was augmented through 165 min (8). Interestingly, the MAP in these subjects was not different than baseline. While this study was a model of OSA, the chronic effect of intermittent hypoxic apnea on chemoreflex control of MAP can not be determined. Therefore, evaluation of chemoreflex gain must be systematically studied in an untreated population of OSA subjects to determine the relationship between chemoreflex gain and blood pressure response to apnea.

It has been demonstrated that altered chemoreflex control of MSNA occurs with untreated OSA; and furthermore, that augmented sympathoexcitatory response mediates the blood pressure response to apnea in this population. Logically, the question follows; could the blood pressure response to apnea be used as an indicator of chemoreflex gain in obstructive sleep apnea patients?

CHAPTER 3

EXPERIMENTAL DESIGN AND METHODS

Three studies are proposed to address the specific aims. These studies focused on answering the following questions: (1) Does hyperoxia, following intermittent hypoxic apnea, reverse the elevation of basal MSNA and the associated altered sensitivity of chemoreflex control of MSNA to voluntary apnea? (2) Can the relation between MSNA response during apnea be directly correlated to chemoreflex gain, and, if so, can the blood pressure response during apnea be directly related to MSNA? (3) Can the blood pressure response to apnea be used as a marker of MSNA and thus, gain of chemoreflex response? The studies, specific aims and hypotheses are numbered in parallel to address these basic questions.

STUDY 1.

Study 1 addressed Specific Aim #1a and Specific Aim #1b by determining whether hyperoxia after a period of intermittent apneas will: a) reverse the basal elevation of MSNA and b) reverse the increased sensitivity of chemoreflex control of MSNA. It was hypothesized that 15 min of hyperoxia will reverse the elevation of basal MSNA and this reversal was sustained throughout recovery. In addition, sensitivity of chemoreflex control of MSNA, following hypoxic apnea and subsequent hyperoxia treatment, will be returned to baseline. Healthy subjects underwent 20 min of intermittent hypoxia apnea

(as described below) with an immediate post-perturbation exposure to hyperoxia (100% O_2) for 15 min followed by a 180 min recovery period.

Subjects: Eight healthy volunteers free of symptoms or history of OSA were studied (as determined by completion of Epworth Sleepiness scale). After giving written, informed consent, each subject completed a medical history questionnaire prior to participation in the study. All subjects were non-smokers, reported no history of cardiovascular, pulmonary or neurological disease and will not currently using medications other than oral contraception. All female subjects took a urine pregnancy test, to insure they were not pregnant, and were not tested during menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume and cardiovascular function. Subjects were instructed to abstain from vigorous exercise and alcohol for 24 hours and from caffeine for 12 hours prior to the start of the study.

Statistical power: Subject numbers were predicted by calculating an estimated effect size of 0.6 from the Morgan *et al.* (40) and Morgan *et al.* (41) studies. With an estimated effect size of 0.6 and an alpha level of 0.05, it was determined that 8-10 subjects would be needed to obtain acceptable statistical power (>0.8).

Experimental Protocol: These studies were performed in the semi-recumbent position in a laboratory with an ambient temperature of 23-24° C. Subjects were instrumented for measurement of HR, BP, respiratory function, Sa_{O2} and MSNA. Prior to instrumentation, all subjects were allowed to use the restroom. Following instrumentation, 5 min of baseline was recorded while participants breathe room air, while wearing the nasal mask. Prior to starting the treatment, participants performed 2-3

hypoxic apneas (as described below) over a range of oxygen saturation of 85-95%. Participants then performed one 30 s hypoxic apnea every 1 min (simulating an apnea/hypopnea index of 60/hr) for 20 min. During the first 10 s of the hypoxic apnea, participants were primed with one to two breaths of 95-100% nitrogen, followed by a 20 s end-expiratory voluntary apnea (lung volume equal to FRC) such that Sa_{O2} reached 85-95%.

Following the initial 20 min intermittent hypoxic apnea exposure, subjects breathed 15 min of hyperoxic (100% O2) gas. Subjects then recovered while breathing room air for 180 min, without the nasal mask. Every 15 min during recovery participants were instrumented with the nasal mask for 2 min. During this 2 min period, HR, BP, respiratory function, Sa_{O2} and MSNA were measured continuously and subjects performed a single 30 s hypoxic apnea (as described above), sufficient to produce an Sa_{O2} between 85-95%.

Data Analysis: Basal MSNA measurements during baseline and recovery time points reflect average values obtained over a 1 min measurement period and are reported as both bursts/min and total activity/min. Total activity for MSNA was obtained as described previously by Smith et al.(57) The MSNA response to a single 30 s hypoxic apnea represents total MSNA during the apnea minus the basal MSNA (total activity) immediately prior to the apnea. Comparisons of baseline to intermittent hypoxic exposure (1 min post intermittent hypoxic apnea), and recovery (every 15 min) MSNA responses to a single hypoxic apnea were related to the magnitude of the hypoxemia during each hypoxic apnea. Hypoxic apneas were matched to a baseline (i.e. pre-

intermittent 20 min hypoxic apnea exposure) hypoxic apnea with a similar nadir Sa_{O2} ($\pm$2%).

All statistical analyses were performed at a significance level (α) of 0.05. Basal MSNA and chemoreflex sensitivity responses to single hypoxic apneas were analyzed using one-way analysis of variance with repeated measures. Where F values revealed differences, *post hoc* analysis was performed by pairwise comparison using the Student-Newman-Keuls method. All data are expressed as means $\pm$ SE.

Expected Results: Consistent with hypothesis #1a and #1b; it was predicted hyperoxic treatment will completely reverse both the elevation of MSNA and the hypersensitization effect on the chemoreflex. It was also predicted that this effect will persist for the full 180 min of recovery.

STUDY 2.

Study 2 addressed Specific Aim #2a, #2b and #2c by determining whether a) the MSNA response during an apnea is directly related to chemoreflex gain (determined by $\Delta MSNA/\Delta Sa_{O2}$), b) the blood pressure responses during apnea are related to MSNA; and thus, chemoreflex gain and c) the MSNA and blood pressure responses during apnea are augmented in untreated OSA subjects as compared to controls. Study 2 tested the hypotheses that a) that the hemodynamic responses to apnea is a function of elevation of MSNA, and thus, can be used as an index of chemoreflex gain, and b) hypoxic end-expiratory apnea will demonstrate an elevation of MSNA which is directly related to altered chemoreflex gain in OSA patients. Effort was made to match groups for similar mean age, Body Mass Index [BMI: calculated as (body weight in kg/(height in cm)^2] and

resting mean arterial pressure. In addition, effort was made to match groups for similar gender differences.

Subjects: Ten healthy volunteers free of symptoms or history of OSA (as determined by completion of Epworth Sleepiness Scale and Spousal Epworth Sleepiness scale, when possible), as well as 9 untreated OSA patients were studied. After giving written, informed consent each subject completed a medical history questionnaire prior to participation in the study. All subjects were non-smokers, with no history of cardiovascular, pulmonary or neurological disease and were not currently using medications other than oral contraception. All female subjects took a urine pregnancy test, to insure that they were not pregnant, and were not tested during menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume and cardiovascular function. Subjects were instructed to abstain from vigorous exercise and alcohol for 24 hours and from caffeine for 12 hours prior to the start of the study.

Statistical power: Subject numbers were predicted by calculating statistical power based on the variance of data sets of several prior studies from this laboratory. Subject numbers required to obtain a power of 0.80 for detecting a 10 % difference between the groups was 8-10/group; thus, an n of 9 per group should assure adequate statistical power.

Experimental Protocol:. These studies were performed in the semi-recumbent position in a laboratory setting with an ambient temperature of 23-24° C. Subjects were instrumented for measurement of HR, BP, respiratory function, Sa_{O2} and MSNA. Prior to instrumentation, all subjects were allowed to use the restroom. Following

instrumentation, 5 min of baseline was recorded while participants breathed room air, while wearing the nasal mask. Participants then breathed, at random, 1 min of normoxic or hypoxic gas (either 21%, 16% or 12% oxygen), followed by a 20 s end-expiratory voluntary apnea (lung volume equal to FRC). A 5 min washout period was obtained between each trial. Three repeat bouts at each level of gas exposure was obtained and mean values for each subject were determined for each level of hypoxic gas.

Data analysis. Basal MSNA measurements during baseline and hypoxic gas exposure reflect average values obtained over a 10 s measurement period and are reported as both total activity/10 s and percent change/10 s. Total activity for MSNA was obtained as described previously by Smith *et al.* (18). The MSNA response to a single 20 s hypoxic apnea represents total MSNA during the apnea minus the basal MSNA (total activity) immediately prior to the gas exposure. MSNA responses to a single hypoxic apnea were related to the magnitude of the hypoxemia during each hypoxic apnea to determine chemoreflex gain.

All statistical analyses were performed at a significance level (α) of 0.05. Chemoreflex gain was determined from a linear regression analysis of the Δ MSNA/ΔSaO_2 for each subject's data set. A Spearman correlation coefficient was estimated for the relationships between chemoreflex gain; peak MAP, peak systolic and peak diastolic arterial pressure response immediately following apnea. This was performed for all subjects collectively, and for each group separately to assure that these relationships hold for each group. A test of normality was performed using a

Komolgorov test. The data sets were normally distributed; therefore group comparisons for each variable were performed using an unpaired Student's T test.

Expected Results: Consistent with hypothesis #2a, #2b and #2c; it was predicted that hypoxic end-expiratory apnea will demonstrate augmentation of MSNA, which is directly related to nadir Sa_{O2}; and thus, chemoreflex gain. In addition, it was predicted that the blood pressure responses to hypoxic apnea are directly related to the elevation of MSNA. In OSA patients, when compared to controls, it was predicted that hypoxic apnea will produce an augmentation of MSNA response which is greater as correlated to change in Sa_{O2}. Furthermore, the blood pressure response in OSA patients will be greater for a given change in Sa_{O2}, thus demonstrating altered chemoreflex gain and control of MSNA in OSA patients.

STUDY 3.

Study 3 addressed Specific Aim # 3 in a clinical setting, by determining blood pressure response to voluntary end-expiratory apnea as a marker of MSNA and chemoreflex gain in normal controls and untreated OSA patients. Study 3 tested the hypothesis that chemoreflex control of arterial pressure and MSNA is augmented in untreated OSA patients. The altered chemoreflex response to hypoxia was therefore hypothesized to produce a greater elevation in peak systolic blood pressure compared to healthy subjects following voluntary apnea. In addition, we believe this response could be used as a non-invasive measure of chemoreflex sensitivity for control of MSNA. An effort was made to match groups for similar mean age, BMI and resting arterial pressure. In addition, effort was made to match groups with similar gender differences, although,

the control group had a slightly lower number of male subjects than the untreated OSA group (7 vs. 9 males, respectively). Additionally, the control group had an increased number of females than the untreated OSA group (17 vs. 11, respectively).

Subjects: Twenty-four healthy volunteers free of symptoms (as determined by completion of Epworth Sleepiness scale and/or Spousal Epworth Sleepiness scale, when possible) or history of OSA, as well as, 20 untreated OSA patients were studied. After giving written, informed consent each subject will complete a medical history questionnaire prior to participation in the study. All subjects reported no history of congestive heart failure, myocardial infarction, endocrine, pulmonary or neurological disease.

Statistical power: Subject numbers were predicted by calculating statistical power based on the variance of data sets of several prior studies from this laboratory. Subject numbers required to obtain a power of 0.80 for detecting a 10 mmHg difference between the groups was 18-20/group; thus, an n of 20 per group should assure adequate statistical power.

Experimental Protocol: These studies were performed in the seated position in a clinical setting. Subjects were instrumented for measurement of heart rate (HR), blood pressure (BP) and Sa_{O2}. Prior to instrumentation, all subjects were allowed to use the restroom. Non-invasive beat-to-beat arterial pressure (photoplethysmography) and arterial oxygen saturation (pulse oximetry) were measured continuously before, during and after a voluntary end-expiratory apnea (lung volume equal to FRC). Peak systolic blood pressure was also recorded with auscultation via sphygmomanometer and

stethoscope at end breathhold. Within 5 seconds to end of a given breathhold, inflation of blood pressure cuff was begun. Peak systolic pressure was recorded as first sound, via auscultation, for each subject within 3-5 seconds of end apnea. Data was recorded for three repeat bouts of voluntary apnea with data acquisition software on a laptop computer. The average value for HR, BP and nadir Sa_{O2} was recorded for each subject and used in the analysis.

Data Analysis: A test of normality was performed using a Komolgorov test. The data sets were normally distributed, therefore, group comparisons for each variable was performed using an unpaired Student's T test. For all analyses, a p value of 0.05 was set for statistical significance. An effort was made to match groups for similar mean age, BMI and resting arterial pressure. In addition, effort was made to match groups with similar gender differences, although, the control group had a slightly lower number of male subjects than the untreated OSA group (7 vs. 9 males, respectively). Additionally, the control group had an increased number of females than the untreated OSA group (17 vs. 11, respectively).

Expected Results: Consistent with hypothesis #3; It was predicted that untreated OSA patients will demonstrate a greater peak systolic pressure response to voluntary apnea and, it follows, that this is indicative of altered chemoreflex control of MSNA.

Measurements and Procedures

Cardiovascular measurements: Heart rate (HR) was measured using standard limb-lead ECG.

Arterial blood pressure (BP) was measured non-invasively using photoplethysmography at the finger (Finapres Blood Pressure monitor 2300, Ohmeda, Inc., Englewood, CO). This method has been shown to be a reliable and valid measure of arterial blood pressure (30, 50). Additionally, Finapres obtained BP was confirmed in Study 1 and 3 with manual auscultation obtained BP during baseline periods. Peak systolic pressure was measured both manually and via Finapres in study 3 for verification of manual accuracy (as described above).

Respiratory measurements: Arterial oxygen saturation (Sa_{O2}) was assessed at the forehead using pulse oximetry (DS-100A Durasensor, Nellcor Puritan Bennett Inc., Pleasanton, CA). Respiration was monitored using a respiratory monitoring band placed around the subject's abdomen (Grass Instruments, West Warwick, RI) and using a low-resistance turbine volume transducer (model VMM, Alpha Technologies, Inc., Laguna Hills, CA) attached to a leak-free nasal mask (connected to a breathing circuit), allowing the investigator to assure that apneas were performed at end-expiration. All apneas were performed at functional residual capacity (FRC) because apneas during OSA occur at end-expiration. The breathing circuit consisted of the nasal mask, a 3-way Rudolph valve, and Douglas bags. End-tidal oxygen and end-tidal carbon dioxide (ET_{CO2}) was measured with mass spectrometry (model MGA 1100B, Perkin-Elmer, St. Louis, MO) via a port at the side of the mouthpiece.

Muscle Sympathetic Nerve Activity: Postganglionic muscle sympathetic nerve activity was directly measured from the peroneal nerve at the popliteal fossa using standard microneurographic techniques (66). Two sterile tungsten microelectrodes (tip

diameter 5-10mm, 35mm long, Frederick Haer and Co., Bowdoinham, ME) were inserted; one serving as a reference and the other inserted into the peroneal nerve for measurement of MSNA. Due to their small size, microelectrodes were inserted without local anesthesia to avoid any effect anesthesia might have on local nerve function. Nerve signals were processed by a preamplifier and an amplifier (nerve traffic analyzer model 662C-3, Department of Bioengineering, University of Iowa, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000 Hz), rectified and discriminated. Finally, a resistance capacitance circuit with a time constant of 0.1 s integrated raw nerve signals. MSNA recordings were confirmed using the following criteria: 1) pulse-synchronous bursts occurring 1.2-1.4 s after the associated QRS complex, 2) reproducible activation during apnea and phase II and III of the Valsalva maneuver, and 3) no activation following a pinch, skin stroking, or startle stimuli (all of which activate skin sympathetic fibers). Amplitude of the integrated MSNA signal is largely dependent of the recording site. Therefore, the variability exists in the absolute amplitude (volts) of MSNA burst between trials, making it inappropriate to compare absolute amplitude (volts) between subjects. However, amplitude of the integrated MSNA signal is important, because changes in firing rate of single fibers and recruitment of additional fibers become evident by changes in the amplitude of the MSNA burst. Therefore, we normalized the sympathetic burst amplitude, allowing comparison between subjects. The following procedures were used to normalize sympathetic burst amplitude. The average burst amplitude voltage during each baseline period was assigned a value of 100 units. The voltage of all bursts during the experimental conditions was normalized to

this value. For example, if the mean baseline burst amplitude is 0.2mV (100 units), then a burst amplitude of 0.3 mV will have a normalized value of 150 units. Smith *et al.* have previously described this procedure (57).

Chemoreflex Control of MSNA: Chemoreflex control of MSNA sensitivity was determined from the MSNA response to hypoxic voluntary breath-hold challenges. In addition, chemoreflex sensitivity was extrapolated via peak systolic blood pressure response to end-expiratory apnea.

Recruitment of Subjects: Subjects were recruited from the surrounding community, area universities, and from specified local family medicine and sleep medicine clinics. The principal investigator or collaborators explained the study to each volunteer prior to enrollment. All subjects gave written informed consent prior to participation in the study. In addition, verbal explanation was given to each subject at the time of testing. Study 1 and 2 included financial reimbursement to subjects for their time and effort during the study.

Protection of Human Subjects: The risks associated with the measurements of blood pressure, heart rate, respiration, and Sa_{O2} are negligible. The sympathetic nerve recording procedure occasionally (3-10%) results in localized soreness or numbness where the electrode was placed. Rarely, the subject will experience some parasthesias in the foot or toes. Typically, each of these side effects resolves within 1-7 days. A follow-up questionnaire is given to each subject and is returned within 1-2 weeks of the study. Exposure to hypoxic gases may cause bradyarrhythmias, lightheadedness, and anxiety. Although no participants were affected by these symptoms in the studies completed. The

laboratory is equipped with an emergency "crash" cart with defibrillator, ventilator and drugs to address medical emergencies. An on-call physician was available on-site if required.

Inclusion of Women and Minorities: There was no exclusion based on gender, race, or religious affiliation. Female subjects of child-bearing age were given urine pregnancy test, and were not studied if pregnant. This exclusion is due to potential confounding effects of pregnancy on blood volume and cardiovascular function, as well as, unnecessary risk of exposure to hypoxic gas. Female subjects were not studied during or within two days of menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume and cardiovascular function.

Inclusion/Exclusion of Children: Children under the age of 18 were excluded from these studies. This exclusion is justified, in part, because the clinical incidence of OSA is very small in younger children and occurs due to different etiologies and secondly for technical reasons. The technical reasons include 1) the need for subjects to remain still for several hours when there is a MSNA recording electrode in place, 2) the protocols for the hypoxia stimulus (breathing gases from a mask) require considerable cooperation from the subject.

Medical Exclusion Criteria: Participants were excluded if they report a personal history of, or have been previously diagnosed with any of the following conditions: 1) coronary artery disease, 2) angina pectoris, 3) prior myocardial infarction, 4) heart failure, 5) diabetes, 6) chronic obstructive lung disease, 7) neuromuscular disease, and 8) seizures. For all studies, multiple blood pressure recordings taken on separate days, when

possible, or the use of a chart review were used to verify subjects' arterial pressure. Normal subjects with either 3 systolic BP recordings over 140 or 3 diastolic BP recordings over 90 will not be used in study #1 and #2, due to possible alteration of chemoreflex function.

Risk/Benefit Assessment: A risk/benefit ratio is difficult to establish for each individual subject. Overall, the risk is low for the subject group, while the potential gain of knowledge is great; the advancement of knowledge far outweighs the risks. The specific benefit to the individual subject is likely to be minimal. The overall clinical benefit has the promise of: 1) developing a better understanding of the cause of significant cardiovascular co-morbid states often observed in OSA, 2) developing a non-invasive tool to screen patients for increased risk of hypertension and 3) the potential development of a treatment modality for the reversal or prevention of chronic sympathoexcitation which is known to provoke and/or exacerbate most cardiovascular diseases.

Confidentiality: All of the medical records will remain confidential, and participants were referred to only by a number or letter in any published reports. Each participant's identity will remain confidential. However, the laboratory staff, representatives of the Institutional Review Board, and federal regulatory personnel will have access to study data and medical records.

CHAPTER 4

HYPEROXIA REVERSES SYMPATHOEXCITATION AND CHEMOREFLEX RESPONSE FOLLOWING INTERMITTENT HYPOXIC APNEA

Janelle M Hardisty, B.S.[1], Michael J Cutler, M.S.[1], Matthew Brothers, B.S.[1], John R Burk, M.D. [1,2], Michael L Smith, Ph.D.[1]

[1] Department of Integrative Physiology

University of North Texas Health Science Center

3500 Camp Bowie Blvd.

Forth Worth, Texas 76107

[2] Sleep Consultants of Texas

1521 Cooper St.

Fort Worth, Texas 76104

ABSTRACT

Background - The mechanisms which link obstructive sleep apnea (OSA) and cardiovascular disease are not fully understood; however, several studies have shown that patients with OSA have sustained daytime elevation of muscle sympathetic nerve activity (MSNA). Moreover, this appears to be due in part to active chemoreflex control of MSNA mediated primarily by hypoxia. Two recent studies from this laboratory showed that 20 min of intermittent apnea produces a sustained sympathoexcitation and associated augmented chemoreflex control of MSNA. Therefore, we hypothesized a converse effect of hyperoxia: that 15 min of hyperoxia following 20 min of intermittent hypoxic apnea can reverse both the basal elevation of MSNA and chemoreflex control of MSNA accompanying the intermittent apneic perturbation.

Methods and Results - Eight healthy subjects were exposed to 20 min of intermittent hypoxic apnea followed by 15 min of hyperoxia. Basal MSNA and chemoreflex control of MSNA was assessed during baseline, 1 min post-intermittent hypoxic apnea, 5 min post-hyperoxia and every 15 min throughout 180 min of recovery. Recovery hypoxic apneas were matched to baseline hypoxic apnea with a similar nadir Sa_{O2}. Consistent with our hypothesis, both basal total MSNA and burst frequency were significantly reduced by hyperoxia compared to post-intermittent hypoxic apnea through 150 min of recovery ($p<0.05$ and $p<0.05$, respectively) and was not different from pre-intervention MSNA through 180 min of recovery ($p=0.940$). Chemoreflex control of MSNA was not different from pre-intervention baseline through 180 min of recovery ($p=0.278$).

Conclusions – These data support the hypothesis that hyperoxia can reverse the basal sympathoexcitation, as well as the augmented chemoreflex control of MSNA, associated with intermittent hypoxic apnea, and thereby support the hypothesis that elevations in MSNA are hypoxia mediated.

INTRODUCTION

Sleep-related breathing disorders, which can range from habitual snoring to frank central or obstructive sleep apnea, are now recognized as major health problems. Obstructive sleep apnea (OSA) has been shown to be associated with several cardiovascular disorders and recent studies have suggested that there might be a causal link of OSA to hypertension (7, 13). Sleep apnea patients have a generalized elevation in risk for most cardiovascular diseases with an increased incidence of dysrhythmias, myocardial infarction, hypertension, stroke, and sudden death (1, 9, 13, 23, 24).

It has been demonstrated in both rat and canine models that chronic intermittent hypoxia leads to a 10-15 mmHg elevation in resting mean arterial pressure (3, 8). The combined chronic elevation of daytime muscle sympathetic nerve activity (MSNA), with elevations in mean arterial pressure has lead some investigators to hypothesize that the chronic elevation in MSNA may contribute to the increased incidence of hypertension (4, 15, 26). Many recent studies have suggested that early recognition and treatment of OSA may improve cardiovascular function, reduce the risk of hypertension, and decrease elevations in muscle sympathetic nerve activity (10, 31, 33). Since increased MSNA also accompanies most cardiovascular diseases, it is probable that the chronic sympathoexcitation found in OSA contributes to the increased incidence and/or risk of cardiovascular disease found in these patients.

Data from several studies suggest that chemoreflex control of MSNA is altered in patients with OSA (5, 20, 25, 28, 29, 32). Collectively, these studies have lead

to the hypothesis that augmented chemoreflex gain (in the control of MSNA) contributes to the chronic elevation of MSNA in OSA patients. Certainly, OSA are exposed to repeated episodes of chemoreflex activation, however, it is not known whether this intermittent perturbation alters the chemoreflex control of MSNA? A human model of intermittent hypoxic apnea was recently used by Cutler *et al.* to determine the relative contributions of hypoxia, hypercapnia, and absent ventilation on MSNA (6). In this model, 20 min of intermittent hypoxic apnea in healthy human subjects produced a prolonged elevation in basal MSNA and they found that hypoxia was the primary mediator of these adaptive responses. Their findings support those of Xie *et al.*, which demonstrated that hypoxia is the primary mediator for the sympathoexcitation (32). In a separate study, Cutler *et al.* demonstrated that chemoreflex control of MSNA was augmented in parallel with the elevation of MSNA after the 20 min intermittent apneic perturbation (5). In another study, Narkiewicz *et al.*, examined the effect on MSNA of breathing 100% O_2 versus room air in untreated OSA patients and matched controls in an effort to deactivate the chemoreflex (20). During chemoreflex deactivation (100% O_2), MSNA and blood pressure were significantly reduced compared to breathing room air only in untreated OSA patients. These data suggest that a potential mechanism for the elevated daytime MSNA in OSA patients is a tonic activation of excitatory afferent chemoreflex neurons. These data are consistent with finding of Smith *et al.*, in which the Sa_{O2} threshold for sympathoexcitation is elevated in OSA patients compared to controls (25). However, it is unknown if hyperoxia following intermittent hypoxic apnea can reverse the documented basal sympathoexcitation.

Therefore, the purpose of the present investigation was to address the following questions: 1) Does 15 min of hyperoxia (100% O2), following 20 min of intermittent hypoxic apnea, reduce the observed elevation in basal MSNA?, 2) What is the time course for the reduction in basal MSNA compared to post intermittent hypoxic apnea? , 3) Can hyperoxia reverse the augmentation of chemoreflex control of MSNA following intermittent hypoxic apnea?, and finally, 4) How long can an attenuation of chemoreflex control of MSNA persist? Accordingly, we assessed both basal MSNA and chemoreflex control of MSNA (microneurography), heart rate (ECG), arterial pressure (photoplethysmography) before, 1 min post intermittent hypoxic apnea, 5 min post hyperoxia and every 15 min during 180 min of recovery from 20 min of exposure to intermittent hypoxic apnea. We hypothesized that 15 min of hyperoxia following 20 min of intermittent hypoxic apnea would reduce basal MSNA to baseline levels throughout the 180 min recovery period and that MSNA chemoreflex sensitivity would be reduced to baseline following hyperoxia exposure.

METHODS

Subjects. This study was approved by the University of North Texas Health Science Center Institutional Review Board. Eight healthy volunteers (6 males, 2 females, ages 22 to 29 years) participated in this investigation. After giving written, informed consent each subject completed a medical history questionnaire prior to participation in the study. All subjects were non-smokers, reported no history of cardiovascular, pulmonary or neurological disease and were not currently using medications other than oral contraception. Female subjects all tested negative for pregnancy and were not tested

during menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume and cardiovascular function. Subjects were asked to abstain from vigorous exercise and alcohol for 24 hours and caffeine for 12 hours prior to the start of the study.

Cardiovascular measurements. Heart rate (HR) was measured using standard limb-lead ECG. Arterial blood pressure (BP) was measured non-invasively using photoplethysmography at the finger (Finapres Blood Pressure monitor 2300, Ohmeda, Inc., Englewood, CO). This method has been shown to be a reliable and valid measure of arterial blood pressure (14, 22).

Respiratory measurements. Arterial oxygen saturation (Sa_{O2}) was assessed at the forehead using pulse oximetry (DS-100A Durasensor, Nellcor Puritan Bennett Inc., Pleasanton, CA). Respiration was monitored using a respiratory monitoring band placed around the subject's abdomen (Grass Instruments, West Warwick, RI) and using a low-resistance turbine volume transducer (model VMM, Alpha Technologies, Inc., Laguna Hills, CA) attached to a leak-free nasal mask (connected to a breathing circuit), allowing the investigator to assure that apneas were performed at end-expiration. All apneas were performed at functional residual capacity (FRC) because apneas during OSA occur at end-expiration. The breathing circuit will consist of the nasal mask, a 3-way Rudolph valve, and Douglas bags. End-tidal oxygen and end-tidal carbon dioxide (ET_{CO2}) was measured with mass spectrometry (model MGA 1100B, Perkin-Elmer, St. Louis, MO) via a port at the side of the mouthpiece.

Muscle sympathetic nerve activity. Postganglionic muscle sympathetic nerve activity was directly measured from the peroneal nerve at the popliteal fossa using

standard microneurographic techniques (30). Due to their small size, microelectrodes were inserted without local anesthesia to avoid any effect anesthesia might have on local nerve function. Nerve signals were processed by a preamplifier and an amplifier (nerve traffic analyzer model 662C-3, Department of Bioengineering, University of Iowa, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-2,000 Hz), rectified and discriminated. Finally, a resistance capacitance circuit with a time constant of 0.1 s will integrate raw nerve signals. MSNA recordings were confirmed using the following criteria: 1) pulse-synchronous bursts occurring 1.2-1.4 s after the associated QRS complex, 2) reproducible activation during apnea and phase II and III of the Valsalva maneuver, and 3) no activation following a pinch, skin stroking, or startle stimuli (all of which activate skin sympathetic fibers).

Chemoreflex control of MSNA sensitivity. Chemoreflex control of MSNA was assessed by comparison of the MSNA response to hypoxic apneas producing similar levels of hypoxemia. Each hypoxic apnea consisted of the following: during the first 10s of the hypoxic apnea, participants were primed with two breaths of 95-100% nitrogen, followed by a 20 s end-expiratory voluntary apnea (lung volume equal to FRC), such that Sa_{O2} reached 85-95%. Baseline, treatment and recovery MSNA responses were related to the magnitude of hypoxemia during the hypoxic apnea. This was accomplished by matching nadir Sa_{O2} during baseline hypoxic apneas to treatment and recovery hypoxic apneas.

Experimental protocol. These studies were performed in the semi-recumbent position in a laboratory with an ambient temperature of 23-24° C. Subjects were

instrumented for measurement of HR, BP, respiratory function, Sa_{O2} and MSNA. Prior to instrumentation, all subjects were allowed to use the restroom. Following instrumentation, 5 min of baseline was recorded while participants breathed room air, while wearing the nasal mask. Prior to starting the treatment, participants will perform 2-3 hypoxic apneas over a range of oxygen saturation of 85-95%. Participants will then perform one 30 s hypoxic apnea every 1 min (simulating an apnea/hypopnea index of 60/hr) for 20 min. During the first 10 s of the hypoxic apnea, participants were primed with one to two breaths of 95-100% nitrogen, followed by a 20 s end-expiratory voluntary apnea (lung volume equal to FRC) such that Sa_{O2} reached 85-95%. Following the 20 min hypoxic exposure (1 min post-exposure), subjects performed a single 30 s hypoxic apnea and were then exposed to 15 min of hyperoxic (100% O_2) gas. Subjects then recovered while breathing room air for 180 min, without the nasal mask. Every 15 min during recovery participants were instrumented with the nasal mask for 2 min. During this 2 min period, HR, BP, respiratory function, Sa_{O2} and MSNA was measured continuously and subjects will perform a single 30 s hypoxic apnea (as described above), sufficient to produce an Sa_{O2} between 85-95%.

Data analysis. Basal MSNA measurements during baseline and recovery time points reflect average values obtained over a 1 min measurement period and are reported as both bursts/min and total activity/min. Total activity for MSNA was obtained as described previously by Smith *et al.* (25). The MSNA response to a single 30 s hypoxic apnea represents total MSNA during the apnea minus the basal MSNA (total activity) immediately prior to the apnea. Comparisons of baseline to intermittent hypoxic

exposure (1 min post intermittent hypoxic apnea), and recovery (every 15 min) MSNA responses to a single hypoxic apnea were related to the magnitude of the hypoxemia during each hypoxic apnea. Hypoxic apneas were matched to a baseline (i.e. pre-intermittent 20 min hypoxic apnea exposure) hypoxic apnea with a similar nadir Sa_{O2} ($\pm$2%).

All statistical analyses were performed at a significance level (α) of 0.05. Basal MSNA and chemoreflex sensitivity responses to single hypoxic apneas were analyzed using one-way analysis of variance with repeated measures. Three of the 8 subjects only completed 165 min of the 180 min recovery period. For statistical purposes only, missing data were replaced using linear interpolation. Additionally, statistical analyses of these data were compared to analyses with removal of the entire data set for the subject's with missing data. Results of the two analyses were not different. Graphical data include only raw data and do not include replaced data because missing values were only replaced for statistical purposes. Where F values revealed differences, *post hoc* analysis was performed by pairwise comparison using the Student-Newman-Keuls Method. All data are expressed as means $\pm$ SE.

RESULTS

Chemical Stimuli. During each minute of intermittent hypoxic apnea exposure, subjects spent an average of 29 $\pm$ 3 s performing the hypoxic apnea and 31 $\pm$ 3 s breathing room air. Nadir Sa_{O2} during the hypoxic apnea periods averaged 86.4$\pm$1.1%. Sa_{O2} values during hyperoxia treatment averaged 100 $\pm$ 0.1%. Finally, Sa_{O2} values

during recovery were not different than baseline (99 $\pm$ 0.7% vs. 100 $\pm$ 0.6%, respectively).

Effect of hyperoxia on basal MSNA following intermittent hypoxic apnea. Figure 1 is a representative tracing of basal MSNA during baseline, 1 min post-intermittent hypoxic apnea, post-hyperoxia treatment and during recovery at 60, and 120 min. Twenty min of intermittent hypoxic apnea was associated with an elevation in basal MSNA compared to baseline, $p<0.05$. This elevation in basal MSNA was *attenuated* within 5 min of hyperoxia exposure and was sustained below post-hypoxic exposure through 150 min of recovery, $p<0.05$ (figure 2). Furthermore, MSNA total activity was not different compared to pre-intermittent apnea baseline through 180 min of recovery following hyperoxia treatment, $p=0.940$. Basal MSNA burst count was attenuated immediately following hyperoxia treatment and remained at or below baseline throughout recovery and was below post-intermittent hypoxic apnea levels through 165 min of recovery ($p=0.637$ and $p\leq0.05$, respectively. See figure 4).

Effect of hyperoxia on chemoreflex control of MSNA. Our laboratory previously showed that the MSNA response to a single hypoxic apnea following 20 min intermittent hypoxic apnea was *augmented* from 30 min through 165 min of recovery when compared to baseline and had not returned to baseline by 180 min (5). Following hyperoxia treatment, the MSNA response to a single hypoxic apnea was not different than baseline through the 180 min recovery period ($p=0.278$) and was *attenuated* through 45 min of recovery when compared to post-intermittent hypoxia, $p<0.05$ (figure 3). MSNA during nadir Sa_{O2} matched apnea were shown to be attenuated throughout the 180 min recovery

period ($p<0.001$), as compared to pre-intermittent apneic baseline. While the immediate post intermittent hypoxic apnea was also attenuated, it has been demonstrated in previous work from our lab that the augmentation of the MSNA response is not apparent until approximately 30 min post intermittent hypoxic apnea perturbation (5).

Effect of hyperoxia following intermittent hypoxic apnea on heart rate and ventilatory rates. Table 1 represents the heart rate and ventilatory changes during baseline, immediately post intermittent hypoxic apnea and at 20, 60, 120 and 180 min of recovery. The present study demonstrated that following intermittent hypoxic apnea and hypoxic exposure, HR was unchanged during both basal time points and during recovery hypoxic apnea as compared to baseline, $p=0.77$ and $p=0.50$, respectively. Ventilatory rate was unchanged as compared to baseline at all time points ($p=0.719$). Additionally, Nadir Sa_{O2} during baseline hypoxic apnea was not different than recovery hypoxic apnea at 20, 60, 120 and 180 min of recovery, $p=0.130$.

DISCUSSION

The present investigation was designed to determine whether 15 min of hyperoxia (100% O2), following 20 min of intermittent hypoxic apnea, can reduce the observed elevation in basal MSNA and reverse the altered chemoreflex response seen in previous studies (5, 6). The primary findings of this study are that 15 min of hyperoxia (100% O2) following 20 min of intermittent hypoxic apnea results in reversal of basal sympathoexcitation for at least 150 min., and prevented the augmentation of the apnea-induced sympathoexcitatory response previously documented. These effects persisted for at least 180 min after the 20 min intermittent apnea intervention. These data are the first demonstration of prolonged reduction in MSNA response following intermittent hypoxic apnea and provide further support that hypoxia importantly determines the adaptation of chemoreflex control of MSNA to prolonged intermittent apnea.

Model of intermittent apnea: A unique intermittent hypoxic apnea model, used previously in our laboratory, demonstrated an acute perturbation of intermittent hypoxic apnea in healthy volunteers can produce sustained elevation in basal MSNA throughout 180 min of recovery (6), as well as an augmented chemoreflex control of MSNA (5) through 165 min of recovery. This model more closely resembles the intermittent apneas that are experienced by OSA patients, and while not completely established, implies that hours of nightly intermittent apneas could be the primary cause of sustained elevation of MSNA in this patient population. The apnea used in this model is voluntary, in comparison to the obstructive apnea seen with obstructive sleep apnea

which may be considered a limitation to the present study. Yet, the sympathoexcitation which occurs during voluntary apnea has been shown in previous unpublished work from our laboratory to be comparable to that which occurs during obstructive sleep apnea. Additionally, it has been reported by Morgan *et al.* that "peak increases in sympathetic outflow and arterial pressure were comparable in obstructive and non-obstructive apneas of the same duration" (19). Therefore, while the mode of apnea in the present investigation is voluntary as compared to obstructive, we believe the data derived from this model provide unique insights into the adaptive responses to intermittent hypoxia and apneas which include a similar prolonged elevation of MSNA as seen in OSA patients. The present study is an attempt to further understand the mechanisms of these adaptations by using this model.

Chemoreflex control in sleep apnea: Investigators have postulated the daytime elevation in MSNA may be mediated by several different mechanisms, including: humoral factors (34), reduction in sensory input from baroreceptors (21, 34), apnea-related hypoxia and arousals from sleep (2, 16), and altered arterial chemoreflex activation (12, 15, 20). As the potential cardiovascular effects of OSA have been established, much work has been undertaken to determine a causal relationship between OSA and chronic elevation of MSNA. While not completely delineated, chemoreflex control of MSNA has been a prime target of investigation for the observed sympathoexcitation. Narkiewicz *et al.* elegantly demonstrated in a recent study that the sustained elevation of MSNA is partially due to an enhanced peripheral chemoreflex response to hypoxia (20) which is similar to what has been demonstrated in mild

hypertensive patients (27). Interestingly, the enhanced peripheral chemoreflex has been also shown to be apparent in normotensive OSA patients (20).

Several recent studies have demonstrated that hypoxia, acting primarily at the level of the peripheral chemoreceptors, may be the primary mediator of the augmentation in MSNA (6, 18, 29, 30, 32). Cutler *et al.* evaluated the MSNA response during recovery from either 20 min of intermittent hypoxic apnea, hypercapnic hypoxia, or isocapnic hypoxia. They determined no difference between the various groups ($p=0.51$) and concluded that hypoxia was the primary mediator for the sympathoexcitation (6). Additionally, Xie et al. compared MSNA during recovery from 20 min of sustained isocapnic hypoxia and normoxic hypercapnia, which demonstrated sustained elevation of MSNA in the hypoxia group only (32). Furthermore, Narkiewicz *et al.* demonstrated that *during* hyperoxia exposure, as compared to room air exposure, untreated OSA patients showed reduced basal MSNA while no change in MSNA was found in healthy controls ($p=0.008$ and $p=0.4$, respectively) (20). The authors postulate that the daytime elevation in MSNA in OSA patients may be explained in part by tonic activation of excitatory chemoreflex afferents (20). Regardless of whether tonic chemoreflex activation has been demonstrated in sleep apnea patients, it is unknown whether hyperoxia can reverse the effects of altered chemoreflex sensitivity past the initial exposure.

Effect of hyperoxia on MSNA and chemoreflex adaptations: The present study followed 20 min of intermittent hypoxic apnea, previously shown to produce sustained basal and chemoreflex elevations in MSNA (5, 6), with 15 min of 100% oxygen. We postulated a reversal of sympathoexcitation would occur and the augmentation in

chemoreflex sensitivity to intermittent hypoxic apnea would be attenuated. Our data support these hypotheses as pre-intermittent apnea baseline MSNA, while elevated following intermittent hypoxic apnea, was attenuated compared to post-intermittent hypoxic apnea for 165 min and reduced to baseline levels through 180 minutes following hyperoxia exposure. Additionally, chemoreflex control of MSNA, while augmented following intermittent hypoxic apnea in previous studies, was attenuated to baseline levels following hyperoxia in the present investigation through 180 min. Therefore, the current study supports recent demonstrations that prolonged elevations in MSNA are mediated importantly by hypoxic stimuli, as hyperoxia effectively reversed the augmentation. Furthermore, these data provide further support that hypoxia importantly determines the adaptation of chemoreflex control of MSNA to prolonged intermittent apnea. In addition, the effect of hyperoxia is long lasting and effectively reverses the chemoreflex adaptation occurring with 20 min of intermittent hypoxic apnea through 180 min of recovery.

Several studies have demonstrated an early depression of chemoreflex response to hypoxia (5, 11, 17). In the present study, hyperoxia treatment was begun within 5 min of intermittent hypoxic apnea and an augmentation of chemoreflex control of MSNA was not seen following post-intermittent hypoxic apnea perturbation. Yet, MSNA is significantly below baseline levels throughout 180 min of recovery ($p \leq 0.001$) and the change in total activity of MSNA is at or below baseline levels through 180 min of recovery (figure 3.) This is in stark contrast to recent reports of elevated chemoreflex

responses within 30 min of intermittent hypoxic apnea which persisted through 165 min (5).

It has been postulated that chronic exposure to hypoxic apnea and the ensuing augmentation of chemoreflex control of MSNA may contribute to the increased incidence in hypertension in the sleep apnea population (5). Therefore, the model and findings in this study, which evaluated hyperoxia effects on sustained elevation in MSNA and chemoreflex control of MSNA, are both clinically relevant and further support the hypothesis that hypoxia mediates elevations in sympathetic outflow.

In conclusion, the present study is the first to show that hyperoxia following intermittent hypoxic apnea can prevent the elevation of MSNA. Additionally, these data demonstrate that chemoreflex control of MSNA remains at or below normal following hyperoxia. These data provide further support of the hypothesis that hypoxia is the primary mediator of the sympathoexcitatory response to intermittent apnea and the postulated that a tonic chemoreflex-mediated sympathoexcitation may be present in patients with OSA.

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FIGURE LEGENDS

Figure 1. Representative 30 sec tracing of basal MSNA during baseline, 1 min post-intermittent hypoxic apnea, post hyperoxia treatment and during recovery at 60, and 120 min.

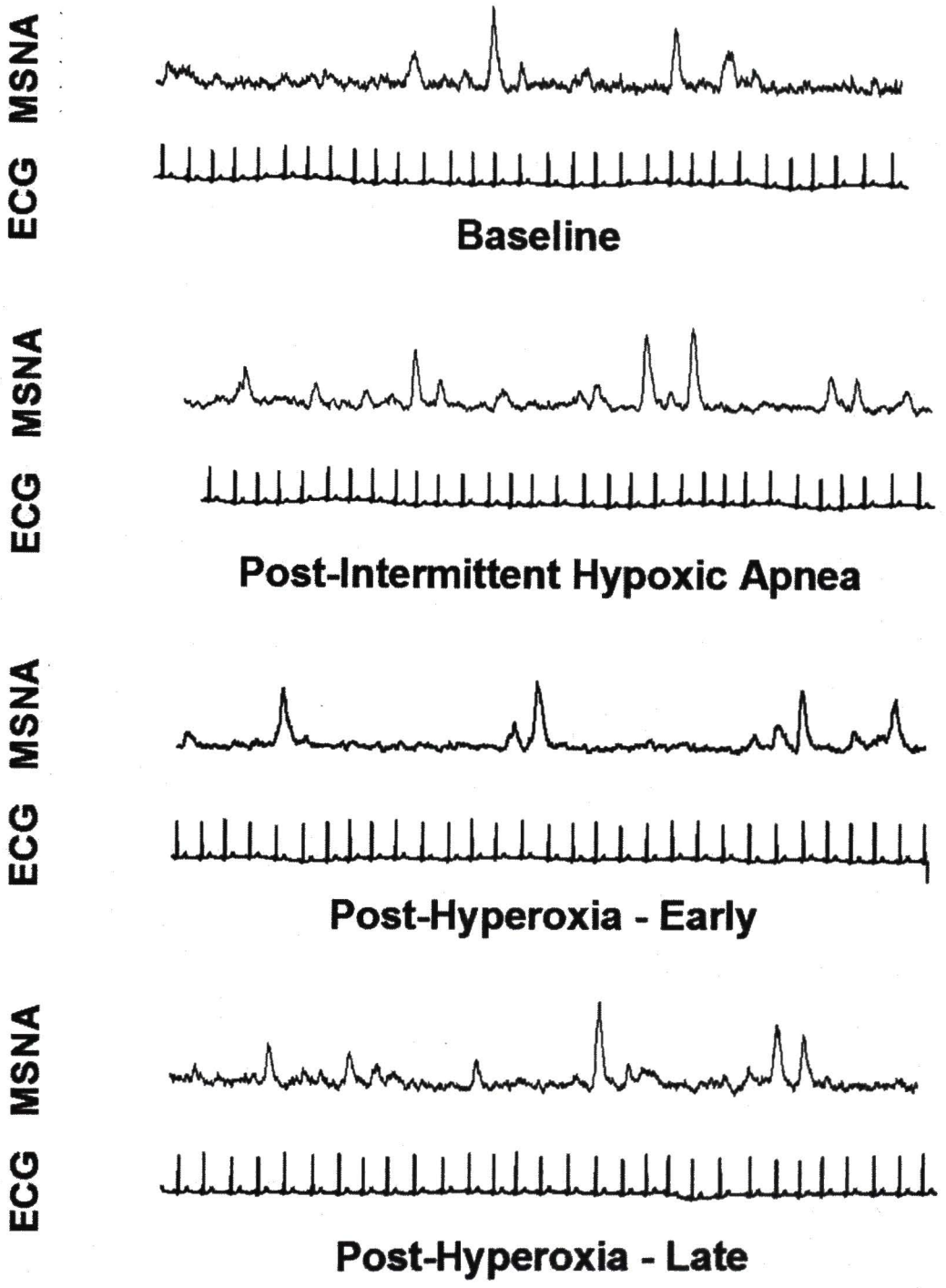
Figure 2. Basal MSNA total activity during baseline, immediately post intermittent hypoxic apnea and during 180 min of recovery. Dotted line represents post 15 min hyperoxia exposure. *$p \leq 0.05$ vs. baseline. #$p \leq 0.001$ vs. post 20 min intermittent hypoxic apnea exposure. +$p \leq 0.05$ vs. post 20 min intermittent hypoxic apnea exposure. Values are means $\pm$ SE; n = 8 subjects.

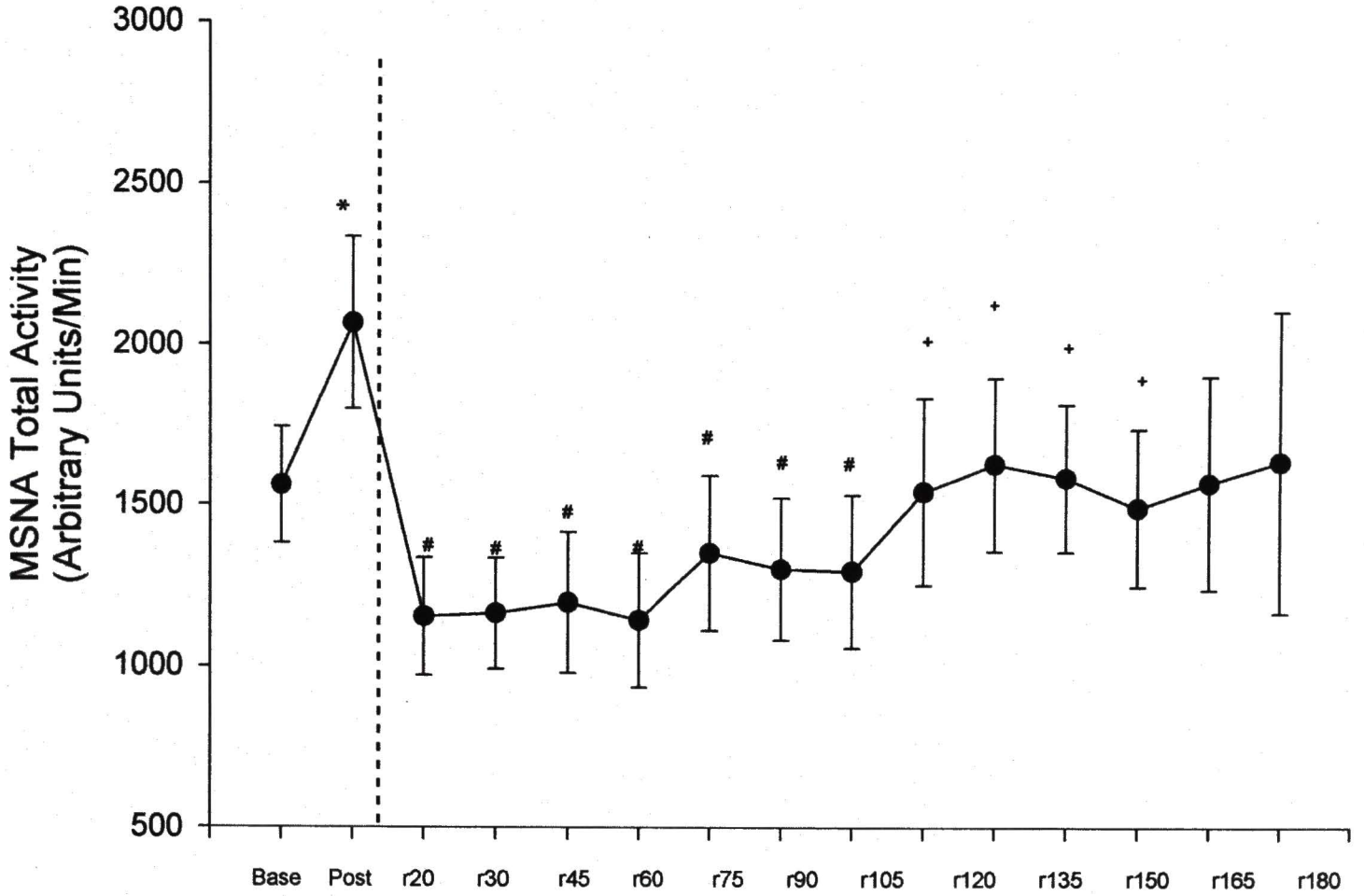
Figure 3. Difference from baseline in the MSNA response to a nadir Sa_{O2}-matched 30s voluntary hypoxic apnea, baseline, 1 min post 20 min intermittent hypoxic apnea exposure, and during recovery from 15 min hypoxia through 180 min. Dotted line represents post 15 min hyperoxia exposure. *$p<0.05$ vs. baseline. Values are means $\pm$ SE, n = 8 subjects.

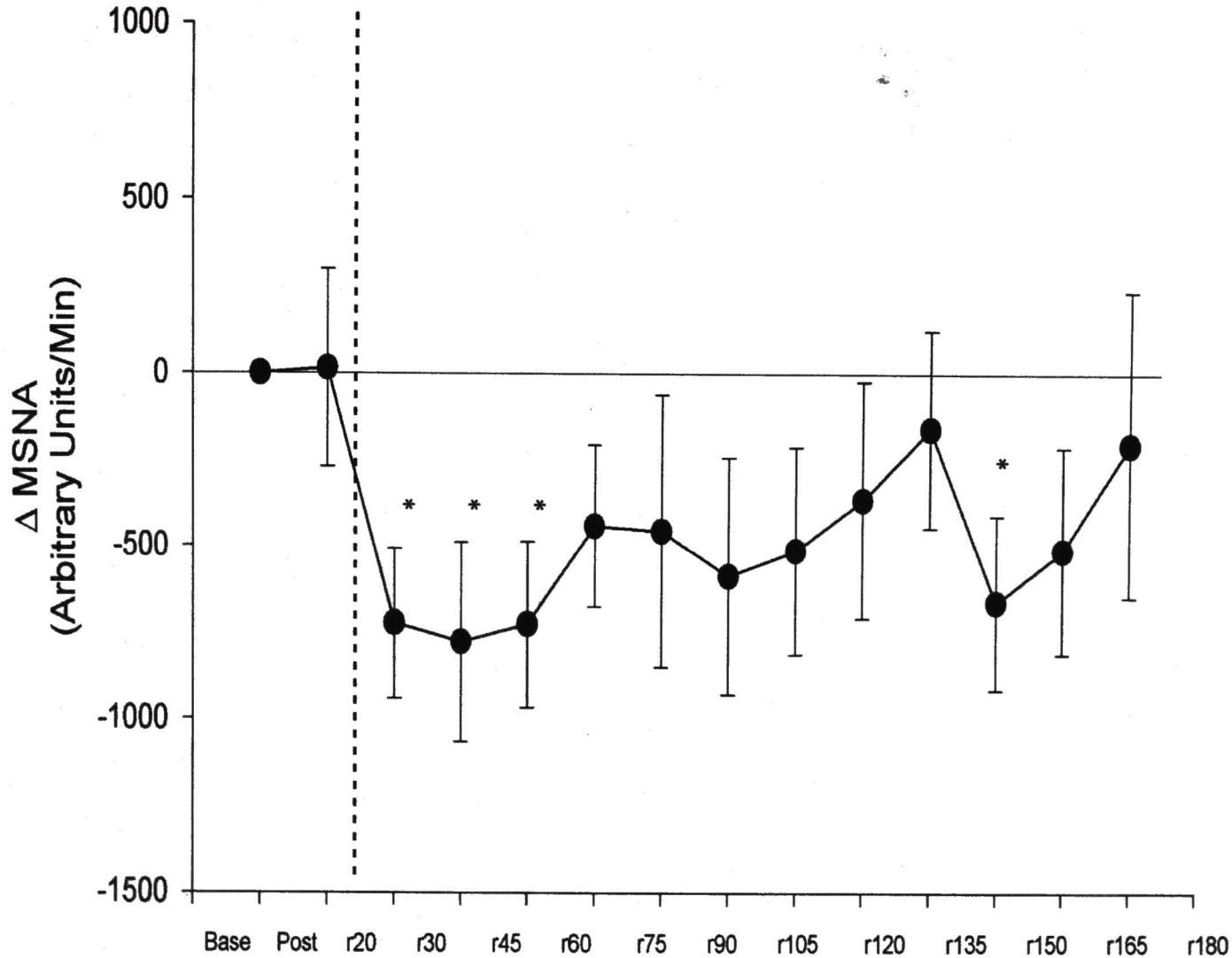
Figure 4. *Top*: Basal MSNA burst frequency (expressed as percent of baseline) 1 min post 20 min intermittent hypoxic apnea exposure, and during recovery from 15 min hypoxia through 180 min. Dotted line represents post 15 min hyperoxia exposure.

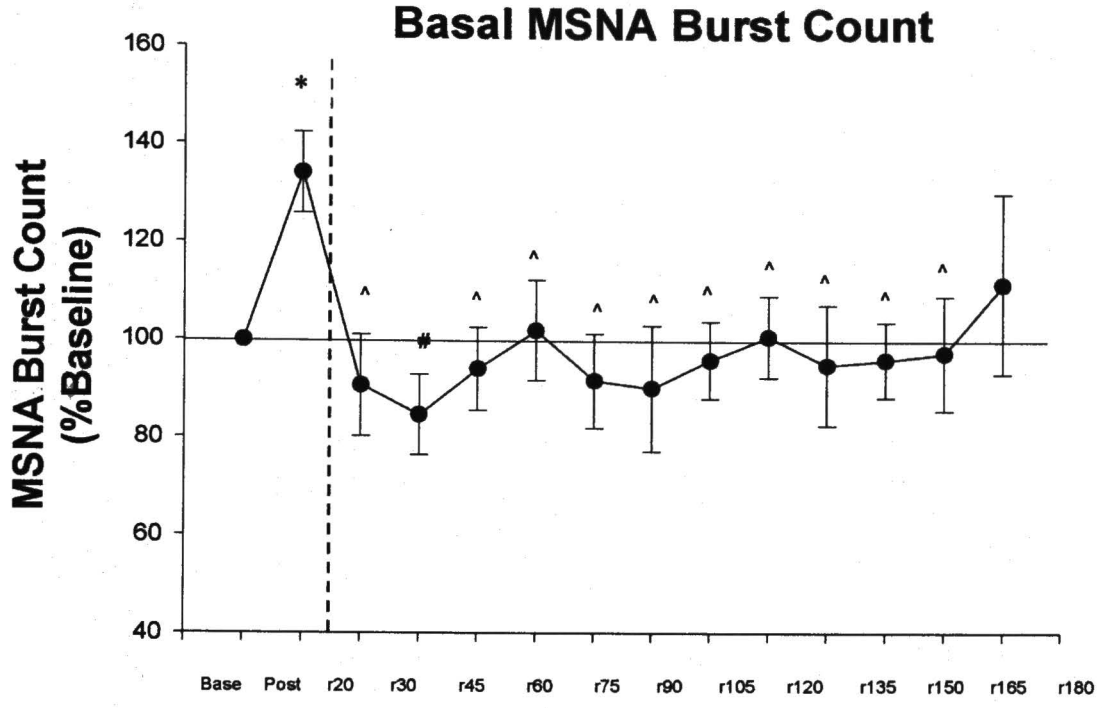
*$p \leq 0.05$ vs. baseline. ^$p \leq 0.05$ vs. post 20 min intermittent hypoxic apnea exposure. #$p \leq 0.001$ vs. post 20 min intermittent hypoxic apnea exposure. Values are means $\pm$ SE, n = 8 subjects. *Bottom:* MSNA burst frequency (expressed as percent of baseline) with single hypoxic apnea at 1 min post 20 min intermittent hypoxic apnea exposure, and during recovery from 15 min hypoxia through 180 min. Dotted line represents post 15 min hyperoxia exposure. *$p \leq 0.001$ vs. baseline. Values are means $\pm$ SE, n = 8 subjects.

Table 1. Cardiovascular and ventilatory data during baseline, post 20 min intermittent hypoxia apnea exposure and following hyperoxia at 20, 60, 120 and 180 min of recovery. Values are means $\pm$ SE, n = 8 subjects.


ECG
MSNA
Baseline
ECG
MSNA
Post-Intermittent Hypoxic Apnea
ECG
MSNA
Post-Hyperoxia - Early
ECG
MSNA
Post-Hyperoxia - Late
10 sec


MSNA Total Activity
(Arbitrary Units/Min)
3000
2500
2000
1500
1000
500
Base
Post
r20
r30
r45
r60
r75
r90
r105
r120
r135
r150
r165
r180
*
#
+


1000
500
0
-500
-1000
-1500
Δ MSNA
(Arbitrary Units/Min)
*
*
*
*
Base
Post
r20
r30
r45
r60
r75
r90
r105
r120
r135
r150
r165
r180


Basal MSNA Burst Count
MSNA Burst Count (%Baseline)
160
140
120
100
80
60
40
Base
Post
r20
r30
r45
r60
r75
r90
r105
r120
r135
r150
r165
r180

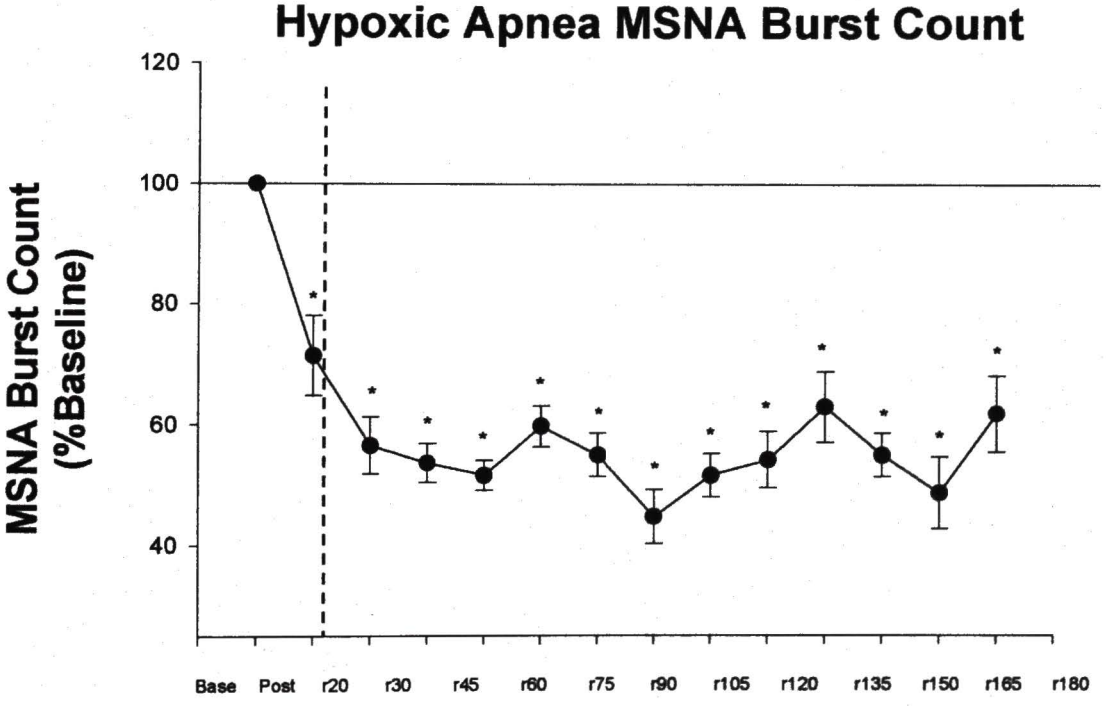

Hypoxic Apnea MSNA Burst Count
MSNA Burst Count (%Baseline)
120
100
80
60
40
Base
Post
r20
r30
r45
r60
r75
r90
r105
r120
r135
r150
r165
r180

Table I. Cardiovascular and ventilatory data during baseline, post intermittent hypoxic apnea and following hyperoxia for 180 min of recovery.

	Baseline	Post 20 min IHA	Post 15 min 100% O_2	Recovery			
				20 min	60 min	120 min	180 min
Basal							
Heart rate, beats/min	66.86±4.23	66.86±3.57	66.00±3.93	68.57±3.17	66.00±3.47	68.57±3.67	71.00±3.60
Ventilatory rate, breaths/min	13.17±1.32	11.79±1.02	13.06±1.05	13.48±0.78	12.31±1.01	13.22±1.09	11.59±1.79
During Hypoxic Apnea							
Heart rate, beats/min	70.71±3.45	66.86±2.76		67.86±1.76	67.71±4.2	68.57±3.90	73.00±3.92
Nadir Sa_{O2}	87.18±0.93	87.80±1.61		89.48±1.42	87.86±1.41	86.41±1.09	86.63±1.84

Values are means ± SE. IHA = Intermittent Hypoxic Apnea exposure.

CHAPTER 5

BLOOD PRESSURE RESPONSE TO APNEA PREDICTS CHEMOREFLEX- SYMPATETIC NEURAL CONTROL IN OBSTRUCTIVE SLEEP APNEA

Janelle M Hardisty, B.S.[1], John R Burk, M.D. [1,2], Michael L Smith, Ph.D.[1]

[1] Department of Integrative Physiology

University of North Texas Health Science Center

3500 Camp Bowie Blvd.

Forth Worth, Texas 76107

[2] Sleep Consultants of Texas

1521 Cooper St.

Fort Worth, Texas 76104

ABSTRACT

Background – Patients with obstructive sleep apnea (OSA) experience repetitive apneic events during sleep with resulting hypoxia and hypercapnia. It has been demonstrated that hypoxia, acting via tonically active chemoreflexes, elicits chronic increases in muscle sympathetic nerve activity (MSNA) and consequent increases in blood pressure. Therefore, we tested the hypotheses that: 1) the degree of hypoxia occurring during an apnea determines the MSNA and resulting blood pressure responses, furthermore these responses are augmented in untreated OSA patients; 2) that altered chemoreflex function in OSA patients is predictive of blood pressure response to hypoxic apnea; and 3) the blood pressure response to voluntary apnea can distinguish patients with OSA in a clinical setting.

Methods and Results – In an experimental setting, 9 subjects with untreated OSA and 10 healthy subjects breathed, at random, 1 min of normoxic or hypoxic gas (either 21%, 16% or 12% oxygen) followed by a 20 s voluntary apnea. MSNA was augmented in OSA subjects ($p \leq 0.05$) at each level of hypoxic apnea, while MAP was augmented at the peak hypoxic exposure ($p \leq 0.05$). Chemoreflex gain was determined to be augmented in OSA subjects ($p \leq 0.05$), while a correlation between chemoreflex gain and MAP response was found for all subjects ($R=0.707$, $p<0.001$). In a clinical setting, 20 subjects with untreated OSA and 24 control subjects performed 3 repeat bouts of 20 s voluntary apnea. Peak systolic blood pressure response was determined to be greater in OSA patients than controls ($p<0.001$)

Conclusions – These data support the hypotheses that the MSNA and blood pressure responses to hypoxic apnea are augmented in OSA. In addition, chemoreflex gain is related to the blood pressure response to apnea. Furthermore, the peak systolic pressure response to voluntary apnea is augmented in untreated OSA patients and could possibly be used as a non-invasive measure of chemoreflex gain and augmented sympathetic activity in OSA.

INTRODUCTION

Over the past twenty years, the medical community has become increasingly aware that obstructive sleep apnea (OSA) is a common disorder, which adversely affects both the respiratory and cardiovascular systems. OSA has been reported to be associated with an increased incidence of dysrhythmias, myocardial infarction, stroke and sudden death (1, 5, 11). Furthermore, OSA patients are at an increased risk for hypertension (5, 17, 36), with reports of up to 40-60% of OSA patients having been diagnosed with arterial hypertension (27). Although the cause of these associations remains unknown, growing evidence has shown that sympathetic activity is chronically elevated (14), and may be related, in part, to augmented chemoreflex sensitivity (22). Successful treatment of OSA with nasal continuous positive airway pressure (nCPAP) has been shown to produce a reduction in severity and/or reversal of hypertension which supports a potential causal relation between OSA and hypertension (20, 31, 34). Nevertheless, understanding of the relationship between these two diseases, and the underlying pathophysiology remains unclear.

Insights into the underlying pathophysiology linking these two diseases may be derived from the control of arterial pressure during sleep apnea. In general, it has become clear that patients with OSA tend to have chronically elevated sympathetic nerve activity as shown by several different methods of measurement (6, 14). During individual apneic events, muscle sympathetic nerve activity (MSNA) and arterial pressure tend to increase significantly. The increase in MSNA is related to the lack of

respiration, and most importantly, to the activation of chemoreceptors due to progressively developing hypoxemia and hypercapnia (31). Several studies have shown that the predominant stimulus for this sympathoexcitatory response is the hypoxemia (3, 22). Nevertheless, demonstration that the MSNA response increases in proportion to the degree of hypoxemia has not been determined. This is the first aim of this study.

The sympathoexcitation occurring during episodes of sleep apnea has been demonstrated to be carried over into wakeful hours and has been postulated to be one of the primary mediators for the increased risk for hypertension (7). Chemoreflex control of MSNA has been a prime target of investigation for the observed sympathoexcitation. Recent studies by Cutler *et al.* and Narkiewicz *et al.* reported that a selective potentiation of peripheral chemoreceptors contribute to increased MSNA (4, 22). Narkiewicz *et al.* demonstrated peripheral chemoreflex deactivation, using 100% oxygen, resulted in decreased MSNA ($p=0.008$) and mean arterial pressure ($p=0.02$) in OSA subjects but not controls (22). Perhaps more importantly, it has been demonstrated that chemoreflex control of MSNA is augmented in OSA patients (22). However, how this affects blood pressure regulation is unclear.

An important answer to this question lies in the mechanism of the elevation of blood pressure occurring at the end of apneic events. Moreover, the hemodynamic response to hypoxia at regional tissues is complex: it involves both direct and reflex effects on the vasculature (8, 9). Obstructive apnea in OSA patients and voluntary apnea in healthy controls have been shown to produce transient increases in arterial pressure with associated elevation of MSNA (9, 15). While, the peak MSNA response to apnea is

greatest at end apnea, arterial pressure has been shown to be greatest several seconds after the resumption of breathing (9, 15, 28). It has been postulated that the peripheral vascular response to hypoxia in OSA patients is abnormal with an augmented blood pressure response demonstrated in several studies. Hedner *et al.* found that OSA subjects have a greater pressor response to hypoxia than controls, yet did not differ in their HR response (13). They suggested that a difference in vascular response to hypoxic stimulation in OSA. Additionally, Remsburg *et al.* reported that exposure to isocapnic hypoxia (Sa_{O2} = 80%) produced a vasodilation (as measured by forearm vascular resistance) in controls, yet was not apparent in OSA subjects (28). These data suggest altered vascular responses to hypoxia in OSA.

The purpose of these studies was to determine: 1) whether the degree of hypoxia occurring during an apnea determines the sympathoexcitatory response and resulting blood pressure response and if these responses are augmented in untreated OSA subjects; 2) altered chemoreflex gain in OSA subjects is predictive of augmented blood pressure response to hypoxic apnea; and 3) whether the blood pressure response to voluntary apnea can distinguish patients with OSA in a clinical setting. Consequently, we assessed MSNA and blood pressures responses as well as chemoreflex gain during various degrees of hypoxic apnea in untreated OSA patients compared to controls. Chemoreflex gain was assessed by the MSNA response to a single voluntary hypoxic apnea and was related to the magnitude of the hypoxemia during the hypoxic apnea. We hypothesized that the degree of hypoxia with apnea would be predictive of the MSNA and blood pressure response. Furthermore, these responses would be increased in untreated OSA subjects as

compared to healthy controls and would be predicted by altered chemoreflex gain. In addition, we assessed the blood pressure response to voluntary end expiratory apnea in a clinical setting to determine if OSA patients would demonstrate an augmented peak systolic pressure response compared to controls. We hypothesized that the peak systolic blood pressure response to voluntary apnea would be greater in the untreated OSA population than in controls.

METHODS

STUDY 1

Subjects. This study was approved by the University of North Texas Health Science Center Institutional Review Board. Nine untreated OSA volunteers (7 males, 2 females, ages 24 to 55 years) and 10 healthy volunteers (8 males, 2 females, ages 23 to 46 years) participated in this investigation. After giving written, informed consent each subject completed a medical history questionnaire prior to participation in the study. All subjects were non-smokers, reported no history of cardiovascular, pulmonary or neurological disease and were not currently using medications other than oral contraception. Female subjects all tested negative for pregnancy and were not tested during menses to eliminate potential confounding effects of menses on fluid metabolism, blood volume and cardiovascular function. Subjects were asked to abstain from vigorous exercise and alcohol for 24 hours and caffeine for 12 hours prior to the start of the study.

Cardiovascular measurements. Heart rate (HR) was measured using standard limb-lead ECG. Arterial blood pressure (BP) was measured non-invasively using

photoplethysmography at the finger (Finapres Blood Pressure monitor 2300, Ohmeda, Inc., Englewood, CO). This method has been shown to be a reliable and valid measure of arterial blood pressure (16, 26).

Respiratory measurements. Arterial oxygen saturation (Sa_{O2}) was assessed at the forehead using pulse oximetry (DS-100A Durasensor, Nellcor Puritan Bennett Inc., Pleasanton, CA). Respiration was monitored using a respiratory monitoring band placed around the subject's abdomen (Grass Instruments, West Warwick, RI) and using a low-resistance turbine volume transducer (model VMM, Alpha Technologies, Inc., Laguna Hills, CA) attached to a leak-free nasal mask (connected to a breathing circuit), allowing the investigator to assure that apneas were performed at end-expiration. All apneas were performed at functional residual capacity (FRC) because apneas during OSA occur at end-expiration. The breathing circuit consisted of the nasal mask, a 3-way Rudolph valve, and Douglas bags containing appropriate gas mixtures. End-tidal oxygen and end-tidal carbon dioxide (ET_{CO2}) was measured with mass spectrometry (model MGA 1100B, Perkin-Elmer, St. Louis, MO) via a port at the side of the mouthpiece.

Muscle sympathetic nerve activity. Postganglionic muscle sympathetic nerve activity was directly measured from the peroneal nerve at the popliteal fossa using standard microneurographic techniques (33). Due to their small size, microelectrodes were inserted without local anesthesia to avoid any effect anesthesia might have on local nerve function. Nerve signals were processed by a preamplifier and an amplifier (nerve traffic analyzer model 662C-3, Department of Bioengineering, University of Iowa, Iowa City, IA) with a total gain of 90,000. Amplified signals were band-pass filtered (700-

2,000 Hz), rectified and discriminated. Finally, a resistance capacitance circuit with a time constant of 0.1 s will integrate raw nerve signals. MSNA recordings were confirmed using the following criteria: 1) pulse-synchronous bursts occurring 1.2-1.4 s after the associated QRS complex, 2) reproducible activation during apnea and phase II and III of the Valsalva maneuver, and 3) no activation following a pinch, skin stroking, or startle stimuli (all of which activate skin sympathetic fibers).

Chemoreflex Gain. Chemoreflex gain was assessed by comparison of the MSNA response to hypoxic apneas at similar levels of hypoxemia. The linear regression relating the change in MSNA per change in O_2 saturation was estimated for each subject.

Experimental protocol. These studies were performed in the semi-recumbent position in a laboratory with an ambient temperature of 23-24° C. Subjects were instrumented for measurement of HR, BP, respiratory function, Sa_{O2} and MSNA. Following instrumentation, 5 min of baseline was recorded while participants breathed room air, while wearing the nasal mask. Participants then breathed, at random, 1 min of normoxic or hypoxic gas (either 21%, 16% or 12% oxygen), followed by a 20 s end-expiratory voluntary apnea (lung volume equal to FRC). A 5 min washout period was obtained between each trial. Three repeat bouts at each level of gas exposure was obtained and mean values for each subject were determined for each level of hypoxic gas.

Data analysis. Basal MSNA measurements during baseline and hypoxic gas exposure reflect average values obtained over a 10 s measurement period and are reported as both total activity/10 s and percent change/10 s. Total activity for MSNA was obtained as described previously by Smith *et al.* (30). The MSNA response to a single 20

s hypoxic apnea represents total MSNA during the apnea minus the basal MSNA (total activity) immediately prior to the gas exposure. MSNA responses to a single hypoxic apnea were related to the magnitude of the hypoxemia during each hypoxic apnea to determine chemoreflex gain.

All statistical analyses were performed at a significance level (α) of 0.05. Chemoreflex gain was determined from a linear regression analysis of the Δ MSNA/ΔSa_{O2} for each subject's data set. A Spearman correlation coefficient was estimated for the relationships between chemoreflex gain; peak MAP, peak systolic and peak diastolic arterial pressure response immediately following apnea. This was performed for all subjects collectively, and for each group separately to assure that these relationships hold for each group. A test of normality was performed using a Komolgorov test. If the data sets were normally distributed, then group comparisons for each variable were performed using an unpaired Student's T test. If a data set was not normally distributed, then a nonparametric analysis using a Mann-Whitney Rank Sum test was performed. An effort was made to match the two groups for similar mean age, body mass index [BMI: calculated as (body weight in kg/(height in cm)^2], and resting arterial pressure. Additionally, effort was made to match groups for similar gender equality.

STUDY 2

Subjects. This study was approved by the University of North Texas Health Science Center Institutional Review Board. Twenty-four volunteers (7 males, 17 females,

ages 26 to 82 years) free of symptoms (as determined by completion of Epworth Sleepiness scale and/or Spousal Epworth Sleepiness scale, when possible) or history of OSA, as well as, 20 untreated OSA patients (9 males, 11 females, ages 25 to 73 years) participated in this investigation. OSA patients were diagnosed (apnea/hypopnea index > 10/hr) following overnight polysomnography at an accredited sleep lab and participated in this study prior to beginning treatment. After giving written, informed consent each subject completed a medical history questionnaire prior to participation in the study. Eight of the 20 OSA participants, and 8 of the 24 control participants, reported a history of hypertension. Two of the 20 OSA participants, and 6 of the controls reported a history of smoking. All subjects denied a history of congestive heart failure, myocardial infarction, pulmonary, neurological or endocrine disease.

Measurements. Heart rate (HR) and arterial oxygen saturation (Sa_{O2}) were assessed at the forehead using pulse oximetry (DS-100A Durasensor, Nellcor Puritan Bennett Inc., Pleasanton, CA). Arterial blood pressure (BP) was measured non-invasively using photoplethysmography at the finger (Finapres Blood Pressure monitor 2300, Ohmeda, Inc., Englewood, CO). In addition, ausculatory blood pressure was taken as a baseline reading and peak systolic pressure within 5 seconds of the end of each voluntary apnea. This consisted of inflation of blood pressure cuff during the last 3 – 5 sec of a voluntary apnea, with peak systolic measurement recorded within 5 sec of resumption of breathing.

Experimental protocol. These studies were performed in the seated position in a clinical setting. Subjects were instrumented for measurement of heart rate (HR), blood

pressure (BP) and Sa_{O2}. Prior to instrumentation, all subjects were allowed to use the restroom. Participants were instructed on study protocol and one practice end-expiratory apnea was performed prior to recording. Non-invasive beat-to-beat arterial pressure (photoplethysmography) and arterial oxygen saturation (pulse oximetry) were measured continuously before, during and immediately following three separate voluntary end-expiratory apnea. A minimum of two minutes elapsed between each apnea. Baseline systolic and diastolic pressures were recorded via sphygmomanometer and stethoscope. Peak systolic blood pressure was also recorded via auscultation within 5 seconds of end apnea. Data was recorded for three repeat bouts of voluntary apnea with data acquisition software on a laptop computer.

Data Analysis. All statistical analyses were performed at a significance level (α) of 0.05. The average value for HR, BP and nadir Sa_{O2} for each subject was used in the analysis. A test of normality was performed using a Komolgorov test. As the data sets were normally distributed, the group comparisons for each variable were performed using an unpaired Student's T test. An effort was made to match the two groups for age, BMI, and resting arterial pressure. In addition, effort was made to match groups with similar gender differences, although, the control group had a slightly lower number of male subjects than the untreated OSA group (7 vs. 9 males, respectively). Additionally, the control group had an increased number of females than the untreated OSA group (17 vs. 11, respectively).

RESULTS

Study 1. Nine untreated, newly diagnosed OSA patients and 10 healthy controls were enrolled as subjects in an experimental setting.

MSNA response to hypoxic apnea. Figure 1a depicts the MSNA response to the various hypoxic apnea perturbations. The change in total activity was greater for all trials in the OSA subjects compared to baseline ($p \leq 0.05$). Controls exhibited elevated MSNA responses for the 16% and 12% hypoxic apnea only, $p \leq 0.05$. OSA subjects, compared to controls, demonstrated augmented MSNA response for all trials ($p < 0.001$). Similar mean nadir Sa_{O2} were found for the two groups during progressive levels of hypoxic gas exposure prior to apnea ($p=0.753$, 21% oxygen exposure; $p=0.336$, 16% oxygen exposure; and $p=0.265$, 12% oxygen exposure, prior to apnea).

Arterial pressure responses to hypoxic apnea. Figure 2 represents arterial pressure responses to various levels of hypoxic exposure and subsequent apnea. A significant main effect was determined for peak MAP and peak systolic pressure responses following hypoxic apnea in OSA, occurring with all levels of progressive hypoxic exposure ($p \leq 0.05$ and $p < 0.001$, respectively). While controls exhibited elevated MAP and peak systolic pressure responses during 16% and 12% gas exposure compared to baseline ($p < 0.001$ and $p < 0.001$, respectively), no change was observed with normoxic gas exposure (21% O_2) prior to apnea ($p=0.88$). Diastolic pressure responses were not different than baseline in OSA following 21% and 16% hypoxic apnea ($p=0.914$ and $p=0.150$ respectively), or controls ($p=0.994$ and $p=0.177$, respectively) yet were elevated

similarly in OSA and controls at the 12% exposure and subsequent apnea ($p \leq 0.05$ and $p \leq 0.05$, respectively).

Blood pressure response to hypoxic apnea as a function of MSNA response. Figure 3a is representative of the mean blood pressure response as a function MSNA response, while 3b is representative of the peak systolic pressure response as a function of MSNA response to hypoxic apnea. Each subjects' data is represented as a mean for each level of hypoxic apnea perturbation. A correlation between ΔMSNA and MAP response to hypoxic apnea was found for both OSA subjects and controls ($R=0.925$, $p<0.001$ and $R=0.628$, $p<0.001$, respectively). In addition there were similar strengths of correlation for ΔMSNA and peak systolic pressure response for OSA and control subjects ($R=0.880$, $p<0.001$ and $R=0.805$, $p<0.001$, respectively). Using an Analysis of Covariance to determine differences between correlations of hemodynamic responses and change in MSNA demonstrated no difference between the slopes for peak systolic pressure responses and ΔMSNA between the two groups ($p=0.937$), while a significant difference was found for the slope analyses for MAP and ΔMSNA between the groups ($p=0.007$).

Chemoreflex gain and relationship to peak blood pressure responses. Chemoreflex gain was augmented in OSA subjects compared to controls following 21% oxygen gas exposure and subsequent apnea ($p<0.001$, figure 1b). Interestingly, augmented chemoreflex gain was also observed in OSA compared to controls during 16% and 12% hypoxic apnea yet were reduced in magnitude compared to the 21% exposure ($p<0.05$). The correlation between chemoreflex gain; mean arterial pressure

response, peak systolic pressure response and peak diastolic pressure response for all subjects is shown in Figure 4. A significant correlation was found between chemoreflex gain and both peak systolic pressure response to hypoxic apnea ($R=0.808$, $p<0.001$) as well as MAP response ($R=0.707$, $p<0.001$). Yet, a correlation could not be determined for chemoreflex gain and peak diastolic response to hypoxic apnea ($R=0.384$, $p=0.102$).

Study 2. Twenty untreated, newly diagnosed OSA patients and 24 controls were enrolled as subjects in a clinical setting.

Baseline measurements and demographic data. Table I reports the mean baseline heart rate, mean arterial pressure, body mass index (BMI), and Epworth scores for the OSA and control subjects. There was no difference in baseline HR ($p=0.624$), mean arterial pressure ($p=0.213$), or BMI ($p=0.067$) between the two groups. While the untreated OSA patients were determined to have higher Epworth score than the controls ($p=0.002$).

Blood pressure response to voluntary end-expiratory apnea. The peak systolic pressure response to voluntary end-expiratory apnea were greater in OSA patients than controls ($p\leq0.05$, Figure 5). This relationship held true for peak systolic pressure measured via finapres, and for ausculatory pressure measurements. Table I includes mean nadir Sa_{O2} data for each apnea and, for the second trial, it was determined that OSA patients desaturated slightly more than controls ($p=0.013$), yet no difference was determined for trial 1 and trial 3 ($p=0.268$ and $p=0.113$, respectively).

DISCUSSION

The purpose of the present investigation was to determine: 1) whether the degree of hypoxia occurring during an apnea determines the sympathoexcitatory response and resulting arterial blood pressure response and if these responses are augmented in subjects with obstructive sleep apnea; 2) whether altered chemoreflex gain in OSA subjects is predictive of augmented blood pressure response to hypoxic apnea; and 3) if the blood pressure response to voluntary apnea can distinguish patients with OSA from healthy normotensive subjects in a clinical setting. Accordingly, these data support the hypothesis that the degree of hypoxia during apnea determines the MSNA response. Furthermore, the MSNA response is predictive of the MAP and systolic pressure responses. In addition, these data support the hypotheses that MSNA and arterial pressure responses to hypoxic apnea are augmented in OSA subjects consistent with augmented chemoreflex sensitivity. Finally, it was determined that, in a clinical setting, the auscultation of the peak systolic arterial pressure response to voluntary apnea is a reliable and reproducible indication of augmented MSNA and possibly could be used as a marker for chemoreflex gain in OSA patients.

Role of hypoxia in sympathoexcitatory and blood pressure response to apnea. Hypoxia has been demonstrated to increase muscle sympathetic nerve activity and numerous studies have reported significant increases in MSNA with exposure to hypoxic gas. (29, 30, 32). Rowell *et al.* (29) investigated the sympathetic effects of exposure to 8%, 10% and 12% oxygen for approximately 20 min. A significant increase in MSNA

occurred during all conditions yet the response was reported sooner during the 8% and 10% oxygen. Additionally, Smith *et al.* (30) investigated the effects of acute hypoxemia, in OSA subjects and controls, whereby subjects breathed 1-4 breaths of hypoxic gas (0-5% oxygen). The degree of hypoxemia was titrated in repeat trials to produce a range of arterial oxygen saturation from 70-95% Sa_{O2}. They reported a 'threshold' for sympathoexcitation which was elevated to higher Sa_{O2} levels in OSA subjects. While these studies examined the effect of hypoxia during spontaneous and controlled breathing (respectively), the sympathoexcitatory effect of apnea superimposed on hypoxia has been shown to be more dramatic.

End-expiratory apnea, similar to what occurs repetitively during sleep in OSA, removes the sympathoinhibitory influence from the lung inflation receptors and may contribute to a greater net sympathoexcitation, yet does hypoxia play a role? Hedner *et al.* (14) reported a larger desaturation during apnea which produced larger increases in MSNA and postulated that hypoxemia superimposed on apnea was mediating the effect. In addition, Hardy *et al.* and Leuenberger *et al.* (12, 19) reported augmented MSNA and MAP responses with hypoxic apnea, as compared to normoxic apnea. Specifically, Hardy *et al.* investigated MSNA and MAP responses to spontaneous breathing of room air, hypoxic gas (10.5% oxygen) and hyperoxic gas (100% oxygen) (12). In healthy subjects breathing spontaneously, MSNA rose during hypoxemia and decreased with hyperoxia while MAP remained unchanged. Compared to spontaneous breathing, voluntary apnea was found to show both increased MSNA and MAP. Furthermore, the MSNA and MAP responses to apnea were augmented during hypoxic apnea and

attenuated following hyperoxia apnea, as compared to normoxic apnea. These data support a role for apnea, superimposed on hypoxia, mediating augmented sympathoexcitatory and pressor responses.

Similar to previous reports, the present investigation determined that controls demonstrated progressively augmented MSNA response to hypoxic apnea following increasing hypoxic gas exposures (16% and 12% oxygen, Figure 1a,). Yet, in contrast with Hardy *et al.* (12), elevation in MSNA during normoxic apnea was not significant. MSNA responses were determined to be progressively elevated above baseline in OSA subjects with each trial (21%, 16%, 12%, respectively). Importantly, elevation of MSNA responses during normoxic apnea was also determined. As compared to controls, augmented MSNA responses to apnea were evident at all levels of gas exposure in OSA subjects. Finally, the trend for increased MSNA was correlated to nadir Sa_{02} for all subjects.

The present study also determined that as the degree of hypoxic exposure prior to apnea increased; mean arterial and peak systolic pressure responses to hypoxic apnea were progressively augmented (Figure 2). Yet, similar to changes in MSNA, controls showed no elevation in blood pressure responses during normoxic apnea. In contrast, obstructive sleep apnea subjects showed a consistent elevation of peak systolic pressure responses compared to controls at all levels of gas exposure, while mean arterial pressure responses were no different than controls until maximum hypoxic apnea trials.

A correlation between ΔMSNA and blood pressure responses were determined in both groups and are represented in Figure 3. Although MSNA responses were

augmented in OSA, the correlation between the blood pressure response and sympathoexcitation were similar in both groups. This demonstrates the significance of the sympathoexcitatory response to apnea as it relates to the subsequent blood pressure response regardless of the group. These data lend further support for a causative relation between MSNA and arterial pressure during voluntary breathholding and likely sleep apnea. The apnea used in this model is voluntary, in comparison to the obstructive apnea seen with obstructive sleep apnea which may be considered a limitation to the present study. Yet, the sympathoexcitation which occurs during voluntary apnea has been shown in previous unpublished work from our laboratory to be comparable to that which occurs during obstructive sleep apnea. Additionally, it has been reported by Morgan *et al.* that "peak increases in sympathetic outflow and arterial pressure were comparable in obstructive and non-obstructive apneas of the same duration" (21). Therefore, these data also suggest that the arterial responses to a voluntary apnea are reflective of the degree of sympathoexcitation occurring during the apneic event. Study 2 addressed the question of whether differences in blood pressure responses can be determined between OSA patients and healthy normotensive subjects.

Altered chemoreflex function and MSNA in OSA. Several recent studies have investigated the hypothesis that altered chemoreflex function could mediate the chronic elevation of MSNA in OSA patients. Narkiewicz *et al.* examined the effect of breathing 100% O_2 versus room air on MSNA in untreated OSA subjects and matched controls, in an effort to deactivate the chemoreflex (22). During chemoreflex deactivation (100% O_2), MSNA and blood pressure were significantly reduced compared to breathing room

air only in untreated OSA subjects. Furthermore, Cutler *et al.* evaluated altered chemoreflex control of MSNA following intermittent hypoxic apnea exposure in a human OSA model (4). It was reported that chemoreflex control of MSNA is augmented through 165 min following 20 min of intermittent hypoxic apnea exposure. These data support both altered chemoreflex function in OSA, as well as, sustained chemoreflex sensitivity in an OSA model, which could lead to the chronic sympathoexcitation evident in sleep apnea.

The present study evaluated chemoreflex function, reported as chemoreflex gain (determined by $\Delta MSNA/\Delta Sa_{O2}$), and found augmentation in untreated OSA subjects compared to controls. Elevations in chemoreflex gain were apparent at all levels of gas exposure. Interestingly, the normoxic gas exposure and subsequent apnea demonstrated the greatest difference in chemoreflex gain between OSA subjects and controls ($p<0.001$). This supports previous data from Smith *et al.* (30) which demonstrated the threshold for sympathetic activation to occur in OSA subjects at higher oxygen saturations. Furthermore, these data support previous reports that a potential mechanism for the elevated daytime MSNA in OSA patients is altered chemoreflex function.

While altered chemoreflex gain has been demonstrated in untreated OSA, can chemoreflex gain be predictive of blood pressure response to hypoxic apnea? The present study determined that a correlation between chemoreflex gain ($\Delta MSNA/\Delta Sa_{O2}$) and peak systolic pressure, as well as, MAP responses following hypoxic apnea were evident in all subjects (Figure 4). This relationship demonstrates that the level of hypoxia and subsequent sympathoexcitation determines blood pressure responses to

apnea. Furthermore, since chemoreflex gain has been shown to be augmented in OSA, this relationship could potentially be used in a clinical setting to evaluate sympathoexcitatory responses to apnea.

Blood pressure response to voluntary apnea in OSA. Sleep apnea has been shown to be associated with several cardiovascular disorders and recently has been recognized by the Joint National Committee on Prevention, Detection, Evaluation and Treatment of High Blood Pressure (JNC7) as an "identifiable cause of hypertension" (2). In addition, sleep apnea patients have a generalized elevation in risk for most cardiovascular diseases with an increased incidence of dysrhythmias, myocardial infarction, stroke, and sudden death (1, 5, 11). Many recent studies have suggested that early recognition and treatment of OSA may improve cardiovascular function, reduce the risk of hypertension, and decrease elevations in sympathetic nerve activity (10, 35, 37). Yet, obstructive sleep apnea is largely under diagnosed and, in many cases, goes unrecognized in a clinical setting.

Some have postulated that the increase in negative intrathoracic pressure during apnea may play a role in raising arterial pressure via redistribution of blood volume. Recent reports by O'Donnell *et al.* (25) and Katragadda *et al.* (18) have demonstrated that the acute pressor response to obstructive apnea is primarily sympathetically mediated and abolished by autonomic blockade. Additionally, Morgan *et al.* reported that chemoreflex stimulation and resulting sympathetic outflow to skeletal muscle is more likely to mediate arterial pressure elevations than negative intrathoracic pressure during obstructive apnea (21). These data support the hypothesis that augmented MSNA is partially responsible

for mediating the cardiovascular effects of sleep apnea and the subsequent altered pressure response to apnea.

Due to the fact that increased MSNA also accompanies most cardiovascular diseases, it has been postulated that the chronic sympathoexcitation found in OSA contributes to the increased incidence and/or risk of cardiovascular disease found in these patients. Several recent studies have demonstrated that successful treatment of OSA with nasal continuous positive airway pressure (nCPAP), can produce a reduction in severity of hypertension while also leading to reductions in daytime MSNA (20, 23, 31, 34). Yet, a direct clinical measure of elevated MSNA is not currently available.

Study 2: Demonstrating augmented blood pressure responses in OSA in a clinical setting. In the experimental setting, the present study 1 demonstrated that untreated OSA subjects have augmented MSNA and blood pressure responses to normoxic, as well as, hypoxic apnea. Additionally, it was determined that chemoreflex gain is augmented in OSA subjects with an increased threshold for activation during normoxic apnea, as compared to controls. Furthermore, it was found that blood pressure responses to hypoxic apnea are correlated to changes in MSNA and chemoreflex gain. We hypothesized that chemoreflex gain, and thus, sympathetic response, is predictive of peak systolic pressure responses to hypoxic apnea as shown in Study 1. Therefore, Study 2 tested in a clinical setting the hypothesis that 20 s voluntary end-expiratory apnea would demonstrate augmented peak systolic pressure responses in untreated OSA patients.

It was determined that the peak systolic pressure response to voluntary end-expiratory apnea was elevated in untreated OSA patients compared to controls. In

addition, it was found that simple auscultation, with use of a sphygmomanometer and stethoscope, yielded peak systolic pressure measurements that were reliable and reproducible, as compared to finapres.

Several limitations of the clinical study need to be addressed. Due to a limited population from which to recruit subjects, there were 8 of the 20 OSA participants, and 8 of the 24 control participants, who reported a history of hypertension and were currently on medication. Data analysis of the groups as a whole showed a significant difference between OSA and controls peak systolic response ($p<0.001$). Yet, separate data analysis (Two-Way Analysis of Variance) adding hypertension as a factor, showed no difference between groups in peak systolic pressure response ($p= 0.074$), although the trend was still apparent. In addition, comparison of the hypertensive patients within the OSA groups and the normotensive OSA patients showed no difference in peak systolic response ($p = 0.242$). Hypertensive controls compared to normotensive controls were also found to have similar peak systolic pressure responses ($p = 0.180$). Furthermore, 2 of the 20 OSA participants, and 6 of the controls reported a history of smoking. The possible effects of smoking on endothelial dependent vasodilation can not be overlooked, yet was not addressed in this study.

An additional limitation must not be disregarded; the control subjects in this study were not systematically evaluated for occult OSA. In a study by Narkiewicz *et al.*, obese OSA patients and obese controls were evaluated to determine if obesity alone could account for chronic elevations if MSNA (24). It was reported that obesity, in the absence of OSA, is not accompanied by increased sympathetic activity. Yet, with the use of

overnight polysomnography in all control subjects, it was found that 9 of 30 obese controls had undiagnosed obstructive sleep apnea. The present study used a subject measure of daytime somnolence to screen for OSA, and while the Epworth scores were significantly different in OSA patients (Table I), the possibility of a small percentage of control subjects having undiagnosed sleep apnea exists.

In conclusion, the present study supports the hypothesis that the degree of hypoxia occurring during apnea determines the sympathoexcitatory response and resulting blood pressure responses, and that these responses are augmented in OSA subjects. OSA subjects were found to have elevations in MSNA, MAP and peak systolic pressure responses during normoxic apnea which suggest a lower threshold for sympathoexcitatory activation. The correlation between MSNA and blood pressure responses were significant and were found to be similar between OSA and controls. This further explains the importance of augmented sympathetic responses during apnea determining the elevated blood pressure response in OSA. Furthermore, the chemoreflex gain was augmented in OSA subjects. The correlation between chemoreflex gain, MAP and peak systolic pressure signify an important relationship which could potentially be used to evaluate augmented chemoreflex function; and thus, elevated MSNA, in a clinical setting. Finally, clinical evaluation of peak systolic pressure responses in untreated OSA patients were determined to be significantly elevated compared to controls. We believe this modest evaluation could be used as a marker for altered chemoreflex gain; and thus, elevated sympathetic responses, to voluntary apnea in suspected obstructive sleep apnea patients.

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FIGURE LEGENDS

Figure 1a. Difference from baseline in MSNA response to apnea, following 1 min exposure to 21%, 16% or 12% oxygen gas. (*$p<0.05$ vs. baseline. #$p<0.001$ vs. baseline. +$p\leq0.05$ vs. controls. Values are means $\pm$ SE, OSA; n = 9 subjects, Control; n=10 subjects)

Figure 1b. Chemoreflex gain (determined by $\Delta MSNA/\Delta Sa_{O2}$) following 1 min exposure to 21%, 16% or 12% oxygen gas and subsequent apnea. (#$p<0.05$ vs. control. *$p<0.001$ vs. control. Values are means $\pm$ SE, OSA; n = 9 subjects, Control; n=10 subjects.)

Figure 2. Mean arterial, peak systolic and peak diastolic blood pressure responses immediately following voluntary end-expiratory apnea in obstructive sleep apnea subjects and controls. One min exposure to 21%, 16% or 12% oxygen gas preceded apnea.. (*$p\leq0.05$ vs. baseline. #$p<0.001$ vs. baseline. +$p\leq0.05$ vs. control, $^{\tau}p<0.001$ vs. control. Values are means $\pm$ SE; OSA; n = 9, Control; n = 10 subjects)

Figure 3a. Difference from baseline in MSNA response to apnea, following 1 min exposure to 21%, 16% or 12% oxygen gas, expressed as correlation to mean arterial pressure response. Values are expressed as means for each subject during all levels of hypoxic apnea. (OSA; n = 9 subjects, Control; n=10 subjects.)

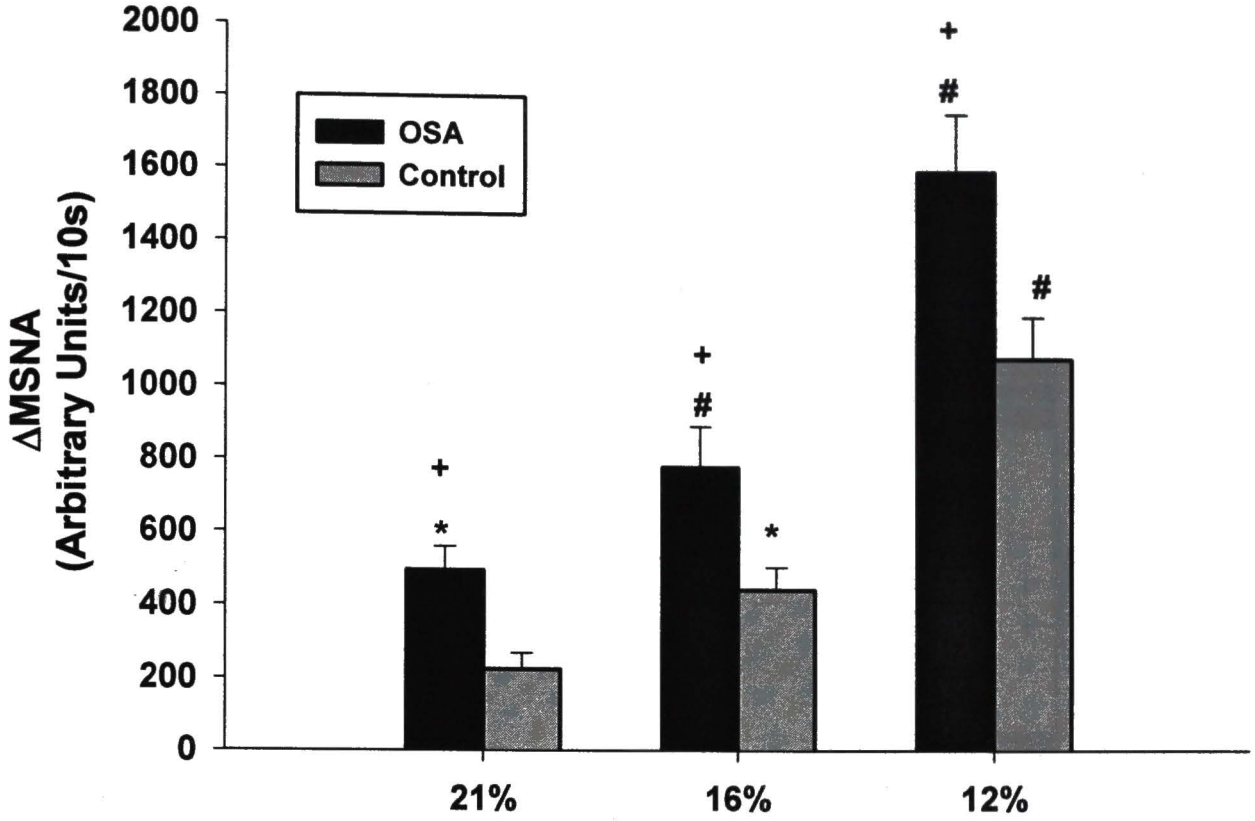
Figure 3b. Difference from baseline in MSNA response to apnea, following 1 min exposure to 21%, 16% or 12% oxygen gas, expressed as correlation to peak systolic pressure response.

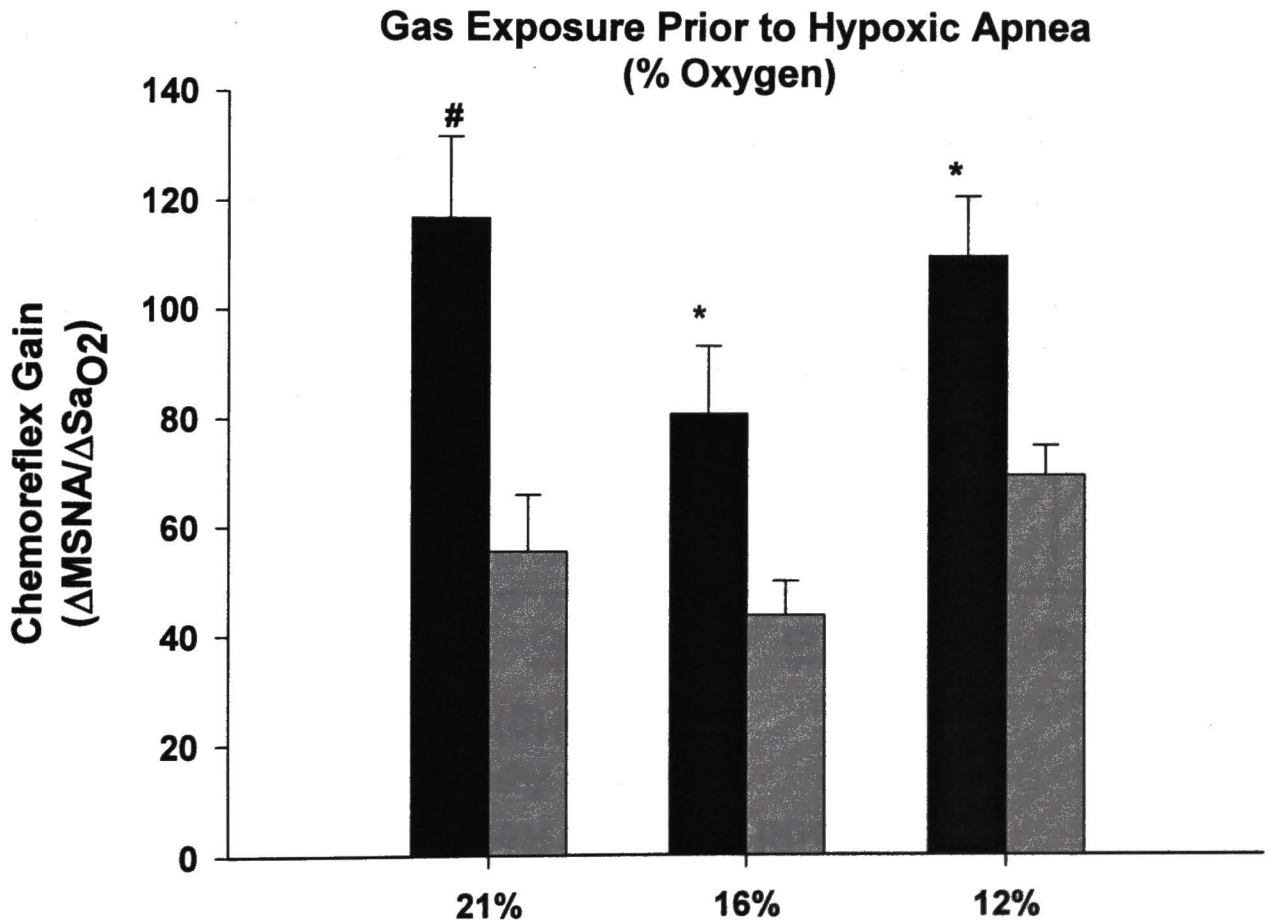
Values are expressed as means for each subject during all levels of hypoxic apnea. (OSA; n = 9 subjects, Control; n=10 subjects.)

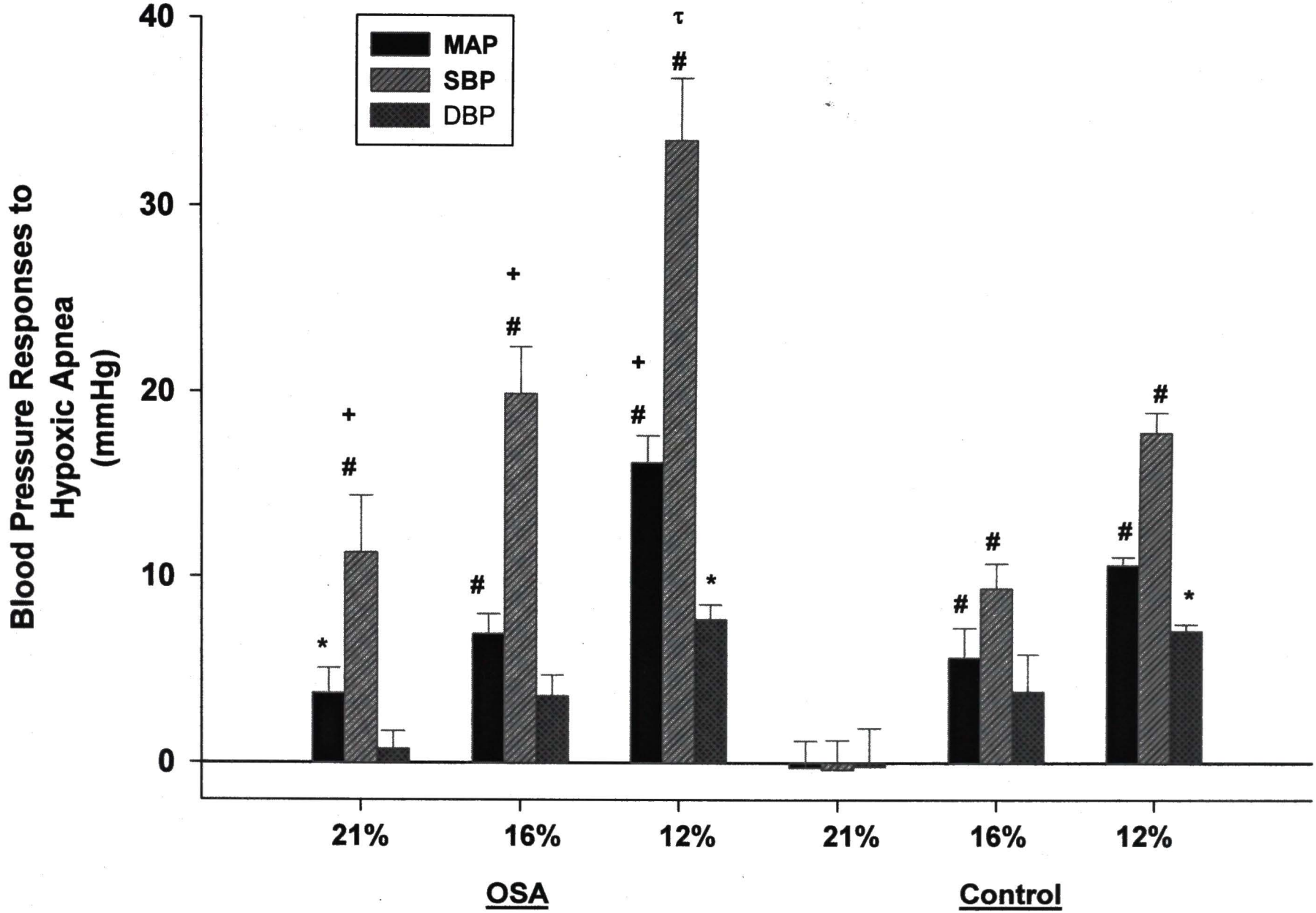
Figure 4. Chemoreflex gain (determined by $\Delta MSNA/\Delta Sa_{O2}$) following 1 min exposure to 21%, 16% or 12% oxygen gas and subsequent apnea, expressed as a correlation to blood pressure responses. Values are expressed as means for each subject at all levels of hypoxia. (n=19.)

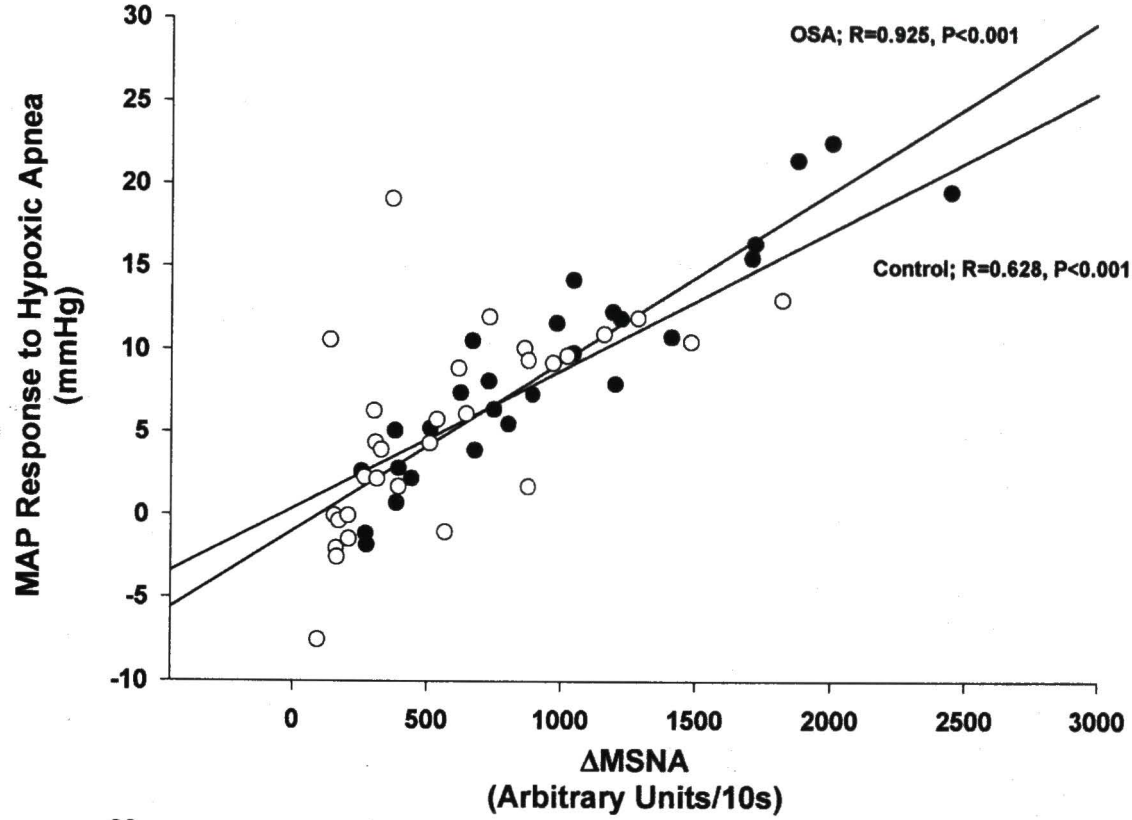
Table I. *Baseline measurements and demographic data.* Mean baseline heart reate, mean arterial pressure, body mass index (BMI), and Epworth scores for the OSA and control subjects. Additionally, nadir Sa_{O2} for each voluntary apnea are reported.

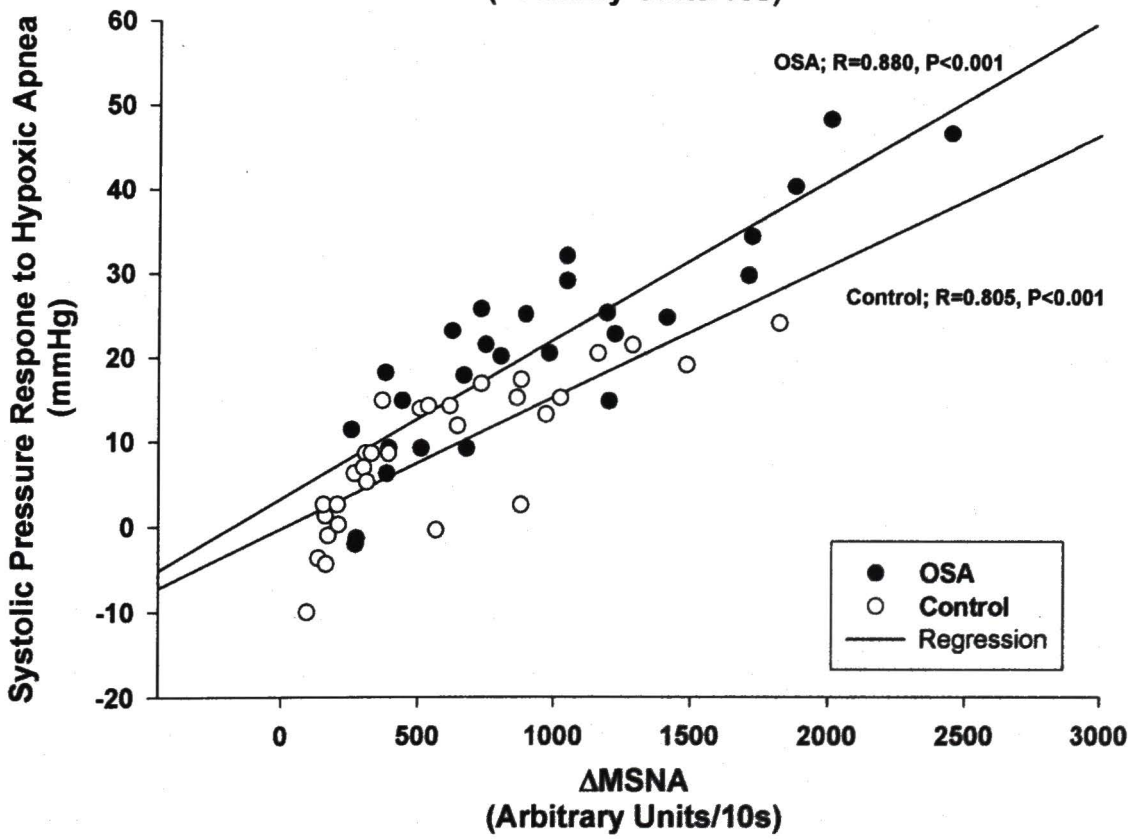
Figure 5. Difference from baseline in peak systolic pressure response during each voluntary apnea trial, reported as finapres measurements and auscultatory measurements separately. . (#$p<0.05$ vs. control. *$p<0.001$ vs. control. Values are expressed as means $\pm$ SE. OSA; n = 20. Control; n = 24.)


ΔMSNA
(Arbitrary Units/10s)
2000
1800
1600
1400
1200
1000
800
600
400
200
0
OSA
Control
+
*
+
#
*
+
#
#
21%
16%
12%


Gas Exposure Prior to Hypoxic Apnea
(% Oxygen)
Chemoreflex Gain
(ΔMSNA/ΔSaO2)
140
120
100
80
60
40
20
0
#
*
*
21%
16%
12%


Blood Pressure Responses to Hypoxic Apnea (mmHg)
40
30
20
10
0
MAP
SBP
DBP
21%
16%
12%
21%
16%
12%
OSA
Control


OSA; R=0.925, P<0.001
Control; R=0.628, P<0.001
MAP Response to Hypoxic Apnea (mmHg)
ΔMSNA (Arbitrary Units/10s)


OSA; R=0.880, P<0.001
Control; R=0.805, P<0.001
OSA
Control
Regression
Systolic Pressure Respone to Hypoxic Apnea (mmHg)
ΔMSNA (Arbitrary Units/10s)

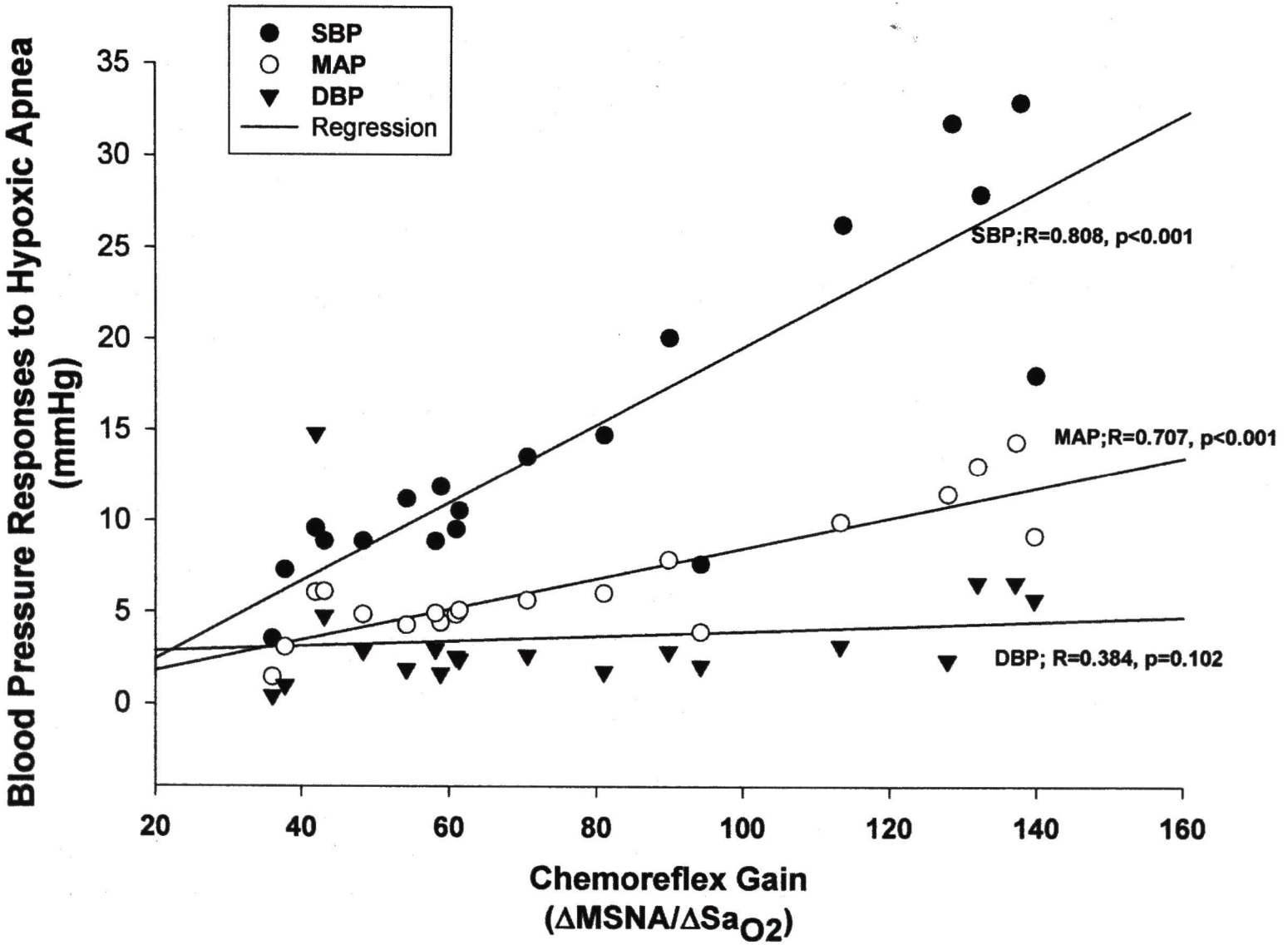
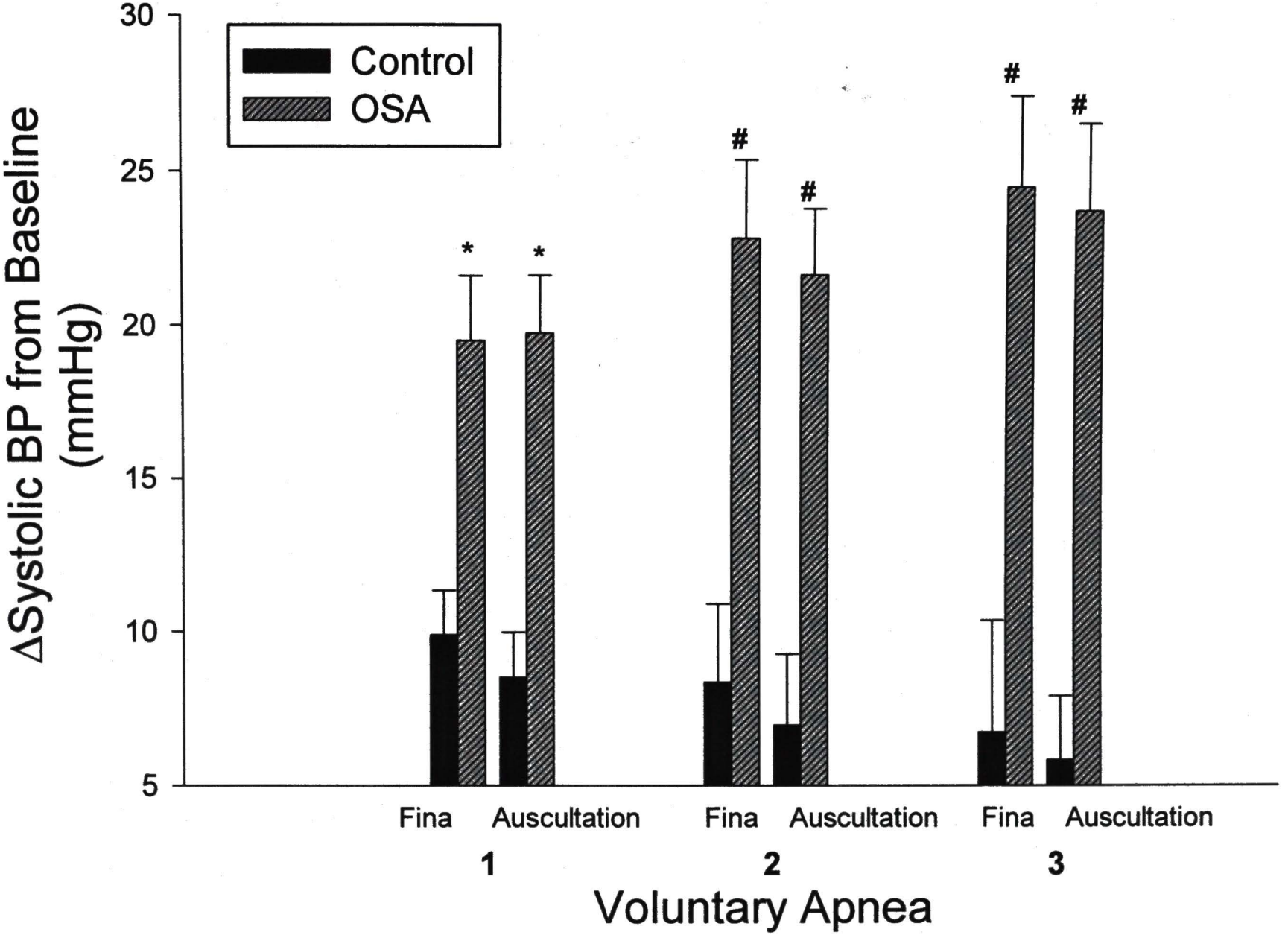

SBP
MAP
DBP
Regression
Blood Pressure Responses to Hypoxic Apnea (mmHg)
35
30
25
20
15
10
5
0
20
40
60
80
100
120
140
160
Chemoreflex Gain
(ΔMSNA/ΔSaO2)
SBP;R=0.808, p<0.001
MAP;R=0.707, p<0.001
DBP; R=0.384, p=0.102

Table I. Baseline cardiovascular measurements, demographic data and mean nadir Sa_{O2} during each apnea.

	Obstructive Sleep Apnea	Control	*p* Value
Baseline Heart Rate beats/min	71.43 ± 3.77	73.71 ± 2.80	0.624
Baseline Mean Arterial Pressure (mmHg)	97.51 ± 1.83	94.31 ± 1.70	0.213
Baseline Systolic Arterial Pressure (mmHg)	131.11 ± 2.80	130.04 ± 3.30	0.804
BMI	29.50 ± 1.01	26.95 ± 0.81	0.067
Age	52.75 ± 3.02	46.54 ± 3.37	0.185
Epworth Score	9.71 ± 1.34	4.83 ± 0.75	0.002
Mean Nadir Sa_{O2}			
Apnea 1	93.95 ± 0.63	94.48 ± 0.76	0.268
Apnea 2	93.35 ± 0.75	95.44 ± 0.55	0.013
Apnea 3	94.11 ± 0.52	95.29 ± 0.51	0.113


Control
OSA
ΔSystolic BP from Baseline (mmHg)
30
25
20
15
10
5
*
*
#
#
#
#
Fina
Auscultation
Fina
Auscultation
Fina
Auscultation
1
2
3
Voluntary Apnea

CHAPTER 6

FUTURE DIRECTIONS

The present investigation demonstrated that hyperoxia, following intermittent hypoxic apnea, can reverse both the basal elevation in MSNA, as well as, augmented chemoreflex control of MSNA to intermittent hypoxic apnea. This supports previous work which suggests tonic chemoreflex activation is a primary mechanism for sustained elevation of MSNA following intermittent hypoxic apnea. Additionally hyperoxia, reversing the effects of altered chemoreflex activation with hypoxic apnea, supports the hypothesis that this adaptation is occurring at the level of the peripheral chemoreceptors. Furthermore, the present study demonstrated the sympathoexcitatory response to apnea is related to the degree of hypoxia and that the MSNA response is predictive of the blood pressure response. In addition, it was determined that the MSNA response to hypoxic apnea and the consequent blood pressure response are augmented in untreated OSA patients. As a correlation between nadir Sa_{O2}, MSNA and blood pressure response was evident, this demonstrates that chemoreflex gain could be assessed from the blood pressure response to hypoxic apnea. Finally, in the clinical setting, it was demonstrated that the peak systolic pressure response to apnea was augmented in untreated OSA patients as compared to controls. We believe this demonstrates altered sympathoexcitatory responses to hypoxic apnea; and therefore, is indicative of

augmented chemoreflex gain. However, many questions remain regarding neural control of cardiovascular responses to hypoxic apnea in obstructive sleep apnea patients.

Is the response to hyperoxia following intermittent hypoxic apnea similar in obstructive sleep apnea patients? Future studies will be needed to determine whether the model used in the present investigation can be extended to the mechanism of altered chemoreflex control of MSNA and MAP in sleep apnea patients. While previous work has demonstrated that tonic activation of excitatory chemoreflex afferents is likely in OSA patients (42), the time course for attenuated basal MSNA and chemoreflex control following hyperoxia in this population is unknown. Additionally, while it has been demonstrated that OSA patients have elevations in MSNA and MAP following hypoxic exposure and decreased MSNA and MAP *during* hyperoxia, the present investigation did not evaluate MAP responses following hyperoxia. Furthermore, previous work in our lab did not show an elevation in MAP following 20 min of intermittent hypoxic apnea in healthy controls (8). Therefore, a systematic evaluation of the effects of hyperoxia on untreated OSA patients is warranted. If it was found that indeed MSNA and MAP were attenuated, this would strengthen the hypothesis that augmented responses to hypoxic apnea in OSA patients are mediated by altered peripheral chemoreflex sensitivity. Yet, the precise mechanism for this adaptation is still to be elucidated.

What is the mechanism of altered blood pressure response to hypoxia in OSA? The present study demonstrated that untreated OSA subjects had both elevations in MSNA and blood pressure responses to graded levels of hypoxic apnea as compared to controls; yet, the precise mechanism for this response has not been decisively

demonstrated. Many studies have demonstrated altered chemoreflex activity in sleep apnea (8, 42, 57) but is this the cause of the apparent altered chemoreflex gain? Could altered vascular responses to hypoxia be the mediator in pressor responses? Future studies investigating the altered pressor responses to hypoxia in relation to changes in MSNA activity are needed. Additionally, is there attenuation in baroreflex activity in long standing sleep apnea such that a given blood pressure is associated with a lesser sympathoinhibition compared to controls? Narkiewicz et al. examined the effects phenylephrine (baroreceptor activation) and nitroprusside (baroreceptor deactivation) infusion in normal controls and sleep apnea patients (44). While no difference was found between the groups in baroreceptor activation, nitroprusside infusion demonstrated decreased elevation in sympathoexcitation. Although this study demonstrates no change in MSNA during baroreceptor activation, hypoxic stimuli were not concurrently studied. Recently, Ziegler et al. reported apneic subjects tended to exhibit lower baroreflex sensitivity in response to hypoxia than controls (76). These data demonstrate that the interaction between hypoxia, MSNA, and reflex control of blood pressure to be complex. Therefore, future studies will need to address the precise mechanism of these interactions.

Can peak systolic pressure response be used as a marker of chemoreflex gain in a clinical setting? Further research will be needed to determine the precise link between chemoreflex gain, altered MSNA response and blood pressure response to apnea in OSA patients. While this study demonstrated in a laboratory setting the augmentation of MSNA and blood pressure responses to hypoxic apnea in untreated OSA subjects, the clinical correlation must be further evaluated. In a study by Narkiewicz *et al.*, obese

OSA patients and obese controls were evaluated to determine if obesity alone could account for chronic elevations if MSNA (43). With the use of overnight polysomnography in all control subjects, it was found that 9 of 30 obese controls had undiagnosed obstructive sleep apnea. The present study used a subject measure of daytime somnolence to screen for OSA, and while the Epworth scores were significantly different in OSA patients, the possibility of a small percentage of control subjects having undiagnosed sleep apnea exists. Furthermore, future studies would need to address the confounding factor of anti-hypertensive medications. As these medications affect vascular smooth muscle function and subsequent blood pressure response, a wash-out period of all medications should be attempted in a controlled setting.

In conclusion, this investigation demonstrated hyperoxia attenuates basal MSNA and chemoreflex control of MSNA following intermittent hypoxic apnea. Furthermore, this study demonstrated a relationship between level of hypoxia and sympathoexcitatory and blood pressure responses which were augmented in untreated OSA patients. These finding provide support for previous work on the adaptations of the cardiovascular system in response to hypoxic apnea, and perhaps have elucidated a potential non-invasive marker of chemoreflex gain in OSA patients.

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