The effect of mandibular advancement devices on sympathetic nerve activity and markers of cardiovascular health in obstructive sleep apnea patients: a prospective case series protocol

Emily King MSc[1]; Sana Ayesha MBBS[2], Craig Steinback PhD[3¶], Carlos Flores-Mir DSc[4¶*],

[1] Former MSc graduate student, School of Dentistry, University of Alberta, Edmonton, AB, Canada

[2] MSc student, Faculty of Kinesiology, Sport, and Recreation, University of Alberta, Edmonton, AB, Canada

[3¶] Professor, Faculty of Kinesiology, Sport, and Recreation, University of Alberta, Edmonton, AB, Canada

[4¶] Professor, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, AB, Canada

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Abstract

Study Objectives: The impact of mandibular advancement devices (MADs) on sympathetic nervous activity (SNA) has not been measured directly through microneurography. Therefore, this protocol aims to determine whether MADs improve SNA, vascular health, blood pressure, and indirect markers of SNA (heart rate variability and concentrations of neurotransmitters in the blood) in adult OSA participants.

Methods: Patients diagnosed with OSA will be referred by multiple dental providers in Edmonton, Alberta, certified to provide MAD therapy. A sample size of fifty participants is planned, considering a 20% dropout rate. Participants will be examined at baseline and again after three and six months of efficacious MAD therapy. The following outcomes will be recorded at each time point: direct SNA via microneurography, heart rate, continuous blood pressure, flow-mediated dilation (FMD); a marker of vascular health), blood concentrations of noradrenaline, apnea-hypopnea index (AHI) via a level three take-home sleep study, and MAD compliance via a scorecard for self-reported nighttime wear.

Clinical Implications: Understanding how direct and indirect measures of SNA relate to OSA therapy may be valuable, as some participants and researchers consider microneurography an invasive and complex technique. Improved SNA and vascular health would further support MADs as an important alternative to no treatment. Finally, research examining the impact of various OSA therapies on the sympathetic influence of blood pressure and the heart is critical to understanding how changes in cardiac autonomic modulation may influence cardiovascular risk.

Keywords: Mandibular advancement device, sympathetic nervous activity, endothelial dysfunction, cardiovascular health, obstructive sleep apnea.

Introduction

Obstructive sleep apnea (OSA) is a type of disordered breathing defined by repetitive airflow obstruction during sleep due to upper airway collapse. The obstruction can be either partial (hypopnea) or complete (apnea), and each obstructive event contributes to decreased blood oxygen (i.e., hypoxia). The severity of OSA has been mainly determined by the apnea-hypopnea index (AHI), which is the average number of airway obstructions experienced per hour of sleep. In middle-aged populations, it is estimated that 17% of women and 34% of men meet the diagnostic criteria for OSA.[1] OSA has been associated with various cardiovascular diseases, including hypertension, stroke, heart failure, and coronary artery disease.[2] A factor in this association may be the decrease in blood vessel health and the marked over-activation of the sympathetic nervous system (SNS) observed in OSA due to intermittent hypoxia.[3-6]

The SNS and the parasympathetic nervous system (PNS) are the two branches of the autonomic nervous system responsible for maintaining cardiovascular balance. The SNS innervates the heart and peripheral vasculature and responds to changes in blood pressure via baroreceptors and alterations in blood gasses like oxygen and carbon dioxide via chemoreceptors.[7] Autonomic responses to intermittent airway collapse in OSA are multifactorial, involving the effects of hypoxia, hypercapnia, inspiration against an obstructed airway and arousal from sleep. These mechanisms each act uniquely to elevate sympathetic nerve activity (SNA) during apneic episodes.[8] Autonomic abnormalities are speculated to link OSA with hypertension and cardiovascular morbidity.[9] Research has found daytime SNA is elevated even in mild, untreated OSA.[10] In addition, AHI has been positively correlated with SNA, suggesting a relationship between autonomic abnormalities and OSA severity.[11]

Continuous positive airway pressure (CPAP) is an effective treatment for OSA and has been shown to improve patients' blood pressure, AHI, and SNA.[12-14] However, the benefits are dose-dependent and rely on compliance. Unfortunately, 30-40% of patients are non-adherent to CPAP therapy over the long term.[15] Mandibular advancement devices (MAD) are an accepted alternative treatment for OSA. Despite their decreased efficacy in lowering AHI, they show similar reductions in blood pressure when compared to CPAP. They are suggested to improve disease progression through increased compliance compared to CPAP.[16,17] A 2013 cohort study of 570 subjects found that compared to patients with severe OSA who received CPAP or MAD treatment, untreated patients had a six times higher risk of dying from a cardiovascular event (2 per every 100 subjects). Additionally, there was no difference in cardiovascular death rate between MAD and CPAP-treated participants.[18] Therefore, understanding the effect of alternative therapies to CPAP on the progression of OSA via changes in AHI and cardiovascular markers is paramount. However, AHI has been criticized for being a poor proxy of nocturnal oxygen desaturation as it fails to quantify the depth and duration of each hypoxic event.[19] This is an essential consideration, as sleep-disordered breathing and low nocturnal oxygen desaturation have been identified as independent risk factors for sudden cardiac death.[20-22] In contrast, SNA is known to respond acutely and chronically to hypoxia.[23]

Studies investigating changes in SNA during MAD therapy have only been attempted using indirect measures like heart rate variability (HRV). HRV is calculated from an electrocardiogram (ECG), the time variation between each heartbeat, reflecting the balance between the SNS and PNS, or cardiac autonomic modulation.[24] Another promising indirect measurement is the use of skin sympathetic activity (SKNA) in OSA patients.[25,26]

A 2018 systematic review and meta-analysis by de Vries et al.,[16] investigated the effects of oral appliance therapy on a wide range of cardiovascular outcomes. They reported that only four studies, two RCTs, have researched the impact of MAD therapy on HRV. They concluded that three months of MAD therapy appears to have some small favorable effect on HRV. However, sample sizes are small, and results are somewhat heterogeneous as varying HRV analyses and parameters have been used to support this conclusion. Furthermore, conclusions could not extend our understanding of the SNS as the current consensus states the HRV relates most clearly to parasympathetic processes, but not necessarily to sympathetic processes.[23, 24, 27] A meta-analysis of HRV was not possible due to differences in units used for HRV parameters.[16] Regarding SKNA in OSA patients, studies by Peng et al.[25,26] have shown that average SKNA is reduced, SKNA frequency shifts, and not all OSA patients have increased sympathetic tone. Therefore, the SNS response to MAD therapy currently needs to be better defined and understood.

Difficulties in accurately assessing the human SNS have been noted in the literature. While no "gold standard" technique exists, the preferred assessment method is microneurography alongside noradrenaline spillover analysis.[27] Microneurography is the only method that directly records efferent post-ganglionic muscle sympathetic nerve activity in real-time as vasoconstricting impulses are sent to the periphery to regulate blood pressure. This is accomplished by inserting a tungsten microelectrode into a nerve fascicle.[28] Substantial and consistent evidence for altered SNA in OSA has come from microneurography, which has been established as an effective tool for measuring acute and chronic changes in SNA.[10-12, 27] Yet, most studies use HRV as a primary indicator for SNA due to its relative simplicity.[29] To our knowledge, no studies directly measure SNA in a MAD intervention with microneurography. Understanding how direct and indirect measures of SNA relate to OSA therapy may be valuable as microneurography is considered an

invasive and complex technique to perform by some participants and researchers.[30,31] Improved SNA and vascular health would further support MADs as an important alternative to no treatment. Finally, research examining the impact of various OSA therapies on the sympathetic influence of blood pressure and the heart is critical to understanding how changes in cardiac autonomic modulation may influence cardiovascular risk.

Methods and analysis

This study was approved by the Health Research Ethics Board-Health Panel, University of Alberta, Edmonton, Canada (Pro00108618).

Study design

A prospective self-controlled case series study is planned to evaluate the effect of MAD therapy in participants with diagnosed OSA on direct and indirect SNA measures, vascular health and other cardiovascular outcomes, including blood pressure and heart rate. A repeated measures design was chosen due to the large number of factors that can cause individual variability in SNA and vascular health. Additionally, using microneurography to obtain SNA outcomes is a highly technical procedure requiring training and resources. Therefore, a self-controlled repeated measures study design allows fewer recruitment requirements making the study more efficient.

Sample definition and eligibility criteria

Participants who fit the following inclusion criteria will be permitted to participate: 1) diagnosis of OSA by a physician, 2) prescription of a titratable MAD from a certified dentist who has followed the standard of care guidelines for appropriate patient selection and multidisciplinary patient management, 3) age of >18 years, 4) AHI > 5/h, 5) BMI < 40 kg/m2.

Participants will be excluded from participation for the following reasons: 1) an inability to breathe comfortably through the nose, 2) poor pulmonary function, 3) failure to obtain an OSA diagnosis from a physician, 4) diagnosis of central sleep apnea or mixed apnea, 5) anticipated medical changes that could alter the severity of OSA, 6) expected to change in body weight by >5% during the study, 7) pre-existing symptomatic non-respiratory sleep disorder (e.g. restless leg syndrome, chronic insomnia), 8) smoking habit or 9) prescription of a non-titratable appliance.

Recruitment and sample size rationale

Dental providers who adhere to the current standard of care guidelines outlined by the College of Dental Surgeons of Alberta for providing MADs will be approached to refer potential participants to our study. This referral process will rely on the clinical judgment of the referring dental professional to select patients for whom the MADs will be effective. To help ensure MAD efficacy, only patients who are prescribed adjustable MADs and agree to adhere to the titration protocol outlined by their dentist will be approached to participate in the study. Participants deemed unsuccessful in MAD therapy by their dental provider will not be retested after baseline.

A 2022 systematic review and meta-analysis revealed inconclusive data regarding the optimal length of intervention for producing a significant change in SNA. Changes in SNA were detected by microneurography after a single month of CPAP use, with similar changes detected after 12 months of CPAP use.[11] Several prospective studies measuring changes in SNA indirectly through HRV found small positive changes in cardiac autonomic modulation after three months of MAD intervention in mild to severe OSA patients.[32-35] Therefore, in the current study, participants with successful MAD titration as per their dental provider will be tested after three and six months of MAD use after reaching device efficacy. Analysis of the difference between SNA at T0, T1, and T2 considering an alpha=0.05, statistical power=0.9, and medium-large effect size (0.7) will

require an estimated minimum sample size of 44. A sample size of 50 subjects will be appropriate, considering a 20% dropout rate. We propose an interim analysis once 10 participants have completed the protocol to calculate the final sample size more accurately.

Primary Outcome

The primary outcome of this study is the effect of three and six months of efficacious MAD therapy on SNA in mild to severe OSA patients as measured directly through muscle sympathetic nerve activity (MSNA) microneurography.

Secondary Outcome

The secondary outcome of this study is the effect of three and six months of efficacious MAD therapy on vascular health as assessed through FMD analysis.

Other Outcomes

We will also explore the results of two indirect SNA measurements, HRV and blood noradrenaline concentration analysis, compared to direct measurement of SNA through microneurography, which is considered a more accurate technique for assessing the SNS.[27] Self-reported MAD wear (in hours per night) and post-treatment AHI will also be recorded to quantify therapy response for analysis and potential subgroup analysis of responders vs. non-responders. Responders will be defined as those who achieve a reduction in AHI by more than 50% and a final AHI of less than 10/h compared to the baseline.[36] Participant age, BMI, pre-treatment AHI, neck circumference and millimeters of mandibular advancement will also be recorded.

Laboratory protocol

After the initial screening and confirmation of written informed consent by a trained researcher, participants arrive at the laboratory for baseline testing before beginning MAD therapy. They will

come in the morning after a light breakfast (e.g., toast and water) and abstain from caffeine, alcohol, and strenuous exercise for 12 hours. Female participants with a regular menstrual cycle will be tested in the early follicular phase of the menstruation cycle. Female participants taking oral contraceptives or using intrauterine hormonal devices will be tested at their convenience. Subsequent testing sessions at three and six months of efficacious MAD wear will be completed simultaneously and under the same conditions as shown above.

In the lab, participants will be seated comfortably in a dental chair reclined 45 degrees, and a blood sample will be obtained to measure the concentration of the neurotransmitter noradrenaline in the blood. Next, participants will be instrumented with a standard lead II ECG (ML 132, ADInstruments, Colorado Springs, CO, USA) to record heart rate, and a blood pressure cuff and finometer to record beat-by-beat by finger pulse photoplethysmography (Finometer pro, Finapres Medical Systems, the Netherlands). All cardiovascular parameters will be acquired using an analog-to-digital converter (Powerlab/16SP ML 880; ADInstruments, Colorado Springs, CO, USA) interfaced with a personal computer. Commercially available software will analyze cardiovascular variables (LabChart V7.1, ADinstruments, Colorado Springs, CO, USA).

Next, a trained technician will record the multi-unit postganglionic MSNA via microneurography using a sterile tungsten recording microelectrode (35 mm long, 200 μm in diameter, tapered to a 1- to 5-μm uninsulated tip) which will be inserted into a muscle nerve fascicle of a sympathetic nerve bundle of the common peroneal nerve (Figure 1). A reference electrode will also be inserted subcutaneously 1–3 cm from the recording electrode. MSNA will be obtained by manually manipulating the microelectrode until the following criteria are confirmed: (1) pulse synchronous activity, (2) sympathoexcitatory response to end-expiratory apnea, and (3) no response to skin stroking or startle stimuli (e.g., sudden shout). Raw nerve signals are amplified (1000x pre-

amplifier and 100x variable gain isolated amplifier), band pass filtered (700-2,000Hz), rectified and integrated (constant decay 0.1s) to obtain an integrated voltage neurogram (model 662C-3; Iowa University Bioengineering, Iowa City, IA, USA). MSNA data will be sampled at 10,000 Hz and stored for offline analysis in LabChart (Powerlab Software, ADInstruments, Chart Pro v8.3.1).

Following instrumentation, participants will rest quietly for five minutes to relax. Next, MSNA, continuous blood pressure and heart rate data will be recorded for 10 minutes. After 10 minutes, a blood vessel health test, known as "FMD" will be completed. This test represents the production and the bioavailability of a potent blood vessel-derived-relaxing factor, nitric oxide (NO), that stimulates vasodilation and provides an excellent indicator of vascular health. This will be assessed in the brachial artery using a sphygmomanometer cuff and a linear array probe attached to a Duplex ultrasound machine (12MHz linear array probe, GE Vivid 7; DV12USB, Epiphan Systems, Mississauga, ON Canada) at an insonation angle of 60°. The sphygmomanometer cuff will be placed on the forearm, and then baseline brachial artery blood flow velocity and diameter will be recorded by an experienced sonographer for 1 minute. Next, the cuff will be inflated to a supra-systolic pressure (250 mmHg) to occlude forearm blood flow for 5 min. Next, the cuff will be rapidly deflated (~1 s) while the sonographer continues to record changes in brachial blood velocity and diameter (Figure 2). Subject preparation will be completed per the suggested guidelines by Thijssen et al., 2019.[37]

Once testing is completed, the participant will be sent home with a level three sleep study to use overnight (ApneaLink Air, ResMed, Sydney, Australia). This will allow the categorization of participants by AHI response for later analysis and discussion. The participant will also be given a scorecard to track the approximate number of hours the MAD is worn to screen for non-

compliance. Compliance will be defined as wearing the MAD for a minimum of ≥80% per night, ≥ five nights per week.[38]

Microneurography

Microneurography is a direct method of assessing the sympathetic outflow to the vasculature of the skin and muscle. Our study protocol focuses on MSNA, which consists of efferent vasoconstrictor impulses to the large peripheral vascular bed in the body's skeletal muscle. Through constricting skeletal muscle, MSNA critically influences blood flow and resistance in the vasculature and plays a critical role in blood pressure regulation and hemodynamic stability.[3] MSNA demonstrates a high intra-individual reproducibility even over years which allows for long-term monitoring of disease processes and therapeutic interventions.[37] However, MSNA has high inter-individual variability and is additionally influenced by short-term factors such as eating a large meal, maintaining a full bladder, experiencing mental stress, and changes in posture or breathing cadence. Long-term modifiers of MSNA include aging, sex and female hormonal cycles, genetics, and obesity. Therefore, the careful study design is critical in keeping short-term factors consistent during repeated measures and considering long-term factors in recruitment and data analysis.[3]

Flow-mediated dilation (FMD)

Endothelial dysfunction can predict the development of atherosclerosis and vascular disease and is a marker of cardiovascular risk. FMD is the most used test to measure an arterial's capacity to dilate by releasing NO in response to stimulating endothelial cells by reactive hyperemia.[40] FMD is a highly reproducible measure, even amongst testing centers, if standardized criteria for participant preparation are followed. However, patients with increased age, hypertension or dyslipidemia have more significant variability in FMD repeated measures.[37]

Blood concentrations of noradrenaline

Measuring noradrenaline spillover from sympathetic nerve impulses into the circulation indirectly assesses overall SNS activity. A significant positive correlation has been established between noradrenaline plasma concentration and microneurography measures of SNA in humans at rest.[41] However, interpretation is sometimes complicated as plasma noradrenaline is the net result of many factors, including noradrenaline clearance, metabolism, excretion, and total plasma volume. Therefore, plasma noradrenaline concentrations may not accurately reflect SNA in every situation.[42]

Heart rate variability (HRV)

HRV indirectly measures cardiac autonomic balance via power spectral analysis of an ECG. This technique produces several mathematical parameters describing time and frequency component variations within each heartbeat cycle. The amount of variation detected reflects the ability of the autonomic nervous system to maintain cardiovascular balance. HRV best describes parasympathetic nerve activity, and its ability to assess sympathetic nerve activity accurately is heavily debated in the literature.[23] Oversimplification during interpretation can lead to unfounded conclusions if standardized protocols are not followed.[24] However, AHI has been negatively correlated with HRV, suggesting a relationship with OSA severity.[6]

Data Analysis Plan

Direct and indirect SNA and FMD values collected at three and six months will be compared with baseline values for self-controlled comparisons. All LabChart and FMD video files will be assigned a random identifier before analysis to blind the analyzing researcher from participant ID and time point. A repeated measures MANCOVA will be used to assess statistical differences

between the mean of the dependent variables AHI, Oxygen desaturation index (ODI), cardiovascular variables, and mean percent change in MSNA variables (burst frequency and burst incidence) and FMD (normalized percent change) after three months of treatment and six months of treatment versus baseline. BMI, neck circumference, sex, and age will be covariates. Within-subject factors will include time (3 levels) to address our primary question and SNA measurement method (3 levels: direct, microneurography, and indirect; HRV and blood noradrenaline) to address our tertiary question of whether indirect methods produce comparable conclusions to direct methods.

Analysis 1

The effect of MAD therapy on MSNA will be determined by identifying and quantifying bursts of sympathetic activity from the integrated neurogram obtained during data collection. Bursts will be initially identified using a semi-automated peak detection algorithm and confirmed by visual inspection by a trained observer using established criteria by Meah et al., 2019,[31] including peak morphology, relation to diastolic blood pressure, and 3:1 signal-to-noise ratio. Sympathetic activity will then be quantified as burst frequency (bursts/min), burst incidence (bursts/ 100 heartbeats), burst amplitude (normalized to largest resting amplitude), and total activity (burst frequency multiplied by mean normalized burst amplitude). A decrease in these quantifications will indicate a positive change in MSNA. If accompanied by a reduction in AHI, sufficient self-reported MAD compliance, and a non-significant change in short-term influencing factors, we may detect an association between successful MAD therapy and decreased MSNA.

Analysis 2

The effect of MAD therapy on vascular health will be determined through FMD analysis. Baseline arterial diameter (D_A – arterial diameter) will be compared to the peak post-occlusion D_A expressed

as the change in arterial diameter (FMD) as well as percent change (FMD%). FMD values will be normalized to vascular shear stress stimulus, calculated as the product of shear rate and blood viscosity leading to peak arterial diameter. Blood viscosity will be measured at a shear rate of 225s^{-1} using a cone-plate rheometer (DVNext Rheometer, Brookfield Ametek, Middleboro, MA, USA) at 36 °C for 1 minute. The shear rate will be calculated as $8Q_A/D_A$; where Q_A is arterial blood velocity.[37] Brachial artery diameter and blood velocity measurements will be obtained from offline ultrasound analysis (Brachial Analyzer, Medical Imaging Applications; qDAT, Penn State). Forearm vascular conductance will be calculated as blood flow velocity divided by mean arterial blood pressure. If a decrease in AHI accompanies an increase in normalized FMD%, we may detect an association between successful MAD therapy and improved vascular health.

Analysis 3

HRV analysis will be conducted on five minutes of artifact-free ECG data to obtain the time-domain parameters RRI (ms), SDNN (ms), RMSSD (ms) and the frequency-domain parameters total power (TP; ms^2), very low frequency (VLF; ms^2: approximately <0.04 Hz), low frequency (LF; ms^2; range 0.01-0.15 Hz), high frequency (HF; ms^2; range 0.15-0.4 Hz), low frequency normalized units (LF_{nu}; LF/(TP-VLF) x 100), and high frequency normalized units (HF_{nu}; HF/(TP-VLF) x 100). Calculations and interpretations will follow the recommendations outlined in the European Society of Cardiology and the North American Society of Pacing and Electrophysiology 1996 Task Force paper on HRV best practices and standards. Doing so will allow us to compare our findings with other works following the same guidelines, as HRV following dissimilar analyses is otherwise incomparable.

Blood samples will be analyzed for noradrenaline concentrations in nanomoles per liter (nmol/L) as an additional indirect measure of SNA. Concentrations of blood noradrenaline and HRV

parameters at three months and six months will be compared against baseline values. Correlations between direct and indirect measures will be explored. Potential differences in significance amongst direct and indirect findings will be compared by including indirect and direct measures as within-subject factors for a within-subject contrast in the MANCOVA. Conflicting results between direct and indirect variables will be discussed.

Limitations

As long as each device reduces OSA signs and symptoms that are meaningful for the patient, as deemed by the provider and the patient, success would be implied. Researchers will not make that decision but will analyze the end-of-treatment data. Nevertheless, due to the expected sample size, well-supported conclusions based on MAD design will be unlikely. A proof-of-concept initial clinical trial is the first step that could or could not justify similar future efforts but focused on specific MAD designs, titration protocols, and initial mandibular position,

The proposed methodology for this clinical trial is based on the dental provider making the decision of who has the proper indications to use a MAD device. To some degree, that is based not only on AHI values but also on symptomatology. The lack of a specific set of more stringent inclusion criteria could homogenize the sample would reduce the external validity or reflection of what is happening in community dental practices. A proof-of-concept initial clinical trial is the first needed step that could or could not justify similar future efforts but focused on more stringent case inclusion criteria.

Clinical judgments play into the definition of appliance success, such as whether patient goals were reached (being able to sleep with a bed partner again) or achieving decent AHI reduction in the face of TMJ symptoms, etc. Such decisions could be perceived as a limitation of this study design. An attempt to control for this is proposed by measuring AHI/ ODI via a Level 3 sleep study

and categorizing participants accordingly. Own objective definitions of success from that data (i.e., 80% reduction in AHI and AHI < 10 or similar) can be attempted thereafter.

Pain is a multifaceted experience that multiple causes can trigger. Hence, it is very tricky to properly measure it. In future studies, a pain index scale could be considered, but such a simplistic approach would not clarify exactly why pain occurs or is triggered.

Public involvement

The findings of this research are unlikely to be of direct significance to patients using MADs. Although OSA modulates SNA in a manner that may lead to a higher prevalence of cardiovascular disease, heart arrhythmia and sudden cardiac death, the clinical significance of changes in SNA during OSA interventions is a topic for future prospective research.[43] However, our findings may provide researchers and clinicians with more information about how MADs affect the pathophysiology of the nervous system in OSA patients. Before developing this study protocol, dental professionals and specialists were consulted regarding their clinical practices in delivering MAD therapy, the number of patients they treat with MADs each month and their perceived clinical benefit of a deeper understanding of the effects of MADs therapy on cardiovascular and neurovascular pathophysiology found in OSA patients.

It appeared that most clinicians follow a similar clinical protocol and that most providers treat 1-2 patients with MAD therapy every 1-3 months. All providers voiced interest in a deeper understanding of the effect of MAD therapy on cardiovascular and neurovascular pathophysiology, which has ultimately driven their willingness to refer patients to our study. Future discussions with dental providers will seek feedback on the relevance of findings to their clinical practice and guidance on conducting a randomized controlled trial once initial data is available to direct resources.

Abbreviations

Apnea-hypopnea index: AHI

Continuous positive airway pressure: CPAP

Electrocardiogram: ECG

Flow-mediated dilation: FMD

Heart rate variability: HRV

Mandibular advancement devices: MAD

Muscle sympathetic nerve activity: MSNA

Nitric oxide: NO

Oxygen desaturation index: ODI

Obstructive sleep apnea: OSA

Parasympathetic nervous system: PNS

Sympathetic nervous activity: SNA

Sympathetic nervous system: SNS

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Figure legends

Figure 1: A microneurography recording from a tungsten microelectrode. The right of the image depicts the resultant filtered and integrated neurograms.

Figure 2: Ultrasound images showing post-occlusion changes in brachial arterial dimension and blood flow after forearm cuff release during flow-mediated dilation test.

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