The Effect of Mandibular Advancement Devices on Sympathetic Nerve Activity and Markers of Cardiovascular Health in Obstructive Sleep Apnea Patients: A Feasibility Trial

by

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Abstract

OBJECTIVE: To determine whether mandibular advancement device (MAD) therapy improves sympathetic nerve activity (SNA) in mild to moderate adult OSA patients, or in those with severe OSA who cannot tolerate CPAP. Secondarily, we will investigate whether MAD therapy improves vascular health and indirect markers of SNA (heart rate variability and concentrations of noradrenaline in the blood).

HYPOTHESIS: There will be a significant reduction in SNA and improvement in blood vessel health in mild to moderate OSA patients who are compliant with and respond to MAD therapy.

DESIGN: This feasibility trial is a prospective case series examining subjects at baseline, three months and again at six months after a MAD intervention. Participants were referred by dental providers who are certified to provide MAD therapy. The following outcomes were recorded at each time point: muscle sympathetic nerve activity (MSNA), heart rate, blood pressure, flow-mediated dilation (FMD; a marker of vascular health), blood noradrenaline, apnea-hypopnea index (AHI) and oxygen desaturation index (ODI) via a take-home sleep study, and MAD compliance via a scorecard for self-reported nighttime wear.

METHODOLOGY: The participants were seated in a chair reclined at 45 degrees. Blood was drawn to measure the concentration of noradrenaline in the blood as an indirect measure of SNA. Next, participants were instrumented with an ECG, finometer, and blood pressure cuff. Next, a trained technician recorded multi-unit postganglionic MSNA via microneurography, which involves the insertion of a sterile tungsten recording microelectrode into a muscle nerve fascicle of a sympathetic nerve bundle in the peroneal nerve. MSNA was obtained through manipulation of the microelectrode until a characteristic nerve recording pattern was observed. Following instrumentation, the participant rested quietly for 10 minutes while MSNA, beat-by-beat blood

pressure and heart rate data were recorded for 10 minutes. After 10 minutes, a standardized test, known as flow-mediated dilation (FMD) began to assess endothelial function as a marker of vascular health. Participants were sent home with a take-home sleep study to measure AHI and ODI for the categorization of patients according to MAD response for data analysis. Participants also kept a scorecard of the number of hours the MAD was worn per night for each 3-month interval between time points.

CONCLUSION: Descriptive statistics are presented (n=4), and suggestions to enhance future trial feasibility are discussed.

Preface

This thesis is an original work by Emily King. Data collection was aided by the members of the neurovascular health lab at the University of Alberta. The research project presented in this thesis received research ethics approval from the University of Alberta Research Ethics Board as "The Effect of Mandibular Advancement Devices on Markers of Cardiovascular Health in Non-compliant Obstructive Sleep Apnea Patients," Pro00108618, approved 4/19/2021.

Acknowledgments

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Although there were some challenges encountered during this project, I'm proud of what was achieved with the help of these amazing people.

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CHAPTER 1: INTRODUCTION

There are three categories of sleep apnea: obstructive sleep apnea (OSA), central sleep apnea (CSA) and mixed apnea, or complex sleep apnea syndrome. OSA is the most common form of sleep apnea and is characterized 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). In middle-aged populations, it is estimated that 17% of women and 34% of men meet the diagnostic criteria for OSA [1].

CSA is defined by a lack of inspiratory effort to breathe. It is less common than OSA and is often seen in patients with heart failure. CSA may be caused by a variety of factors, such as brainstem abnormalities or underlying neurological conditions. Mixed or complex sleep apnea is a combination of OSA and CSA [2]. The work of this thesis will focus on OSA. The main risk factors for the development of OSA include male sex, obesity, increased age, and genetic factors [3, 4].

A diagnosis of sleep apnea is confirmed by a level 1 nocturnal polysomnography (PSG) conducted in a laboratory or by an unattended level 2 or level 3 take-home sleep test [5]. PSG remains the gold standard test for sleep apnea; however, due to the expense and limited availability of PSG in some centers, take-home sleep studies are a suitable alternative if properly interpreted by a licensed practitioner [5, 6]. The severity of sleep apnea is typically measured using either the apnea-hypopnea index (AHI) or the respiratory disturbance index (RDI). The AHI is the number of apneas and hypopneas per hour of sleep, while the RDI includes the number of additional respiratory events, such as snoring and respiratory effort-related arousals per hour of sleep. The AHI is the most used metric for quantifying sleep apnea severity.

Generally, an AHI or RDI of 5 to 15 events is considered mild, 16 to ≥ 30 is considered moderate and ≥ 30 is considered severe [7]. The American Academy of Sleep Medicine defines OSA as having an AHI of 15 events/hr (with or without symptoms such as excessive daytime sleepiness, etc.) or 5 events/hr with OSA symptoms [8]. An advantage of using AHI or RDI to measure sleep apnea severity is that they are objective measures easily obtained from a sleep study and used in research to categorize disease states. However, a large limitation is the failure to account for the severity or duration of individual apneic events. An apnea that lasts for 30 seconds may have a greater impact on sleep and oxygenation than an event that lasts for 5 seconds [9]. Additionally, the AHI and RDI do not consider other factors of disease severity, such as blood oxygen levels, the level of arousal from sleep or the degree of patient symptoms.

Another measure of sleep apnea severity is the oxygen desaturation index (ODI), which measures the number of times oxygen levels drop below a certain threshold during sleep. The ODI can provide additional information about the impact of sleep apnea on oxygenation, but AHI is reported most commonly. Symptoms of sleep apnea that may help to quantify the severity of the disease include snoring, daytime sleepiness, waking up frequently to urinate, headaches, dry mouth, and restless sleep.

Sleep apnea can have significant societal implications for affected individuals. Daytime sleepiness, difficulty concentrating, and irritability may negatively influence quality of life and workplace productivity [10]. It has been associated with numerous comorbidities, including diabetes, depression, and metabolic syndrome, all of which may burden an affected individual with higher healthcare costs and financial strain [11]. Treating sleep apnea can have positive impacts on an individual's quality of life and overall health, as well as potentially reducing costs and risks to society through reducing accidents and missed days of work due to illness [10, 11].

Sleep-disordered breathing and lowest nocturnal oxygen desaturation have also been identified as independent risk factors for death [12-14]. OSA specifically has been associated with increased cardiovascular morbidity and mortality and plays a role in the pathophysiology of cardiovascular disease (CVD) [3, 13, 15], including stroke, heart failure, and coronary artery disease [16]. However, it is difficult to distinguish whether OSA is involved mainly in the progression of CVD as a comorbidity or in the initiation of CVD as a trigger factor [3, 17]. Studies have shown that OSA leads to increased sympathetic nerve activity (SNA), metabolic dysregulation, inflammation, oxidative stress, and vascular endothelial dysfunction, all of which may play roles in developing CVD [17, 18]. There is also strong evidence for a relationship between OSA and the development of hypertension, a common precursor to CVD [3, 19]. The Wisconsin Sleep Cohort Study suggested that persons with mild and severe sleep-disordered breathing have two and three times, respectively, increased odds of presenting with hypertension four years later, independent of known confounding variables [19]. A factor in these associations could be the marked over-activation of the sympathetic nervous system observed in OSA [20-23] and the resultant dysregulation of the cardiovascular autonomic nervous system (ANS), which is responsible for maintaining hemodynamic homeostasis [3, 20, 22-24].

The cardiovascular ANS regulates heart rate and blood pressure in response to changes detected by arterial baroreceptors, cardiopulmonary receptors, and chemoreceptors [25]. The ANS consists of the parasympathetic and sympathetic nervous systems, which work together to adjust cardiovascular function through the vagus nerve and adrenoreceptors in the heart and blood vessels. Parasympathetic activity slows heart rate, while sympathetic activity increases heart rate and contractility. Sympathetic activation can also cause increases in vasoconstricting signals to the peripheral blood vessels, resulting in substantial increases in blood pressure and

blood catecholamines [24, 26]. When a receptor detects a change, the ANS adjusts its stimulation of certain heart and blood vessel tissues, causing changes in tissue response and contractility to ensure adequate cardiac output to vital organs [27].

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 SNA during apneic episodes [28]. Research has found daytime SNA is elevated even in mild, untreated OSA [29]. In addition, AHI has been positively correlated with SNA, suggesting a relationship between autonomic abnormalities and OSA severity [30].

In OSA, the intermittent cessation of airflow leads to decreased oxygen levels and increased carbon dioxide levels in the blood, activating the chemoreceptor reflex and increasing SNA. This results in peripheral arterial vasoconstriction. When apnea ends, there is a sudden increased cardiac output against a periphery of vasoconstricted arteries, leading to a surge in blood pressure [3]. Repeated intermittent hypoxia may alter the chemoreceptor reflex and arterial baroreflex leading to chronic excitation of the sympathetic nervous system [15]. This may explain why studies comparing weight-matched controls with OSA patients have found markedly increased SNA at night and during wakefulness, in the absence of hypoxia, even in newly diagnosed patients [26, 31].

Increased SNA may be an early marker of OSA's pathological effects, but it is not the only negative consequence of the condition that can contribute to CVD. Other effects of OSA include the production of reactive oxygen species, which are increased in OSA, and can cause endothelial cell dysfunction, reducing the production of nitric oxide and vasodilation [15, 21]. A dose-response relationship between OSA severity, SNA, markers of endothelial health and

hypertension appears to exist [30, 32, 33]. If endothelial dysfunction and increased SNA exist together in OSA, these interactions are a likely mechanism for developing hypertension and CVD.

There are several treatment options for OSA which aim to maintain or improve airway patency during sleep to reduce AHI and nocturnal oxygen desaturation [34-40]:

1. Continuous positive airway pressure (CPAP) is the first-line therapy for patients with moderate to severe apnea and excessive daytime sleepiness. CPAP involves the use of a machine and facemask to deliver a continuous airflow, carefully titrated to maintain airway patency.
2. Mandibular advancement devices (MADs) are a type of oral appliance used to reposition the mandible forward to prevent collapse between the oropharynx and the base of the tongue. Forward posturing of the mandible also increases genioglossus and supra and infrahyoid muscle tonus, resulting in an increased pharyngeal air space [41, 42].
3. Weight loss can reduce the severity of OSA as weight gain, particularly around the neck, can enhance external pressure on the airway.
4. Lifestyle changes, such as changes in sleep posture, quitting smoking and avoiding alcohol and sedatives, may also improve OSA symptoms.
5. Hypoglossal nerve stimulation to increase genioglossus activation, which is the main pharyngeal dilator muscle.
6. Surgical interventions for adult patients, including maxillomandibular advancement, to increase the size of the upper airway at the pharyngeal level.

Continuous positive airway pressure (CPAP) is an effective treatment for OSA and has been shown to improve blood pressure, AHI, endothelial function, and SNA in patients [26, 43-46]. 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 [47]. MADs are an accepted alternative treatment for OSA and are suggested to improve disease progression through increased compliance compared to CPAP [48, 49]. Recent guidelines consider CPAP and MADs as equally accepted first-line therapies in mild to moderate OSA or in severe OSA in adults who cannot tolerate CPAP or refuse surgery [35, 50]. In severe OSA, MADs may be less effective as a standalone treatment and may be prescribed in combination with other therapies such as CPAP or surgery [50].

MAD therapy is a type of oral appliance therapy (OAT), which is an umbrella term that includes other appliances like palate lifters, tongue-retaining devices and snoring aids. MADs rest on the occlusal surfaces of teeth and posture the mandible forward. Many different designs of MADs are commercially available, from prefabricated, store-bought devices to customized, laboratory fabricated devices prescribed by a healthcare provider. Current guidelines recommend the use of custom titratable devices. MADs may be further categorized into two categories, 1) monobloc and 2) duobloc. Monobloc appliances consist of upper and lower occlusal splints, joined by a ridged connector that is not removable or adjustable. Therefore, Monobloc appliances are fabricated to a predefined amount of mandibular protrusion. Duobloc appliances have upper and lower occlusal splints that are connected by bars, elastics, telescopic rods or lateral fins used to advance the lower splint in relation to the top splint [1].

MADs may also be categorized as titratable or non-titratable, where titratable appliances are typically custom made for a patient and allow the healthcare provider to adjust the amount of

mandibular advancement slowly until the ideal position is found. This is desirable as titration allows the provider to find the amount of mandibular protrusion that will optimize AHI reduction, but also be tolerable for the patient to enhance compliance [93]. Titratable duobloc devices may be further categorized as “midline traction” or “bilateral thrust” designs, which denotes where titratable connectors are located on the device [94]. Titratable appliances often allow more freedom for vertical and lateral movement of the mandible, whereas monobloc appliances restrict mandibular movement, which may cause discomfort for some patients [93].

MAD design is an important factor for patient comfort and compliance. Maximizing tongue space and allowing for some vertical and lateral movement of the mandible may be preferred by patients [95]. Lateral fins may allow more comfort by allowing mouth opening; however the fins must be designed in such a way that prevents the mandible from returning to a backwards position during sleep. Elastics may be used to aid in this issue and encourage mouth closure into the correct posture. Another consideration, is the tendency for duobloc appliances to break at their connecting components, requiring frequent adjustments [95].

A 2021 systematic review and metanalyses found that AHI reduction favoured a monobloc design, however, differences in comparison to a bilateral thrust design were small and effect size was low. The difference in AHI reduction between monobloc and bilateral thrust design may have been that the monobloc design does not allow mouth opening and thereby prevents autorotation of the mandible to a backwards position. Midline traction duobloc devices were found to have a more favourable effect on AHI reduction in comparison to monobloc and bilateral thrust duobloc designs. However, this difference was again small. Therefore, it was concluded that a clearly superior design cannot be identified and that device selection should be

based on provider knowledge and aspects of the device such as cost, patient comfort and number of return visits required to fit the device [94].

There are two main mechanisms by which MADs are proposed to prevent upper airway collapse. One is the advancement of the mandible and adjacent soft tissues, increasing posterior airway space. Greater mandibular protrusion has been associated with more considerable AHI reduction. Second is the stimulation of the pharyngeal motor system, increasing genioglossus and upper airway muscle tonus [42].

Regarding OAT compliance, a systematic review by Ferguson et al. found that MAD compliance monitor data reported an average of 6.8 hours of use per night for 7.7 months. Studies using self-reported data, over a duration between 2 and 5 years, found appliance adherence rates ranged from a median of 48% up to 90% in the best cases. Adverse events during OAT that may contribute to poor compliance include temporomandibular joint (TMJ) pain, TMJ sounds, myofascial pain, gagging, tooth pain, salivation, and occlusal changes (incisor inclination and reduction in overbite). While there are potential adverse side effects of OAT, most were reported to be minor or temporary [42].

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 [51]. Therefore, understanding the effect of alternative therapies to CPAP on the progression of OSA via changes in AHI and cardiovascular markers is paramount.

In comparing MAD efficacy to CPAP, Ferguson et al. found that CPAP was better at reducing AHI in 52% of patients. Still, both MADs and CPAP provided similar reductions in

subjective symptoms such as sleepiness. Although MADs have higher compliance rates and are a recognized alternative treatment for mild to moderate OSA, they are not widely used. While the reason is not fully understood, the reported decreased efficacy in lowering AHI compared to CPAP therapy and the lack of specific selection criteria to identify those who may benefit from MADs are likely factors [52].

Furthermore, this lack of accepted selection criteria for patients who may respond positively or negatively to MAD therapy suggests that most trials are conducted in unselected patients, many of whom may not respond ideally. A 2022 systematic review identified that responders to oral devices are younger in age, with lower BMI and neck circumference. Responders also had a shorter distance from the hyoid bone to the third cervical vertebra, a lower anterior and posterior facial height, a shorter maxillary length, a shorter airway, greater stability of ventilatory control, lower pharyngeal collapsibility, and a higher minimum oxygen saturation at night. This meta-analysis suggests that predictive variables are emerging in the literature and should be a focus of future research. Another finding was the heterogeneity of definitions for "successful" appliance therapy. They suggested that achieving an AHI <10 and a reduction in AHI >50% was the most commonly accepted definition of success [53].

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 [53, 54]. This is an essential consideration, as sleep-disordered breathing and lowest nocturnal oxygen desaturation have been identified as independent risk factors for death [12-14]. The European Respiratory Society 2021 guideline on non-CPAP therapies concluded that future research should investigate outcomes other than AHI, specifically cardiovascular outcomes in patients with varying comorbidities and severities of OSA [35].

Studying SNA in those receiving treatment for OSA may provide important insight into the mechanisms of therapy as SNA is known to increase in response to hypoxia, hypercapnia and inspiration against an obstructed airway [28, 55]. However, difficulties in accurately assessing the human sympathetic nervous system have been noted in the literature. While no "gold standard" technique exists, the preferred assessment methods are microneurography alongside noradrenaline spillover analysis [56]. Microneurography is the only method that directly records efferent post-ganglionic muscle sympathetic nerve activity (MSNA) 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 [57]. Substantial and consistent evidence for elevated MSNA in OSA has come from microneurography, which has been established as an effective tool for measuring acute and chronic changes in MSNA [26, 30, 43, 56]. Meanwhile, few studies have been conducted to assess changes in endothelial health during a MAD intervention, with only one control trial finding a significant positive change in endothelial health after two months of MAD therapy [32, 58, 59].

To our knowledge, no studies have measured MSNA in a MAD intervention with microneurography. In addition to endothelial health, we would like to directly measure MSNA before and after a MAD intervention to determine if MADs can improve sympathetic overdrive. Improved MSNA and endothelial function would further support MADs as an important alternative to no treatment. Indirect markers of SNA (heart rate variability (HRV) and blood noradrenaline concentrations) will be measured alongside microneurographic readings [60, 61]. Understanding how both direct and indirect measures of SNA are affected by OSA therapy may be valuable as microneurography is considered an invasive and difficult technique to perform by some participants and researchers [44, 62, 63].

CHAPTER 2: SCOPING REVIEW

Introduction

OSA has been associated with various cardiovascular diseases [16]. A factor in this association may be the marked elevation in SNA observed in OSA [20-23]. AHI has been negatively correlated with HRV and positively with SNA, suggesting a relationship between autonomic abnormalities and OSA severity [17].

HRV has been used widely in the literature to indirectly reflect the balance between cardiac sympathetic and parasympathetic activity or cardiac autonomic modulation. HRV is calculated from an ECG and is the variation in time between each heartbeat [64, 65]. A narrative review by Vanderveken *et al*. in 2011 investigated the broad cardiovascular implications of several OSA treatments, including CPAP, MADs, and electrical stimulation of the hypoglossal nerve [66]. Based on one study assessing the effect of 3 months of MAD therapy on HRV [67], they concluded that oral appliances could impact autonomic function and that this should be a subject of future study. A 2018 systematic review and meta-analysis by de Vries *et al*. reinvestigated the effects of oral appliance therapy on a wide range of cardiovascular outcomes. They reported that only four studies, two of which are RCTs, have researched the effect of MAD therapy on HRV. They concluded that MAD therapy appears to have some favourable effect on HRV, but data remains too heterogeneous to support firm conclusions. Meta-analysis of HRV was not possible due to differences in units used for HRV parameters [68].

Substantial and consistent evidence for elevated SNA in OSA has come from microneurography, which has been established as an effective tool for measuring acute and chronic changes in SNA [26, 30, 43, 56]. To our knowledge, no studies have directly measured SNA in a MAD intervention with microneurography. Therefore, we conducted a scoping review

of studies measuring direct or indirect markers of autonomic nervous activity in OSA patients managed with MAD therapy. A scoping review is indicated when a broad understanding of the available evidence is sought to map every related attempt to investigate a topic. This scoping review aimed to identify all published articles measuring changes in cardiac autonomic activity to determine the extent of our current knowledge regarding changes in SNA in patients using MADs.

Definitions

Indirect markers of SNA were defined as blood or urine norepinephrine or epinephrine concentrations. Heart rate variability was defined as an indirect marker of both sympathetic and parasympathetic activity. Direct assessment of SNA was defined as measuring sympathetic vasomotor activity to muscle (MSNA) using microneurography [69].

Methods

The five-step method for scoping reviews published by Arksey and O'Malley was followed to map the key concepts in the literature surrounding how MAD therapy may influence autonomic activity and identify the available evidence.

Step 1: The research question

Our research question was: *Is there any literature assessing the effect of MAD therapy on SNA directly using microneurography in OSA patients? If not, what is known in the existing literature on the impact of MAD therapy on the autonomic nervous system in mild to severe OSA patients? What measurement tools have been used to quantify autonomic changes during MAD therapy? Does adequate literature exist to justify a formal systematic review and meta-analysis?*

Our primary aim was to complete a formal search strategy to identify any potential publications using microneurography. However, the parasympathetic and sympathetic

components of the autonomic nervous system may also be assessed using various prospective or retrospective, direct or indirect techniques. Therefore, our secondary aim was to identify all existing literature to inform our response to whether a systematic review would provide a meaningful conclusion regarding the effects of MADs on autonomic nervous activity.

Step 2: Identifying relevant studies

Our search strategy involved searching several electronic databases, reference lists and grey literature (google scholar). Due to time and resource constraints, unpublished and non-English studies were excluded from our search and hand-searching of key journals was also not performed. A librarian from the University of Alberta worked with us to identify relevant databases and aided in building an initial search strategy. The initial search strategy was refined under librarian guidance, considering initial search results. The final search was applied in Ovid MEDLINE In-Process & Other Non-Indexed Citations (1946 to the present) (Table 1) and was then revised to appropriate syntax and database-specific subject headings for Ovid Embase (1974 to the present) (Table 2), CINAHL Plus with Full Text (1937 to the present) (Table 3), Scopus (Table 4), Cochrane Library (Table 5) and Google Scholar (Table 6). Each search strategy was reviewed with the librarian. Searches were updated in April 2022. After removing duplicates, all citations were entered into Endnote and Covidence to facilitate data management and extraction.

Table 1: Ovid Medline <1946 to April 05, 2022>
Date searched: 05-Apr-2022
Results: 11

1. sleep apnea syndromes/ or exp sleep apnea, obstructive/ or OSA
2. Snoring/
3. (obstructive sleep adj3 (apnea or apnoea or hypopnea or hypopnoea)).mp.
4. ("upper airway resistance" or "SDB" or "sleep disordered breathing" or snor*).mp.
5. or/1-4
6. Mandibular Advancement/
7. oral appliance therapy.mp.
8. (panthera or prosomnus or Orthoapnea or BTI or Narval or Noa or somnodent or snore rx or HERBST).mp.
9. (mandibular adj3 (advanc* or reposition*) adj3 (appliance* or therap* or device*)).mp.
10. or/6-9
11. 5 and 10
12. parasympathetic nervous system/ or ganglia, parasympathetic/ or parasympathetic fibers, postganglionic/ or exp vagus nerve/ or exp ganglia, sympathetic/ or sympathoadrenal system/ or exp vasomotor system/
13. (parasympathetic or vagus nerve or sympathetic or sympathoadrenal or vasomotor).mp.
14. heart rate variability.mp.
15. exp Autonomic Nervous System/ and exp Arrhythmias, Cardiac/
16. (Cardiac autonomic adj2 (dysfunction or activity or neuropathy)).mp.
17. (sympathoexcitation or sympathetic activation).mp.
18. Norepinephrine/an, bl, ur or catecholamine.mp.
19. (mSNA or microneurography).mp.
20. or/12-19
21. 11 and 20

Table 2: Ovid Embase <1974 to April 05, 2022>
Date searched: 05-Apr-2022
Results: 53

1. OSA.mp.
2. Snoring/
3. (obstructive sleep adj3 (apnea or apnoea or hypopnea or hypopnoea)).mp.
4. ("upper airway resistance" or "sleep disordered breathing" or snor*).mp.
5. or/1-4
6. sleep apnea appliance/ or sleep apnea device/
7. oral appliance therapy.mp.
8. (panthera or prosomnus or Orthoapnea or BTI or Narval or Noa or somnodent or snore rx or HERBST).mp.
9. (mandibular adj3 (advanc* or reposition*) adj3 (appliance* or therap* or device*)).mp.
10. or/6-9
11. 5 and 10
12. cholinergic system/ or autonomic nervous system/ or parasympathetic ganglion/ or parasympathetic nerve/ or vagus nerve/ or vasomotor reflex/ or vasomotor system/ or adrenergic system/ or sympathetic ganglion/ or sympathetic innervation/ or sympathetic nerve/ or sympathetic trunk/
13. (parasympathetic or vagus nerve or sympathetic or sympathoadrenal or vasomotor).mp.
14. heart rate variability.mp.
15. heart disease/ or cardiac autonomic neuropathy/ or ecg abnormality/ or heart arrhythmia/ or heart stress/ or major adverse cardiac event/ or myocardial disease/
16. (Cardiac autonomic adj2 (dysfunction or activity or neuropathy)).mp.
17. (sympathoexcitation or “sympathetic activation”).mp.
18. (noradrenalin or catecholamine or catecholamine).mp.
19. (mSNA or microneurography).mp.
20. or/12-19
21. 11 and 20

Table 3: CINAHL Plus with Full Text <1937 to April 03, 2022> Date searched: 05-Apr-2022 Results: 10
S22 S20 AND S21 S21 S5 AND S9 S20 S10 OR S11 OR S12 OR S13 OR S14 OR S15 OR S16 OR S17 OR S18 OR S19 S19 (MH "Norepinephrine/BL/UR/DU") S18 (MH "Heart Rate Variability") OR (MH "Heart Rate+") S17 “microneurography” S16 “sympathoexcitation” S15 (MH "Vagus Nerve+") OR parasympathetic or “vagus nerve” or sympathetic or sympathoadrenal or vasomotor S14(MH "Parasympathetic Nervous System+") S13 (MH "Sympathetic Nervous System") OR sympathetic or sympathoadrenal or vasomotor OR (MH "Receptors, Adrenergic+") OR (MH "Autonomic Nervous System Diseases+") OR (MH "Epinephrine+") S12 "Cardiac autonomic dysfunction" S11 (MH "Autonomic Nervous System+") S10 "muscle sympathetic nerve activity" S9 S6 OR S7 OR S8 S8 panthera or prosomnus or Orthoapnea or BTI or Narval or Noa or somnodent or "snore rx" or HERBST S7(MH "Orthodontic Appliances+") OR "oral appliance therapy" OR (MH "Surgery, Oral+") S6 (MH "Device Removal+") OR (((Mandibular advance*) OR (mandibular reposition*)) N2 (device* OR appliance OR therap*)) OR mads S5 S1 OR S2 OR S3 OR S4 S4 sdb OR “sleep disordered breathing" S3 (MH "Snoring") S2 "OSA" S1(MH "Sleep Apnea, Obstructive") OR (MH "Sleep Apnea Syndromes")

Table 4: Scopus Date searched: 05-Apr-2022 Results: 49
((TITLE-ABS-KEY ("mandibular advancement device" OR "mandibular advancement devices" OR "mads" OR "mandibular advancement appliance" OR "mandibular advancement therapy" OR "mandibular repositioning device" OR "mandibular repositioning appliance" OR "mandibular repositioning therapy" OR panthera OR prosomnus OR orthoapnea OR bti OR narval OR noa OR somnodent OR "snore rx" OR herbst) AND TITLE-ABS-KEY ("obstructive sleep apnea" OR "osa" OR "obstructive sleep apnea syndrome" OR "osas" OR "sleep disordered breathing" OR "sdb" OR "upper airway resistance syndrome" OR "uars"))) AND ("autonomic nervous system" OR "sympathetic nervous system" OR "heart rate variability" OR "norepinephrine" OR "microneurography" OR "msna" OR "heart rate" OR "cardiac autonomic dysfunction" OR "cardiac autonomic activity" OR "cardiac autonomic neuropathy" OR "sympathoexcitation" OR "sympathetic activation" OR "parasympathetic nervous system")

Table 5: Cochrane Library Date searched: 05-Apr-2022 Results: 10
#1 “Obstructive sleep apnea” OR osa OR “sleep disordered breathing” OR SDB #2 (((Mandibular advance*) OR (mandibular reposition*)) NEAR/2 (device* OR appliance* OR therap*)) OR mads OR panthera OR prosomnus OR orthoapnea OR bti OR narval OR noa OR somnodent OR "snore rx" OR herbst #3 "autonomic nervous system" OR "sympathetic nervous system" OR "heart rate variability" OR "norepinephrine" OR "heart rate" OR "cardiac autonomic dysfunction" OR "cardiac autonomic activity" OR "cardiac autonomic neuropathy" OR "sympathoexcitation" OR "sympathetic activation" OR "parasympathetic nervous system" #4 microneurography OR mSNA OR "muscle sympathetic nervous activity" #5 #1 AND #2 AND (#3 OR #4)

Table 6: Google Scholar Date searched: 05-Apr-2022 Results: 388*
With all of the words: autonomic “mandibular advancement” With the exact phrase: obstructive sleep apnea With at least one of the words: sympathetic Since 2018**

*Only the first 100 results were downloaded as search specificity was limited in Google Scholar.
**Articles were restricted to 2016-2022 in the Google Scholar search as a systematic review was found in previous searches published in 2018 and was presumed to contain relevant articles from dates later than 2016.

Step 3: Study selection

The initial selection was extended to articles whose title or abstract mentioned the use of MADs in mild to severe OSA patients who were at least 18 years of age and included the assessment of any autonomic nervous activity, including parasympathetic and sympathetic, by indirect or direct methods. Studies that were explicitly about individuals with cardiovascular, pulmonary, neurologic, or kidney disease were excluded unless they contained an OSA control group free of these fully diagnosed comorbidities. These conditions were excluded as they are known to influence SNA. Two researchers independently appraised all identified citations using the inclusion and exclusion criteria. If uncertainty arose regarding the relevance of a citation, the complete article was obtained for further discussion until consensus was reached. After the initial selection, copies of all the selected articles were obtained. Two researchers then independently

reviewed these complete articles to finalize the selection. If disagreement was encountered, the study was discussed with a third researcher until consensus was reached.

Step 4: Charting the data

Two independent researchers recorded the following data from the selected studies into the data abstraction table: Author(s); year of publication; sample size, age and sex; study aim; study design, instruments or approaches used to assess autonomic nerve activity in subjects; arms of the intervention, if applicable, and results.

Step 5: Collating results

The data abstraction table and initial drafts of the review were circulated to all research team members for continuous revision until all agreed that it reflected the results of the reviewed articles.

Results

A total of 233 citations were identified, and 150 were reviewed after removing 83 duplicates (Fig. 1). One hundred and twenty-six citations did not meet the inclusion criteria and were excluded. The full articles of the remaining twenty-four citations were reviewed, including their references. Two additional articles were identified from the references, and a total of eighteen articles were excluded after a full-text review. Eight remaining articles with original data were included for review and are summarized in Table 7.

Among the eight studies included with original data, there was one randomized cross-over single-blind controlled trial [70], one randomized cross-over trial [71], two non-randomized

experimental trials [67, 72], two retrospective studies [65, 73] and two unpublished pilot studies [74, 75]. A total of 227 adults were included in these studies. The severity of OSA at baseline was assessed through AHI in seven of the papers (34.1±12.2) and through the respiratory disturbance index (RDI) in Shiomi *et al*. (29.6±16.5). While the two unpublished pilot studies did not describe their population in detail, the remaining six studies had a mainly male population (151 males to 28 females) with an unweighted average age of 50.3±10.2 and BMI of 27.1±2.9. The sample sources varied across the studies, including sleep centers and, university clinics, public and private clinics. Among the studies that presented a control or comparison group to MADs, the intervention varied from placebo oral appliance (POA) to CPAP and sleep surgery. The earliest paper was published in 1996, followed by several in the 2006-2016 range and several in the 2018-2021 range.

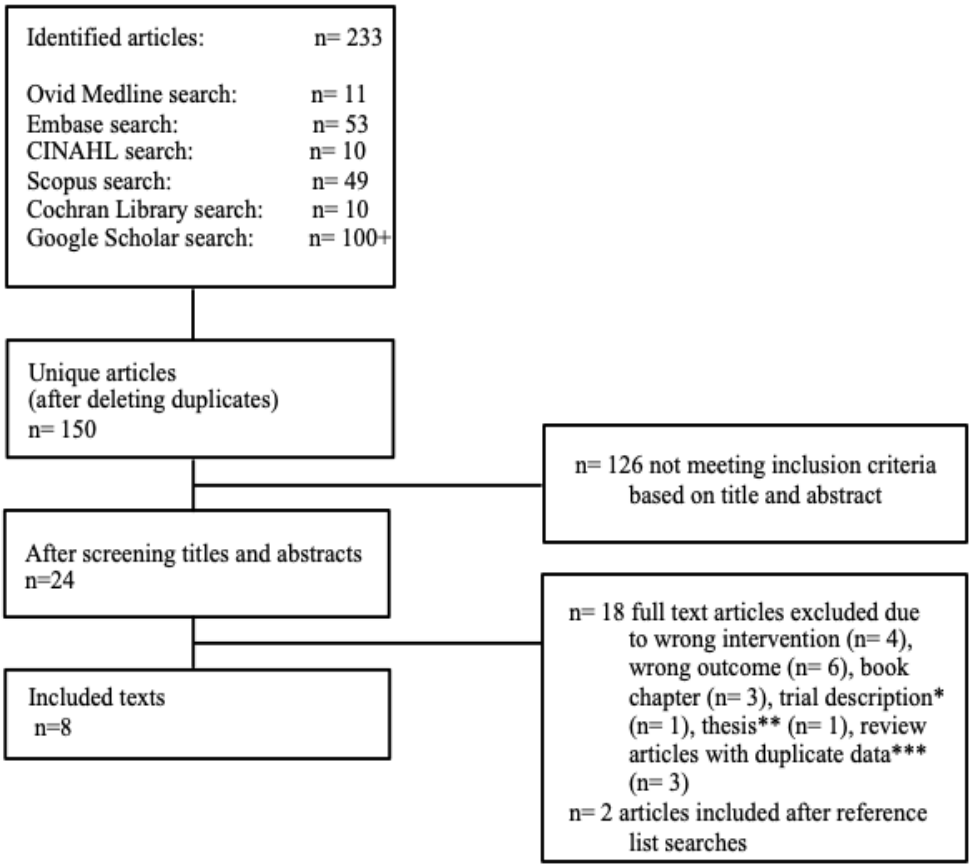

Figure 1: Study selection procedure.

Table 7: Summary of included papers with original data.

Citation	Country	Study design	Sample and inclusion/ exclusion criteria	Instrumentation/ Methodology used to assess autonomic function	Results/ Conclusions
Coruzzi et al. 2006	Italy	Non-randomized experimental study	*n = 20 adults* (10 consecutive OSA+ patients referred for MAD therapy and 10 healthy matched controls). *Inclusion criteria*: AHI >5, free of comorbid disease, no medications, no smoking, consume no more than two espressos per day. *Exclusion criteria*: orthodontic assessment in all spatial planes showed no "significant occlusion defects."	HRV analysis was performed on 20 mins of ECG data collected during supine wakefulness. Data collection was performed at baseline and after three months of MAD therapy. *Indices included:* RRI (ms), RRI variation (ms^2), LF (ms^2), LF (NU), HF (ms^2), HF (NU), and LF/HF ratio.	HF (ms^2) significantly increased ($p<0.001$) after three months of MAD therapy. A significant difference in RRI was no longer seen between groups after MAD therapy. However, within the OSA+ group, the difference in RRI after MAD therapy was not statistically significant compared to the baseline. *Conclusion*: MAD therapy is effective in improving cardiac autonomic modulation, as reflected by changes in HRV.
Dal Fabbro et al. 2014	Brazil	Randomized, crossed-over, single-blind, and controlled trial	*n = 29 patients* from a sleep-disordered breathing clinic (three treatment arms, MAD n = 6, placebo oral appliance (POA) n = 9, CPAP n =14). *Inclusion criteria:* AHI >20, BMI<35, 25-65 years of age, adequate dentition, minimum mandibular protrusion of 7mm. *Exclusion criteria:* periodontal disease, TMD, other sleep disorders, disturbances interfering with CPAP use, alcohol or drug abuse, previous treatment for OSA.	Nocturnal HRV analysis was performed on ECG data obtained from PSG. Two five-minute intervals were analyzed from REM and slow wave sleep (SWS). PSG was done at baseline, after one month of either MAD or CPAP, before switching therapies, and again after one month of the alternative therapy. *Indices included*: LF, HF, TP and the index of sleep autonomic variation ("ISAV": the difference between REM-LF and SWS-LF).	Nocturnal TP and the index of sleep autonomic variation significantly reduced for the MAD group compared with the POA group ($p<0.05$). *Conclusion*: Although CPAP was more effective at attenuating OSA, better compliance was found in the MAD group, favouring autonomic modulation during sleep.

Giannasi et al. 2011	Brazil	Pilot study abstract	*n = 8 patients* who reported snoring, nocturnal breathing arrests, tiredness upon awakening, and difficulty concentrating. *Inclusion criteria:* minimum of 7.0-mm maximum protrusion on the MAD device, 40.0 mm of mandibular opening, eight to ten teeth in each arch, and periodontal health. *Exclusion criteria:* severe temporomandibular dysfunction, clinically significant coexisting disease (e.g., diabetes, hypertension) and predominant central sleep apnea on a sleep study.	A non-invasive computer-based system called Nerve Express System (NES) was used for HRV analysis. The assessment was done during a 5-minute orthostatic test, where participants changed from supine to upright positions. The test was done at baseline and again after six months of MAD therapy. *Indices included:* inconclusive.	Frequency domain parameters improved significantly for the "parasympathetic area" ($p<0.05$). The RR interval improved but was not significant. *Conclusion*: MAD therapy improved cardiac autonomic modulation, as reflected by changes in HRV. Larger samples are needed.
Glos et al. 2016	Germany	Randomized crossover trial	*n = 40 patients* with OSA confirmed by a 6-channel ambulatory sleep apnea monitoring device (Embletta). *Inclusion criteria:* AHI >5, age > 18, patients with "clinical symptom complex" and suffering from lack of refreshing sleep. *Exclusion criteria:* drug abuse, any medication that could influence sleep, medication for OSA, participation in pharmacological trials up to four weeks before the study, sleep disorders other than OSA, prior use of PAP of pharyngeal surgery, psychiatric or neurologic disease, atrial fibrillation, craniomandibular disorders with restricted mobility of the	Cardiac autonomic function tests were performed the morning after each overnight PSG, at baseline, before initiating either 12 weeks of CPAP or 12 weeks of MAD therapy and again after using the alternative therapy for 12 weeks. Cardiac autonomic function tests consisted of beat-by-beat continuous blood pressure recordings (Finapres Medical Systems) in parallel with ECG (lead II), respiration effort belt and nasal airflow recordings from a PSG recorder (Embla systems). During each test, participants were instructed to a) spontaneously, b) at a fixed rate of 6/min, c) at a fixed rate of	Compared to baseline, BRS, RRI, SDNN, LF, and LF/HF values did not differ with either MAD or CPAP therapy for all four breathing variations. During 12/min breathing, HF increased significantly for MAD compared to baseline ($p<0.05$), while 15/min breathing had a trend for increased HF for MAD compared to baseline. CPAP showed no significant improvements for HF compared to baseline. MAD showed no changes for SBPV, while CPAP showed an increase in SBPV LF for only 6/min breathing ($p<0.05$). *Conclusion*: It is unclear why the effects were significant for only some breathing variations. However,

			mandible, acute to subacute dental treatment requirements, <8 stable teeth per jaw, acute periodontal disease, class III dental relationship with an anterior crossbite, those in orthodontic retention for <6 months, and discontinuation of therapy or interruption of therapy for more than one week.	12/min, and d) at a fixed rate of 15/min for five-minute intervals with 2-minute breaks in between each variation. *Indices included:* RRI, SDNN, RMSSD, LF, HF, LF/HF, baroreceptor sensitivity (BRS) and systolic blood pressure variability (SBPV; LF, HF, LF/HF).	changes in cardiac autonomic modulation were observed after 12 weeks of both MAD and CPAP.
Kim et al., 2020	South Korea	Retrospective	*n = 58 adults* who visited the Seoul National University Bundang Hospital Sleep Center because of snoring and sleep apnea. *Inclusion criteria:* age ≥ 18 years, AHI > 5/h, MAD therapy (SomnoDent) and patients with baseline PSG and PSG after three months of MAD therapy. *Exclusion criteria:* significant arrhythmias; low-quality data (artifact> 20% of total sleep time); total sleep time less than 5 h; awake more than 30 minutes from midnight to 5 am; combined sleep disorders; habitual use of sedatives and hypnotics and history of specific pathology related to HRV changes (i.e., MI, diabetic neuropathy, cardiac transplantation).	Nocturnal HRV was analyzed from ECG data exported from commercially available PSG software (RemLogic 3.0 HRV analyzer; Embla Systems). Analysis was performed on ECG recordings from midnight to 5 A.M. by a blinded researcher. Standards of measurement were adapted from the European Society of Cardiology Task Force and North American Society of Pacing and Electrophysiology paper. *The indices included* RRI, SNDD, RMSSD, NN50, pNN50, VLF, LF, LFnu, HF, HFnu, LF/HF, and TP.	When all 58 participants were analyzed, the NN interval significantly increased (p=0.001), SDNN decreased (p=0.042), LFnu decreased (p=0.022), and HF increased (p=0.022). Subgroup analysis was also performed for "responders" (n=34) vs "non-responders" (n=24) and "success" (n=24) vs "failure" (n=34). Within the "responders" vs "non-responders" analysis: the NN interval increased, and TP, VLF, LF, LF/HF and LFnu significantly decreased. Interestingly, HFnu increased (p=0.015). Within the "success" vs "failure" analysis: both the success and failure groups had significant increases in NN intervals, failure group also had a significant decrease in SDNN. Only the success group had significantly decreased TP, decreased VLF, decreased LF and LFnu and increased HFnu. *Conclusion*: 3 months of MAD therapy significantly changed

					cardiac autonomic modulation as represented by nocturnal HRV.
Lee et al., 2020	South Korea	Retrospective	*n = 60 patients* who had sleep surgery (n=30) or MAD therapy (n=30) from 2013-2017, matched for age, BMI and baseline AHI by a blinded researcher. *Inclusion criteria:* age > 18, diagnosed with OSA AHI >5/h, baseline and follow-up PSG at three months post-treatment available. *Exclusion criteria:* Significant arrhythmias, low-quality data (artifacts exceeding 20% of total sleep time), total sleep time < 5 h, awakening more than 30 min from midnight to 5 am, patients with combined sleep disorders, habitual use of sedatives and hypnotics, and history of specific pathology related to HRV changes.	Nocturnal HRV was analyzed from ECG data exported from commercially available PSG software (RemLogic 3.0 HRV analyzer; Embla Systems). Analysis was performed on ECG recordings from midnight to 5 A.M. Standards of measurement were adapted from the European Society of Cardiology Task Force and North American Society of Pacing and Electrophysiology paper. *Indices included*: RRI, SNDD, RMSSD, NN50, VLF, LF, LFnu, HF, HFnu, LF/HF, and TP.	In the MAD and surgery group, the following significant changes were found, NN interval increased, LF/HF decreased, LFnu decreased, and HFnu increased. These changes were not significantly different between groups after adjusting for age, BMI, and baseline AHI. *Conclusion*: Both therapies were considered helpful for cardiac autonomic modulation.

Popovic et al. 2008	United States	Prospective clinical trial (conference abstract)	*n = 50 adults* with OSA (n=40 OSA+ and n=10 healthy controls). *No inclusion or exclusion criterion is listed*.	HRV was analyzed from ECG recordings taken sitting at rest, with eyes open and with eyes closed (length of time not specified). ECG recordings were performed at baseline, at one month and at three months of MAD therapy. Analysis was completed on 5 min segments of artifact-free ECG. *Indices included*: LF, HF, and LF/HF.	In the OSA group, LF and LF/HF significantly decreased with MAD use ($p<0.05$, $p<0.01$, respectively), which coincided with a decrease in AHI ($p<0.01$). These effects were seen after one month of treatment, and the trend of decreased slowed afterwards. Post-treatment LF/HF was still higher in the OSA group compared to healthy controls ($p<0.05$), but the difference in LF was not statistically significant. HF did not change significantly with treatment. *Conclusion*: MAD therapy can ameliorate the increased sympathetic nervous system activity in OSA patients.
Shiomi et al. 1996	Japan	Prospective clinical trial	*n = 12 patients* diagnosed with OSA by PSG. *Inclusion criteria*: no evidence of autonomic dysfunction or neuropathy; no clinical presence of neurological disease or lung disease; normal spirometry and arterial blood gases and normal Valsalva maneuver responses. *No exclusion criteria are listed*.	24-hour ambulatory recordings of Holter ECG (using a Marquette 8500T two-channel recorder), nocturnal airflow and pulse oximetry taken before and after 3+/-1 months of MAD therapy. ECG signals were evaluated semiautomatically on a Marquette 8000 system. Two cardiologists, blinded to the condition, identified very low-frequency peaks. Average 24-hour values of frequency analysis were used for statistical analysis. *Indices included*: SDANN, SD, RMSSD, pNN50, RRI, SDNN, HF, LF, LF/HF, and VLF. VLF peaks.	VLF changed significantly decreased after three months of MAD therapy ($p<0.01$). All other parameters did not change substantially. *Conclusion*: the very low-frequency peak seen before treatment resolved after MAD therapy in patients with a low AHI (cut-off point of about ten events per hour) following treatment.

Table 8: Definitions of statistical parameters most commonly used to describe heart rate variability (HRV).

Analysis Type	Parameter (units)	Definition
Time Domain	NN or RRI (ms)	The "normal-to-normal" interval, which is the time interval between each successive QRS complex or between each R-R wave of the ECG, is often calculated as a mean.
	SDNN (ms)	The standard deviation of the NN interval. This is the square root of the variance, which reflects all the cyclic components responsible for variability in the heartbeat cycle.
	SDANN (ms)	The standard deviation of the average NN interval. This is calculated over short 5-minute segments to estimate the changes in heart rate due to cycles longer than 5 minutes.
	RMSSD (ms)	The square root of the mean squared differences of successive NN intervals
	NN50 count	The number of pairs of adjacent NN intervals that differ by more than 50ms in the entire recording.
	pNN50 (%)	NN50 is divided by the total number of NN intervals.
Frequency Domain	TP (ms^2)	Total power, or the variance of all NN intervals (approx. <0.4 Hz, may be analyzed from 5-minute or 24-hour ECG recording)
(or spectral analysis)	ULF (ms^2)	Power in "ultra-low frequency" (range <0.003 Hz, must be analyzed over an entire 24-hour ECG recording).
	VLF (ms^2)	Power in "very low frequency" (approximately <0.04 Hz, may be analyzed from 5-minute or 24-hour ECG recording)
	LF (ms^2)	Power in "low frequency" (range 0.04 – 0.15 Hz, may be analyzed from 5-minute or 24-hour ECG recording)
	LF_{nu} (normalized units)	LF/(TP-VLF) x 100
	HF (ms^2)	Power in "high frequency" (range 0.15 – 0.4 Hz, may be analyzed from 5-minute or 24-hour ECG recording)
	HF_{nu} (normalized units)	HF/(TP-VLF) x 100
	LF/HF	The ratio of [LF (ms^2)/ HF (ms^2)] is taken from a 5-minute ECG recording.

Methods used to describe cardiac autonomic modulation

No papers using microneurography or urine, or blood catecholamines as markers of SNA during a MAD intervention were identified. Only Shiomi *et al*. mentioned microneurography as a measure of SNA as a point of discussion. All eight papers used HRV as the main method to quantify changes in autonomic balance. In addition to HRV, Glos *et al*. 2016 used baroreceptor sensitivity (BRS) as a marker of cardiac autonomic function and systolic blood pressure variation (SBPV) during various breathing conditions as a marker of sympathetic activity. Three papers assessed nocturnal HRV from ECG data obtained during overnight PSGs [65, 70, 73]. A detailed explanation of HRV, BRS and SBPV analysis and interpretation is outside the scope of this review; however, a brief review to place findings into context is provided below.

Heart Rate Variability

Briefly, HRV is based on observing the continual fluctuation in heartbeat length around the mean value as sympathetic and parasympathetic mechanisms alter heart and respiratory rates to reflect the needs of an individual. Time-domain and frequency-domain analysis of an ECG signal produce mathematical indices that are taken together to describe the simultaneously cyclical and chaotic physiological systems that influence a heartbeat cycle. These are listed and defined in Table 8.

Time and frequency domain analysis can be derived from a 24-hour or 5-minute ECG sample. HRV indices derived from 24-hour or 5-minute ECGs are not comparable, as a change in the recording length greatly influences the findings. For example, 24-hour ECGs will capture circadian rhythms, metabolic processes and other processes that alter heart rate, which a 5-

minute recording will not include. Therefore, HRV indices may only be compared when derived from the same length of ECG recording.

Time-domain indices of HRV quantify the amount of variability in the time between successive heartbeats. An increase in the variation of the time interval between heartbeats is generally considered a positive finding. Frequency-domain indices quantify the estimated distribution of signal energy across four frequency bands present in a patient's ECG. These four frequency bands include ultra-low frequency (ULF), very-low frequency (VLF), low frequency (LF) and high frequency (HF). Each of these frequencies originates from the oscillations of cyclical mechanisms that influence heart rate. Although the exact physiological origins of ULF, VLF and LF are still debated in the literature, it is accepted that HF is representative of respiratory and parasympathetic vagal influences on the heart. LF is mostly accepted to represent a mix of parasympathetic and sympathetic activity [69, 76].

Baroreceptor Sensitivity (BRS)

Baroreceptor sensitivity (BRS) is a non-invasive method of measuring autonomic nervous activity based on the activity of the baroreflex feedback loop. Baroreceptors are nerve endings in the walls of the carotid sinus and aortic arch, which provide the central nervous system with a continuous stream of information on changes in blood pressure. Efferent autonomic neural activity is modulated in accordance with this information on a second-by-second basis, responding to fluctuations in blood pressure with corresponding changes in heart rate to maintain stable ambulatory blood pressure [77, 78]. Impairment of the baroreceptor-heart rate reflex contributes to the reduction of parasympathetic activity and the increase in sympathetic activity. Slower baroreflex control of the heart in units of milliseconds per millimeters of mercury (msec/mmHg) has been reported in various cardiovascular diseases.

Therefore, it is an accepted tool for assessing the autonomic control of the cardiovascular system [79].

Systolic Blood Pressure Variability

Systolic blood pressure variability (SBPV) is another non-invasive method of assessing autonomic activity. For the low-frequency range, SBPV is likely related to sympathetic activity, while for the high-frequency range, it is related to the mechanical effects of respiration. Blood pressure variability includes analysis of rhythmic and nonrhythmic fluctuations by spectral analysis. Most studies on spectral analysis of blood pressure variability select data segments 3 to 5 minutes long derived from recordings obtained under standardized laboratory conditions after the removal of artifacts. It has been established that analysis of blood pressure variability by the spectral approach, as well as by time-domain techniques, represents a useful tool for the study of the mechanisms involved in cardiovascular regulation in both normal and diseased conditions. However, as with HRV, blood pressure variability may not consistently represent sympathetic regulation of the vasculature [80].

Findings in the Literature

HRV: Time-Domain Indices

A significant increase in the mean NN interval or RRI (a reduction in heart rate) was reported in two of the retrospective studies and in one of the prospective studies after three months of successful MAD therapy ($p=0.003$, $p=0.002$ and $p<0.02$, respectively) [8, 10, 19]. Coruzzi et al. found a significant increase in RRI variation which was calculated as an index of the total variance in milliseconds squared ($p<0.02$). Giannasi et al.'s pilot study reported a trend toward increased mean RRI. Glos et al. reported an improvement large enough that no difference in RRI was found between the MADs group and healthy controls, although this change was not

significant when comparing pre and post-intervention values within the MAD group [17]. In each study, these changes were taken as an indicator of successful therapy and improved HRV. In one of the retrospective studies, SDNN and RRI were found to decrease in those who did not have successful MAD therapy ($p=0.035$ and $p=0.010$). The meaning of this finding was not explained [8]. No other study noted a significant change in SDNN. No significant changes in RMSSD were noted by either of the two retrospective studies. However, Glos et al. noted a significant increase in RMSS for the MAD therapy group during 12 breath per minute breathing condition ($p<0.05$) and a trend towards increased RMSSD in the 15-breath-per-minute breathing condition ($p<0.1$) when compared to baseline values. The authors were unable to explain why RMSSD changed for these breathing patterns and not the others; however, they took these findings as an indication of a small, positive response in HRV to MAD therapy.

HRV: Frequency-Domain Indices

A significant increase in HF power was observed by Coruzzi *et al*. after three months of MAD therapy compared to baseline values ($p<0.001$). This was interpreted as a positive change in parasympathetic cardiac autonomic modulation. A significant increase in HF power was also observed by Glos *et al*. after three months of MAD therapy during the 12-breath-per-minute breathing condition when compared to baseline ($p<0.05$), while HF in the 15-breath per-minute breathing condition trended towards improvement ($p<0.1$). These changes were interpreted as an indication of a small, positive response to MAD therapy. However, LF and LF/HF did not significantly change in any of the breathing conditions in the MAD group compared to baseline.

Dal-Fabbro *et al*. found no significant changes in nocturnal HF or LF; however, they observed a decrease in total power in the MAD and CPAP groups compared to the placebo oral appliance group ($p<0.05$ for both groups). They suggested that this showed a positive effect on

cardiac autonomic modulation in both therapies. When MAD was compared to baseline, there was only a significant improvement in the index of sleep autonomic variation ("ISAV;" difference between REM-LF and SWS-LF) ($p<0.05$). From these findings, they suggested that MAD therapy reduces sympathetic activity "at each [obstructive] event" while CPAP may reduce sympathetic activity in "a wider way."

In the retrospective study by Kim *et al*., those who had a successful response to three months of MAD (defined as achieving a reduction in AHI by more than 50% and a final AHI of less than 20/h compared to baseline) had a significant increase in nocturnal HF_{nu} (but not HF) ($p=0.015$), while nocturnal VLF, LF, LF_{nu}, LF/HF and TP all significantly decreased compared to baseline ($p=0.010$, $p=0.004$, $p=0.015$, $p=0.031$ and $p=0.007$ respectively). However, once they adjusted for age, BMI and sex, only TP and LF showed significant changes compared to the nonresponse group. They concluded that three months of MAD therapy significantly changed cardiac autonomic modulation. In the retrospective study by Lee *et al*., three months of MAD therapy was found to significantly change nocturnal HRV, evidenced by a decrease in LF/HF ($p=0.025$), LF_{nu} ($p=0.024$) and an increase in HF_{nu} ($p=0.024$). The authors concluded that these changes were indicative of an increase in parasympathetic activity and a decrease in sympathetic activity in response to MAD therapy.

In their prospective trial, Shiomi *et al*. found no significant change in LF, HF or LF/HF after three months of MAD therapy compared to baseline; however, a significant reduction in VLF was observed ($p<0.01$). They concluded that MAD therapy reduced VLF peaks in those participants who achieved a respiratory disturbance index (RDI) less than 10/h post-intervention with a resultant decrease in nighttime hypoxemia.

In their conference presentation, Popovic *et al*. reported that prior to MAD therapy, OSA patients had higher LF and LF/HF than healthy controls ($p<0.01$). After one month of treatment, the OSA group had a significant decrease in LF and LF/HF ($p<0.05$ and $p<0.01$, respectively). After three months of MAD therapy, these changes were still significant; however, the trend of decrease slowed. Post-therapy LF/HF was still higher in the OSA group compared to healthy controls ($p<0.05$); however, the LF was no longer significantly different. HF did not change with treatment. The authors concluded that LF represents sympathetic activation and that MAD therapy resulted in a decrease in sympathetic activation and an improvement in sympathetic–parasympathetic balance, as shown by LF/HF.

Lastly, a pilot study published by Giannasi *et al*. in a supplemental abstract in the Sleep Breath Journal found the frequency domain parameters were significantly improved in the "parasympathetic area" after six months of MAD therapy compared to baseline. To describe this result, they prove two sets of values, each showing a decrease in value from pre- to post-intervention, each with a p-value of <0.05 for significance. However, the exact HRV indices they are referring to was left ambiguous. They conclude that their custom MAD device effectively improved cardiac autonomic modulation in OSA patients; however, a larger sample is needed.

Baroreceptor Sensitivity

Glos *et al*. used spectral analysis of spontaneous heart and systolic blood pressure changes to calculate spontaneous BRS at rest by applying the square root of the ratio of RR interval and systolic blood pressure (SBP) power spectra. BRS values were expressed in milliseconds per millimeter of mercury (msec/mmHg). The cardiac autonomic function test was performed in the morning while awake and abstaining from caffeine and vasoactive drugs. Patients were studied in a 45° head-up position and were trained in metronomically controlled

breathing at varying respiration frequencies for a duration of 5 min in each case. Non-invasive continuous blood pressure was recorded with the Portapres® system (Finapres Medical Systems, Amsterdam, The Netherlands). This is a valid method for time- and frequency-domain analysis of blood pressure variability and BRS. Glos *et al*. found that analysis of the autonomic activity in the frequency domain showed no effects on BRS. BRS did not change in the daytime after three months of both CPAP and MAD therapy.

Systolic Blood Pressure Variability

Glos *et al*. applied fast Fourier transformation to calculate the power spectra of HRV and SBPV in the frequency domain. They found that at forced breathing at 6/min after the use of CPAP, SBPV-LF values increased by 0.324 mmHg ($p < 0.05$), and SBPV-LF/HF values demonstrated a trend toward higher values. Baseline mean values of SBPV-HF did not differ between the two treatment groups. In addition, only small changes were seen in HRV-HF (with MAD) and in SBPV-LF (with CPAP). They commented that although the changes in HRV and SBPV indicated positive treatment effects, their patients were without elevated cardiovascular risk, and no marked elevations of HRV, BPV, or BRS were present upon selection inclusion. Based on these findings, they concluded that changes in these parameters during daytime and an improvement in these parameters with therapy were evident, presumably only in patients with severe sleep apnea or in patients with severe comorbidities.

Discussion

This scoping review aimed to map all available evidence measuring changes in cardiac autonomic activity, specifically SNA using microneurography, in patients using MADs. Several methods of direct and indirect measurements exist for assessing SNA, so a secondary goal of our review was to map which methods have been used.

It was identified that no studies had been conducted using the direct method of microneurography or the indirect method of urine or blood catecholamines to assess SNA during a MADs intervention. The most used method for assessing cardiac autonomic activity was HRV, followed by BRS and SBPV, which are all indirect methods derived from ECG or beat-by-beat blood pressure readings.

Among the eight primary studies, there were two randomized trials, four non-randomized trials, two retrospective studies and two unpublished pilot studies. An assessment of the quality of data was not included in our review. The unpublished data must be interpreted cautiously as we did not gain access to the entire data set from the authors, and the studies were not peer-reviewed. These articles were included in the review to give readers a sense of the work completed toward answering the research question.

Various techniques were used to assess HRV; most notably, some authors investigated nocturnal HRV from PSG-derived ECGs, while others investigated HRV during wakefulness or under varying breathing conditions or positions (seated at a 45-degree angle or supine). According to the European Society of Cardiology and the North America Society of Pacing and Electrophysiology 1996 Task Force paper on HRV standards of measurement, HRV represents a promising marker of autonomic activity. However, the apparent ease of HRV has made it a widespread but perhaps oversimplified tool, creating the potential for "excessive or unfounded extrapolations" and incorrect conclusions. Recognition of this issue resulted in the publication of the 1996 Task Force paper, which aimed to standardize HRV nomenclature and definitions, create standard measurement methods and define physiological correlates of HRV components. A summary of their recommendations is found in Table 9.

Table 9: Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommendations for standards of measurement, physiological interpretation and clinical use of heart rate variability.

Recording requirements and recommendations to minimize error	**Time and Frequency- Domain Measurement and Interpretation**	**Physiological correlates and Interpretation**
During clinical trials, ECG recordings should be taken in similar conditions and environments across participants and time points.	It is incorrect to compare measures obtained from recordings of varying durations as the total variance of HRV increases as the recording period increases.	HF mainly speaks to the vagal activity of the parasympathetic nervous system.
The optimal sampling rate of the ECG recording is 250-500 Hz or higher. A lower rate may require interpolation to refine R-wave fiducial point.	Ectopic beats, missing data and noise may alter the power spectral density of frequency-domain analysis. Short-term recordings free of these complications are preferred; however, the inclusion of only ectopic-free recordings may introduce bias. Interpolation should be used when ectopy is present, and authors should consider how ectopy or missing data may have affected the results. The relative number of RR intervals omitted and interpolated should be quoted.	The interpretation of LF has yet to be agreed upon. When expressed in normalized units, it may be a marker of sympathetic modulation. Others view LF as a reflection of both sympathetic and vagal modulation. Interpretation of VLF and ULF requires further investigation. The existence of a specific physiological process attributable to VLF has been questioned.
An accurate method of identifying and removing ectopic beats.	LF and HF values are affected by changes in TP. Therefore, they should always be reported in absolute values of power (ms^2) and normalized units (LFnu, HFnu) in conjunction with TP values for ease of interpretation.	HRV speaks to the degree of autonomic modulation to the heart and does not speak to the mean level of autonomic tone in a subject.

Three of the nine papers referenced the 1996 Task Force publication, Glos *et al*. 2006, Kim *et al*. 2020, and Lee *et al*. 2020. However, Kim *et al*. and Lee *et al*. were the only two papers to clearly state they had followed the Task Force recommendations in their methodology section. Unfortunately, a general lack of methodological disclosure was noted when each article was assessed for adherence to Task Force guidelines. All but one paper failed to mention the ECG data sampling rate, all but two articles disclosed the method of R-wave fiducial identification, only three papers disclosed their criterion for identifying ectopic heartbeats or artifacts for exclusion from analysis, and only one paper revealed the relative number of R-R intervals omitted. Therefore, it is difficult to comment on the quality of HRV data obtained from

each paper due to a lack of detailed methodology. The Task Force paper also notes that conclusions drawn from HRV depend highly on the analysis and that conclusions drawn from differing methodologies are not comparable. Table 10 outlines the adherence to Task Force recommendations for each paper, including abstracts, although they are expected to give limited information.

Despite methodological differences, all nine papers found that after three months of MAD therapy, several HRV indices changed significantly. All authors interpreted these changes as a marker of positive cardiac autonomic modulation. The HRV indices used to justify these conclusions varied, but most papers found a change in frequency domain parameters. In contrast, only two retrospective studies and one prospective study found significant changes in the time domain parameter RRI. All authors interpreted a decrease in VLF and LF as positive findings indicating decreased sympathetic activity. An increase in HF was interpreted as a positive finding in all the papers except for Giannasi *et al*. 2011 whose abstract appeared to interpret a decrease in HF as a marker of improved parasympathetic activity. However, findings from Giannasi *et al*. 2011 should be interpreted with caution as we did not gain access to the full paper or data set.

Table 10: Adherence to Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology recommendations for measurement of heart rate variability.

Author and Year	ECG recording device used	Sampling frequency	Method of analysis	Method of ectopic beat or artifact identification	Interpolation	The length of the ECG segment was analyzed and standardized among participants
Coruzzi 2006	Unclear whether ECG was recorded separately or derived from PSG (StarDust; Respironics)	Not stated	ECG data were analyzed offline using commercial software MARS 5000; Marquette Hellige; Freiburg, Germany	Not stated	Data were interpolated and resampled at 2.5 Hz	Yes
Dal Fabbro 2014	Derived from overnight laboratory PSG (Somnologica Science system; Embla, version 3.3.1; Flaga Inc, Reykjavík, Iceland)	Not stated	Not stated	Not stated	Not stated	Yes
Giannasi 2011	Not explicitly stated; it appears PSG was used to derive ECG	Not stated	Nerve Express System (NES) - a fully automated computer-based system	Not stated	Not stated	Not stated
Glos 2016	ECG (lead II) recorded using PSG recorder (Embla systems, Broomfield, CO, USA)	200 Hz	MATLAB® software (The Math-Works Inc., Natick, MA, USA), with algorithms as "previously described" in the literature. RR interval detected using a peak detection algorithm	Offline filtering (Butterworth band pass 0.3-70 Hz)	The time series were interpolated at 4 Hz using a cubic spline interpolation algorithm	Yes

Kim 2020	Single lead ECG data from commercially available PSG software (RemLogic 3.0 HRV analyzer; Embla Systems, San Carlos, CA).	Not stated	Commercial software RemLogic 3.0 HRV analyzer	NN intervals of more than 2,400ms and less than 400ms were omitted	The signals were interpolated and resampled at 5.0Hz	Yes
Lee 2020	Single lead ECG data from commercially available PSG software (RemLogic 3.0 HRV analyzer; Embla Systems, San Carlos, CA).	Not stated	Commercial software RemLogic 3.0 HRV analyzer	NN intervals of more than 2,400ms and less than 400ms were omitted	The signals were interpolated and resampled at 5.0Hz	Yes
Popovic 2008	ECG (lead number not specified, brand not specified)	Not stated	Spectral analysis; software not specified	Not stated	Not stated	Yes
Shiomi 1995	Marquette 8500T two-channel recorder (CM5 lead ECG)	Not stated	Commercial software Marquette heart rate variability, version 002A. Semi-automatic peak detection using the Marquette 8000 system and verified by two cardiologists blinded to outcome.	Semi-automatic using the Marquette 8000 system	Not stated	Yes

Most of the papers were careful not to overextend their findings to mean MAD therapy altered autonomic tone and recognized that HRV is only a marker of cardiac autonomic modulation. However, Popovic *et al*. and Shiomi *et al*. commented that changes in LF could speak to changes in global sympathetic tone. The thought that LF may represent peripheral sympathetic tone is highly debated in the literature [56, 81].

Glos *et al*. was the only paper to use SBPV and BRS to assess cardiac autonomic activity. On analysis, they found only a minor increase in SBPV-LF after CPAP, while no change was seen in the MAD group. However, they found no difference in BRS between the MAD and CPAP treatment groups.

In summary, each study concluded that MAD intervention positively impacted HRV and cardiac autonomic activity. However, the strength of this conclusion must be received cautiously, as the methodology used to obtain results varied and was not fully disclosed by each author as per the 1996 Task Force guidelines.

Sample sizes were generally small, apart from Glos et al. (n=40) and the retrospective papers by Lee et al. (n=60) and Kim et al. (n=58). Other literature does not fully support some conclusions about sympathetic tone [81]. Future papers should aim to include more female participants and larger prospective samples.

No paper used microneurography to measure SNA during a MAD intervention. The only paper to mention microneurography as a measure of SNA was a narrative review by Ucak *et al*. (2021); however, they critique it as an invasive and time-consuming measure requiring specialized skills compared to HRV. Their narrative review paper supported machine learning and automated classification systems over manual ones for HRV analysis and systems that do not require high-quality ECG signals to compute HRV [15]. Although the 1996 task force paper cautions against using a purely automated approach to calculating HRV, new analyses of HRV are becoming a focus of current literature [82].

This review is the first to identify what is known regarding sympathetic changes after MAD therapy in OSA patients. However, several limitations exist. First, due to a general scarcity

of evidence, we aimed to map all attempts to answer our research question. Therefore, this review included published and unpublished work without an assessment of bias. Since sympathetic activity is often difficult to assess in practice, we felt including all data, even where limited, provided value towards understanding limitations in the current literature and the number of barriers that must be overcome to answer our research question. One of those barriers is oversimplification and misinterpretation of HRV indices. A second limitation of this review (as it is with any review) is the possibility that articles were missed. A librarian reviewed our search strategy and published it in the University of Alberta electronic research database before article selection to mitigate error and increase transparency.

Conclusion

While some participants and researchers consider microneurography an invasive and challenging technique, it provides an essential level of physiological understanding [69]. Future papers using HRV should closely adhere to Task Force recommendations for ease of comparison amongst publications and transparency of analysis, interpretation, and results.

Finally, direct and indirect measures of cardiac autonomic activity may not correlate in diseased states as in health [56]. Therefore, additional investigation on how both direct and indirect measures of SNA relate to OSA therapy may be of value. Prospective studies should examine how MAD therapy affects SNA using microneurography alongside noradrenaline spillover analysis and HRV, as no single method is without limitations.

CHAPTER 3: PROTOCOL PROPOSAL

Study Design

We propose a prospective self-controlled case series to evaluate the effect of MAD therapy on direct and indirect SNA measures and vascular health in participants with mild to moderate OSA or in those with severe OSA who cannot tolerate CPAP. A repeated measures design was chosen to control for 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 criteria will be eligible 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) expected to change in body weight by >5% during the study, 6) pre-existing symptomatic non-respiratory sleep disorder (e.g. restless leg syndrome, chronic insomnia), 7) smoking habit or 8) prescription of a non-titratable appliance.

Recruitment and sample size rationale

In Alberta, the referral pathway for MADs is from a physician to dentists who provide MADS, or alternatively, patients are identified by dentists and referred to the physician to assess

multidisciplinary management with MADs. Therefore, we approached dental providers who adhere to the current standard of care guidelines outlined by the College of Dental Surgeons of Alberta for providing MADs 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 which the MADs will be appropriate. Participants will follow instructions from their provider regarding titration protocol and when titration is complete. 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 MSNA were detected by microneurography after a single month of CPAP use, with similar changes detected after 12 months of CPAP use [30]. 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 [67, 70-72]. Therefore, in the current study, participants who successfully complete MAD titration as per their dental provider's prescription will be tested after three and six months of wearing the MAD at its final position. Participants will be compensated $200 for their time upon return of the take-home sleep study after the completion of the second time point. Analysis of the difference between MSNA 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 MAD therapy on MSNA in mild to severe OSA patients as measured directly through microneurography.

Secondary Outcome

The secondary outcome of this study is the effect of three and six months of 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. 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. Participant age, BMI, pre-treatment AHI, and neck circumference will also be recorded.

Definitions

Our study will define "responders" to MAD therapy 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 [83]. "Non-responders" will be defined as anyone not meeting this definition. Compliance has been defined by expert consensus as MAD wear for ≥80% of the night, five nights a week [84]. Our study will use a subjective compliance scorecard to report hours of MAD wear. Therefore, will define compliance as 4.5 hours of MAD wear per night, five nights a week, assuming the average participant will sleep 6 hours per night.

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 (ex: 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 will be tested during the placebo phase, and those using intrauterine hormonal devices will be tested at their convenience. Subsequent testing sessions at three and six months of titrated MAD wear will be completed under the same conditions 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 electrocardiogram (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 muscle sympathetic nerve activity (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). MSNA data will be sampled at 10,000 Hz and stored for offline analysis in LabChart (Powerlab Software, ADInstruments, Chart Pro v8.3.1).

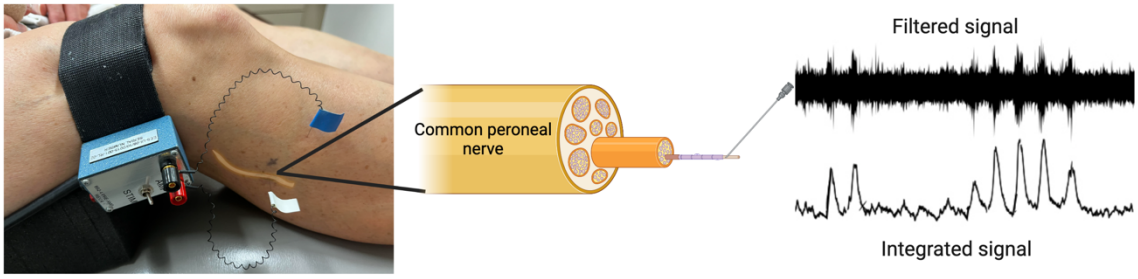

Figure 2: A microneurography recording from a tungsten microelectrode inserted into a muscle nerve fascicle of a sympathetic nerve bundle of the common peroneal nerve. The right of the image depicts the resultant filtered and integrated neurograms. Created with BioRender.com.

Following instrumentation, participants will rest quietly for 5 minutes to relax for physiological variables to stabilize. 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 flow-mediated dilation or "FMD" will be completed. This test causes an endothelial-dependent increase in the bioavailability of the potent blood vessel-derived-relaxing factor, nitric oxide (NO), that stimulates vasodilatation 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) 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 (the stimulus) and resulting changes in diameter (the outcome) (Figure 2). Subject preparation will be completed per the suggested guidelines by Thijssen *et al*., 2019 [85].

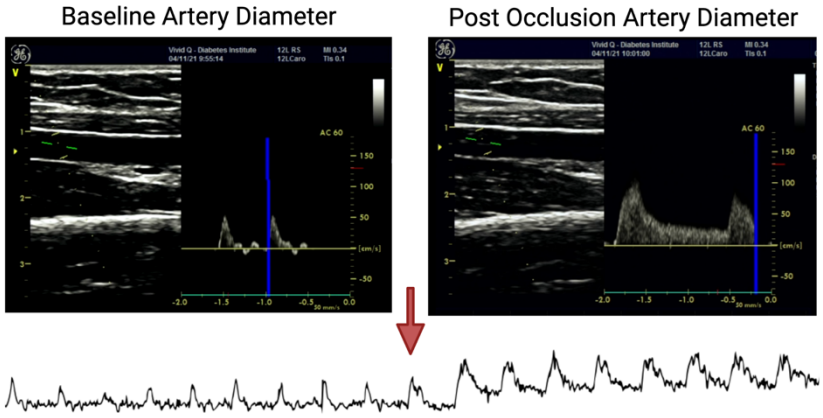

Figure 3: Ultrasound images showing post-occlusion changes in brachial arterial dimension and blood flow after forearm cuff release (red arrow) during flow-mediated dilation test.

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 [84].

Methods

Microneurography

Microneurography is a direct method of assessing the sympathetic outflow to the vasculature of the skin and muscle. Our study protocol focuses on muscle sympathetic nerve activity (MSNA), which consists of efferent vasoconstrictor impulses to the large peripheral vascular bed found in the body's skeletal muscle. Through constricting blood vessels within skeletal muscle, MSNA critically influences blood flow and resistance and plays a critical role in blood pressure regulation and hemodynamic stability [20]. MSNA demonstrates a high intra-individual reproducibility even over years, which allows for long-term monitoring of disease processes and therapeutic interventions [86]. 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 [20].

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 commonly used test to measure an arterial's capacity to dilate by releasing NO in response to stimulating endothelial cells by reactive hyperemia [87]. 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 [85].

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 [88]. 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 [89].

Heart rate variability (HRV)

HRV indirectly measures cardiac autonomic balance via power spectral analysis of an electrocardiogram (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 control of the heart, and its ability to accurately assess sympathetic nervous control of the heart is heavily debated in the literature [15]. Oversimplification during interpretation can lead to unfounded conclusions if standardized protocols are not adhered to [81]. 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 prior to analysis to blind the analyzing researcher from participant ID and time point. Repeated measures MANCOVA will be used to assess statistical differences between the means of the dependent variables AHI, ODI, cardiovascular variables,

and mean percent change in MSNA variables (burst frequency, burst incidence, burst amplitude and total activity) and FMD (normalized percent change) after three months of treatment and six months of treatment versus baseline. Neck circumference, sex and age will be used as covariates.

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, 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, ODI, 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) will be compared to the peak post-occlusion D_A expressed as the absolute change in arterial diameter (FMD) as well as percent change (FMD%). FMD values will be normalized to vascular shear stress stimulus, which will be 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) at 36 °C for 1 minute. The shear rate will be calculated as $8Q_A/D_A$; where Q_A is arterial blood velocity [85]. 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 (FVC) will be calculated as blood flow velocity divided by mean arterial blood pressure (MAP). If an increase in normalized FMD% is accompanied by a decrease in AHI, we may detect an association between successful MAD therapy and improved vascular health.

Analysis 3

HRV analysis will be conducted on 5 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), 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 using ELIZA techniques for noradrenaline concentrations in nanomoles per litre (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.

Ethics and Dissemination

This study was approved by the Health Research Ethics Board-Health Panel, University of Alberta, Edmonton, Canada (Pro00108618); trial registration number NCT05387122. The findings will be presented to sleep medicine, orthodontic and dental professionals through peer-reviewed journals and conference presentations to enhance their understanding of MAD therapy in research and clinical settings.

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 [90]. 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.

The clinicians we consulted follow a similar clinical protocol and most providers reported treating 1-2 patients with MAD therapy every 1-3 months. Several 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.

Discussion

To our knowledge, our protocol outlines the first attempt at directly assessing sympathetic nervous changes through the technique of microneurography. Due to a small pool of patients receiving MADs being further reduced by the COVID-19 pandemic and the bankruptcy of a popular MAD and titration system, we are unable to rely on a single provider. Dental providers may have varying titration protocols and may prescribe different amounts of mandibular advancement based on treatment goals established with the patient. Our study will account for this by defining successful MAD therapy based on self-reported compliance and changes in AHI obtained from level three sleep studies. Participants will be identified as compliant or non-compliant and as responders or non-responders according to our predefined definitions for potential subgroup analysis.

Our protocol could be enhanced by recruiting from dental providers prescribing MADs with devices to measure objective compliance or from providers who use titration systems to identify responders vs non-responders prior to MAD delivery and study enrolment. However, these options were not available within our recruitment pool.

The best study design to assess changes in SNA during a MAD intervention is a randomized control trial (RCT), but due to the lack of preliminary evidence regarding changes in MSNA in this population, a prospective case series is an appropriate initial study. Our protocol has been designed using a convenience sample, increasing the risk of bias in our design. However, our primary goal is to gather evidence regarding the optimal amount of time between

testing points to see changes in MSNA and the ideal sample size prior to conducting an RCT. Microneurography is a technical procedure requiring training and specialized equipment. Therefore, there is value in collecting preliminary data to use resources most effectively in an RCT design.

CHAPTER 4: FEASIBILITY TRIAL

Recruitment

Nine potential referral bases were contacted, eight of which were dental providers suspected to provide MADs, and two of which were physicians that occasionally refer patients to dental providers for MADs were appropriate. Seven dental providers responded to our messages; one stated they do not provide MADs, and one declined to aid in referrals due to personal reasons. The remaining five providers agree to aid in referral to our study. However, due to the bankruptcy of Zephyr technologies, providers who used their MATRIX titration system did not refer to our study. To increase recruitment potential, the international research community was contacted via email flyers to enquire about collaboration with a center providing a higher volume of MAD therapy. A Seattle dentist was interested in collaboration; however, they were later unable to assist due to a staffing shortage. Ultimately, two dental providers were the main source of referrals for our study.

A total of ten patients were referred, eight of which were referred by one dental provider. Two patients did not return our follow-up messages; one was ineligible due to previous MAD wear, two were ineligible due to starting their initial MAD therapy prior to attending baseline testing, and one was ineligible due to a diagnosis of mixed apnea.

Case Descriptions

All data are reported as mean ± SD unless otherwise stated. Four participants were included in the study, three females and one male, with an average age of 59.8±10.5 and BMI of 31.2±1.6 (table 12). Three participants had mild OSA, and one had moderate OSA (AHI 14.13±1.12; ODI 17.07±3.11) as diagnosed by their physician with a level three take-home sleep study. All four participants were from the same dental provider and were all prescribed the same Panthera brand appliance. A list of medications and medical history for each participant is provided in Table 11.

ID	Sex	Occupation	Supplements	Medications	Medical History
1	F	Casual education assistant; Singer	Multi-Vitamin, Vitamin C, D, B6, B12, Zinc, Omega3, Evening primrose oil, Blueberry, Vitalux, Calcium, Magnesium, Allegra 24 hr	Venixxa	Allergic to dust and snow mold
2	F	Volunteer	Cal/Mag/Vit D, Glucosamine Chondro 1500mg, Magnesium 250mg, Omega 3 900mg, Vitamin D 800IU, Melatonin 10mg, CBD oil	7 Align Probiotic, Acetylsalicylic Acid, Atorvastatin, Materna, Escitalopram, Levothyroxine, Muro 128 eye gel, B12 injections	Heart murmur, Stroke, Osteoarthritis, Temporomandibular joint disorder, Uterine ablation, Hypothyroidism, Depression, Anxiety
3	M	RCMP officer		Tylenol arthritis, Synthroid, Warfarin, Perinopal, Amlodipine, Cymbalta,	Hypertension, Hypothyroid, Osteoarthritis

				Pantoprazole, Remeron, Repatha, Ozempic	
4	F	Retired	Vitamin C + D	Indapamide, Candesartan, Cipralex, Pantoprazole	Hypertension, Anxiety, Chronic leg/hip pain, Hemochromatosis, Mastectomy of the left breast

Table 11: Summary of participant's demographic and medical information.

Two participants were on two antihypertensive medications to control their blood pressure. The etiology of participants one and three's OSA was unknown. The etiology of participant two's OSA was an intubation accident after suffering a stroke, and it was unknown whether the accident caused hard or soft tissue damage. Participant two did report success with CPAP; however, it gave them anxiety to wear the device, and their sleep was suffering. The etiology of participant four's OSA was suspected to be partially due to alcohol use and obesity. Participant four declined CPAP and chose MAD therapy as their first option, perceiving that it would be easier to wear than CPAP. Participant three had attempted CPAP therapy as their first treatment option but could not tolerate it due to night terrors.

	Pre-Intervention	
	Mean	**SD**
Age, years	59.75	10.47
BMI, kg/m²	32.18	1.61
Neck circumference, cm	40.53	2.97
AHI	14.13	1.12
ODI	17.07	3.11

HR, bpm	69.83	11.42
SBP, mmHG	119.65	11.20
DBP, mmHG	78.66	3.51

Table 12: Participant anthropometric, sleep study and cardiovascular data at baseline testing. (Heart rate; HR, systolic blood pressure; SBP, diastolic blood pressure; DBP).

Assessment of Intervention

Two participants completed the entire protocol. Participant four dropped out of the study after baseline testing, citing MAD intolerance due to gingival and mucosal ulcers. Participant three completed two time points and agreed to participate in the third time point; however, they were later excluded due to their provider prescribing a combination of MAD and CPAP therapy. On average, it took participants 99.7±66.2 days to complete device titration. On average, 148.2±45.1 days passed between time points, with a shorter number of days between the second and third-time points after titration was completed (105±0 days, Table 13). Participant one reached efficacy the fastest, without any complications. Participant two was delayed in beginning titration for a month, as the MAD was lost in the mail. Once they began therapy, titration was slow due to new pain onset from a previous history of controlled temporomandibular joint disorder (TMD). There was a communication error regarding the completion of titration, and participant two was tested for the second time point after wearing the MAD at its final position for only one month.

Participant three had a challenging time adjusting to their MAD due to continued snoring and a tendency to sleep with their mouth open. They began their titration on June 20th and, on July 15th, reported via email that they had worn their CPAP on several occasions. Prior to baseline testing they reported they had discontinued the CPAP completely due to the inability to

comply with nightly wear. By August 11th, they were progressing through MAD elastic sizes and hopeful about settling on the final position soon. On November 28th, they reported they had been using the MAD appliance at efficacy for three months (since September 1st) and their follow test was booked for December 7th.

ID	Time Point	Days to complete titration	Days between time points
1	1	47	
	2		147
	3		105
2	1	174	
	2		209
	3		105
3	1	78	
	2		175
Mean ± SD		99.7±66.2	148.2±45.1

Table 13: Summary of days to MAD efficacy and days in between time points.

Assessment of Protocol and Outcome Measures

Updating the medical questionnaire and anthropometric data, performing the blood draw, and allowing participants to change into loose-fitting clothing for testing took approximately 15-30 minutes per time point. After participant instrumentation, each recording session took 112.8±25.9 minutes (ranging from 146 to 71 minutes), including an average of 40.3±17.7 minutes (ranging from 24 to 57 minutes) to find a MSNA signal. On average, 3.3±2.4 sites were accessed with the microelectrode per session, ranging from 1 to 7 sites (table 14). MSNA signals were found in three of the four participants; however, two signals became unusable due to

participants' inability to stay still. Participant two had a chronic injury in the right hip, causing them to have a difficult time being seated for long periods of time. At all three time points the signal was lost in this participant because of toe wiggling to prevent discomfort. On average, there were 7.4±10.8 pain-related comments (ranging from 0 to 27) and 5.7±5.0 sensation-related comments made by participants during MSNA searching.

ID	Time Point	Total time (min)	SNA search time	SNA search sites	SNA quality
1	1	85	24	1	Strong
	2	135	50	7	None
	3	93	15	1	Strong
2	1	126	38	2	Poor, lost during patient movement
	2	131	57	6	None
	3	101	23	1	Poor, lost during patient movement
3	1	146	54	4	None
	2	71	N/A	N/A	N/A
4	1	127	61	4	Poor, lost during patient movement

Table 14: Summary of testing sessions and MSNA searching data.

Participants were encouraged to vocalize every sensation during testing as this feedback aids the search as well as maintaining participant comfort. Comments were recorded into LabChart in real time using the participants' own words. Examples of pain comments included “very painful,” “bad pain,” “nervy pain,” “local pain,” “pain radiating down my leg,” “electric,” “intense,” “jolts down the back of my leg.” All pain immediately resided after the microelectrode manipulation stopped; no lasting pain was experienced by any participant after electrode removal

to continue searching at another site. Examples of sensation comments included “cramp in calf,” “tingling” down the shin, in the toes, ankle, back, groin or top of the foot.

Participant two was the only participant to experience a feeling of panic and light-headedness during the initial minutes of the first search at time point one. They also experienced moments of moderate to severe “nervy” pain that radiated down the leg to the toes at all time points. An average of 8.1±11.5 pain-related comments (ranging from 9-27) were recorded over participant two’s three time points. During each session they were agreeable to continuing the search and their pain immediately discontinued after ceasing electrode manipulation. In comparison, participants one, three and four made few pain-related comments (the number of pain comments ranged from 0-2). Participant four experienced “jolts” down the back of his leg ten times but did not explicitly describe the sensation as painful.

Due to the COVID-19 pandemic, blood sampling and processing supplies were unavailable during several tests. Therefore, two blood draws were not possible as supplies were unavailable. Noradrenaline analysis has not yet been completed for processing supply reasons.

In three tests equipment malfunctions lead to data loss during the FMD protocols for participant two. Specifically, the rheometer and finometer malfunctioned, leaving one test without a blood viscosity reading to calculate shear stress stimulus and another without mean arterial blood pressure to calculate forearm vascular conductance. Finally, the qDAT cord was damaged and prevented the recording of blood flow into LabChart for participant two’s final time point. This data will be recovered through another more complicated means of publishing. In each test the outcomes of FMD and FMD% were obtained, although two time points cannot be adjusted for shear stress stimulus. All participants tolerated the FMD procedure well;

interestingly, two participants commented that the forearm occlusion portion of the procedure was more uncomfortable than the MSNA searching.

Each participant lived multiple hours outside of the Edmonton city center, where the laboratory is located. Therefore, arranging the return of level three sleep study devices was not always feasible. Device pick-up was offered and accepted by participant four, but all other participants declined. To accommodate participant two, who could not drive due to blindness, we ordered sleep studies from a commercial sleep clinic near her home that also uses the ApneaLink Air device. After their final timepoint, participant one returned an incomplete sleep study of one hour and thirty-seven minutes instead of the minimum four hours. Unfortunately, we were unable to arrange another sleep study due to the participant living hours away.

No adverse effects were observed in any participants after testing. Each participant that was eligible agreed to participate in the third time point after receiving monetary compensation ($200.00) after the second time point.

Participant Assessment

Each participant was contacted to participate in an interview to understand their experience in the study. Each interview began with the same question "*would you participate in our study again?*" Participant two's response was as follows:

> *"Definitely, yes."*
>
> *[Paraphrased response:] It was comforting how you told me you all had experienced the procedures you were about to do to me, so I knew it wasn't something crazy.*
>
> *I felt like a participant and not a subject because if there was something weird going on, like that nerve ticking sound, you addressed me and said, "you're the one making that noise; if you can relax the leg it will leave" instead of saying to each other "oh I think*

she is nervous because her nerve is clicking." I felt like I was a part of the team, not someone you were doing something to.

[You experienced a lot of discomfort during your test. Do you have any comments about that?]

I have a very high pain tolerance, so other people might not have been able to handle it if they haven't experienced that nervy pain before like I have. But I did panic the first time because the pain was bad, and I got scared that it would trigger more pain like I've had in the past, and I thought, what if this never goes away? But then it went away and three months after, still nothing and then I was comfortable coming back.

[Were there any barriers to participating?]

Not really. I am well connected with friends and family who can drive me, parking was easy, and I like to walk around the campus and come to the city for new lunch places.

[Did anything about the study surprise you?]

I didn't realize you had a whole team that wanted to do whatever they could to answer their research questions. [Coming to the lab] wasn't like going to the doctor's office where there is no enthusiasm to investigate an issue.

[Is that something that made you want to come back?]

Oh definitely, I really feed off of the enthusiasm and excitement. You could just feel it in the team. I am really going to miss you guys, even the guy that squeezed my arm.

Participant three's response was via email:

"Yes, I would participate again – it is a very thorough study and I felt comfortable throughout."

Participants one and four did not respond to our interview request.

Analysis and Statistical Testing

There was insufficient sample data to conduct meaningful analysis and statistical testing in this report. Future analysis of a full data set will be conducted by a researcher who is blinded to both participant ID and time points. Outlier data will be assessed under the guidance of expert investigator C.D.S. Statistical analysis will be performed as outlined in the protocol chapter.

Results

Participant one had an improvement in AHI and ODI from MAD therapy. Of note, their third time point sleep study was only one hour and 37 minutes in duration and therefore, the final AHI and ODI are likely inflated. A decrease in heart rate (HR) was also observed, however systolic and diastolic blood pressure (SBP and DBP) increased. FMD values increased from baseline to time point two and again from time point two to time point three but to a greater extent. However, when adjusted for changes in shear stress stimulus in between timepoints (FMD/AUC, mm; FMD/AUC, %), no change in FMD values were seen. Finally, a decrease in burst frequency and burst incidence was seen from baseline to time point three. Data is summarized in Table 15.

	Pre Intervention	**T2**	**T3**
Participant 1	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Compliance, hrs		6.60	6.30
AHI	13.30	1.20	3.70
ODI	18.50	2.00	3.60
HR, bpm	69.96 ± 2.47	58.35 ± 4.75	60.9 ± 3.85
SBP, mmHG	107.00 ± 2.24	133.14 ± 3.62	131.4 ± 4.63
DBP, mmHG	82.35 ± 1.69	93.42 ± 1.60	90.88 ± 2.74

FMD, mm	0.00	-0.01	0.37
FMD, %	0.09	-0.29	10.92
FMD/AUC, mm	0.00	0.00	0.00
FMD/AUC, %	0.00	0.00	0.00
BF, bursts/min	29.83		27.12
BI, bursts/100 heartbeats	42.68		40.69

Table 15: Summary of participant one's self-reported MAD compliance (hrs), AHI, ODI, cardiovascular variables (HR, SBP, DBP), vascular health variables (FMD, FMD%, FMD/AUC, FMD/AUC%) and MSNA (BF, BI) at each time point.

From baseline to the final testing point, participant one showed an increase in Lf_{nu}, and an increase in Hf_{nu} and RRI, with little change in LF/HF (Table 16). These frequency domain findings do not align well with the suggestion that SNA decreased, as shown through BF and BI reduction. However, time domain analysis shows an increase in the standard deviation of the RR interval, suggesting an increase in variability.

Participant 1	**Pre Intervention**	**T2**	**T3**
RRI (ms)	858.9	1036.0	994.1
SDNN (ms)	32.8	87.5	62.6
Lf_{nu}	51.4	65.6	53.7
Hf_{nu}	49.3	32.6	45.42
LF/HF	1.0	2.0	1.2

Table 16: Summary of participant one's HRV data. Time domain analysis: RRI (ms) and SDNN (ms). Frequency domain analysis: Lf_{nu}, Hf_{nu}, LF/HF.

Participant two had no improvement in AHI and a small decrease in ODI. An increase was observed in HR, while SBP and DBP decreased from baseline values. FMD values (not adjusted for sheer stress stimulus) increased from baseline to time point two but decreased from

time point two to time point three (Table 17). Participant two lost their compliance scorecard but estimated an average of eight hours of wear per night in between time points two and three.

	Pre-Intervention	T2	T3
Participant 2	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**
Compliance, hrs		9.40	
AHI	15.40	15.40	15.10
ODI	19.20	15.60	17.10
HR, bpm	58.35 ± 1.86	58.55 ± 1.78	65.99 ± 1.68
SBP, mmHG	123.65 ± 3.34	116.24 ± 3.85	113.36 ± 3.04
DBP, mmHG	82.35 ± 1.69	70.43 ± 1.62	77.98 ± 1.11
FMD, mm	0.34	0.62	0.50
FMD, %	8.85	16.17	12.38

Table 17: Summary of participant two's self-reported MAD compliance (hrs), AHI, ODI, cardiovascular variables (HR, SBP, DBP) and vascular health variables (FMD, FMD%) at each time point. *FMD/AUC data is not available due to equipment malfunctions described in the text.

Participant two had an increase in Lf_{nu} and LF/HF and a decrease in Hf_{nu}. RRI interval increased, and SDRR remained relatively unchanged (Table 18). These data suggest an increase in SNA, although HRV is not the best measure of the sympathetic nervous system.

Participant 2	Pre-Intervention	T2	T3
RRI	1030.0	1026.0	1074.0
SDRR	28.9	30.1	29.9
Lfnu	28.3	30.6	61.1
Hfnu	71.4	68.1	43.7
LF/HF	0.4	0.4	1.4

Table 18: Summary of participant two's HRV data. Time domain analysis: RRI (ms) and SDNN (ms). Frequency domain analysis: Lf_{nu}, Hf_{nu}, LF/HF.

Participant three had a decrease in AHI and ODI from baseline to time point two. A decrease was seen in HR, SBP and DBP from baseline to time point two. No change was seen in FMD values adjusted for shear stress stimulus in between time points (Table 19).

	Pre-Intervention	**T2**
Participant 3	**Mean ± SD**	**Mean ± SD**
Compliance, hrs		6.56
AHI	25.6	10.90
ODI	15.3	12.50
HR, bpm	81.18 ± 1.86	79.08 ± 2.81
SBP, mmHG	128.29 ± 4.51	126.28 ± 5.82
DBP, mmHG	75.37 ± 1.85	69.43 ± 1.37
FMD, mm	0.51	0.30
FMD, %	11.82	6.89
FMD/AUC, mm	0.00	0.00
FMD/AUC, %	0.00	0.00

Table 19: Summary of participant three's self-reported MAD compliance (hrs), AHI, ODI, cardiovascular variables (HR, SBP, DBP) and vascular health variables (FMD, FMD%, FMD/AUC, FMD/AUC%) at each time point.

Participant three had an increase in RRI and SDRR, suggesting an increase in variability. An increase in Hf_{nu} and a decrease in Lf_{nu} and LF/HF was also seen, suggesting an improvement in sympathetic and parasympathetic balance (Table 20).

Participant 3	Pre-Intervention	T2
RRI	738.5	760.6
SDRR	14.9	21.5
Lfnu	76.0	59.3
Hfnu	24.0	40.5
LF/HF	3.2	1.5

Table 20: Summary of participant three's HRV data. Time domain analysis: RRI (ms) and SDNN (ms). Frequency domain analysis: Lf_{nu}, Hf_{nu}, LF/HF.

Discussion

To date, SNA has been measured by indirect methods during MAD interventions, resulting in inconclusive evidence regarding how the intervention impacts the sympathetic overdrive observed in OSA. This trial demonstrates the ability to use microneurography to measure changes in MSNA as a feasible method to quantify sympathetic outcomes. However, the feasibility of our study could be enhanced through several steps including, 1) reducing travel time and the number of appointments for participants, 2) enhancing communication with dental providers regarding the timing of participant lab visits, and 3) reducing the length of laboratory sessions.

First, operating within the clinic of a single dental provider or university facility that provides MADs, or at minimum, within proximity to a single dental provider, could allow participants to be tested on the same day of their dental appointments, reducing travel. Both referring dental providers reported that a significant barrier to patients' willingness to consider entering our study was traveling to our laboratory. Second, there was also the issue of participants beginning to wear their devices before baseline testing despite adequate communication. Testing participants on the same day the impression is taken for device fabrication would help to avoid this issue. This would require closer communication with dental providers, ensuring that the recruitment flyers are delivered at the initial exam and not at the

impression appointment. This would also require participants to contact our study prior to the impression appointment. Again, operating the study from within a clinic providing MAD therapy would eliminate these steps. This would also prevent participants from taking additional time away from work for both laboratory and dental visits. Lastly, this would allow study coordinators to follow MAD titration more closely and schedule follow-up testing more precisely.

Finally, reducing the length of laboratory visits would enhance the feasibility of our study. This will naturally be achieved as the study progresses and as the laboratory team completes the study protocol more routinely. Establishing a routine during the pandemic was challenging as the participants were few, and sessions were spread apart. Pilot testing was explored as an option to combat this; however, recruiting pilot participants was also a challenge. Participant instrumentation and equipment calibration time may be shortened through routine practice by study members. However, MSNA searching time may not be as it is a tedious and technique sensitive measure to perform. Two factors that may improve MSNA search time are provider experience in using the technique and the ability of the participant to tolerate and cooperate with holding still for longer periods of time.

Due to a relatively small pool of potential participants and COVID-19 pandemic restrictions with accompanying financial and staffing challenges, we were unable to rely on a single dental provider for recruitment. This begs the question of whether having multiple providers could negatively affect our results. To account for varying provider definitions of titration endpoints and different vertical and protrusive set points of different brands of MADs, we chose to measure subjective hours of MAD compliance and AHI to categorize participants into definitions of compliant and non-compliant, responders and non-responders. In the

literature, multiple definitions of these categories exist, and changing the definition will alter conclusions. We adopted a definition of responders from the literature 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 [83]. This appears to be a feasible practice for understanding how SNA and vascular health may change in response to MAD therapy of varying success. However, there remains a risk of patients returning for testing prior to reaching the full AHI, reducing the potential of an appliance if varying definitions of titration completion are followed. This could result in the testing of more non-responders than responders, lessening our ability to answer our research question.

Both dental providers who referred to our study reported that they consider titration to be complete when positive changes in quality of life are reported by the patient. Some also considered the resolution of symptoms as an indication of treatment efficacy or success, even in the absence of AHI reduction. Ultimately, the amount of device titration is a clinical decision as providers must balance patient goals, comfort, and comorbidities such as temporomandibular joint disorders, dental changes and pain. For these reasons, the definitions of device response and success used for research will inevitably vary from definitions used for clinical purposes. An awareness of these nuances is required in order to translate scientific results into a clinical context and vice versa.

It is unknown how changes in symptoms may impact SNA. Current literature has suggested that restoring unobstructed breathing at night may not be sufficient to reduce elevated SNA if sleep fragmentation continues [91]. More research is needed to investigate the effect of MADs on the restoration of uninterrupted sleep and the effect this may have on SNA, independent of AHI reduction. Participant related outcomes to therapy, such as excessive

sleepiness, may be included in future studies to relate their potential effect on MSNA and cardiovascular outcomes [92].

A limitation of this study is the large amount of time between test points. Participants were asked prior to recruitment whether they planned to make significant lifestyle changes or whether they anticipated a change in weight greater than 5%. However, natural changes in daily stress and activity with seasonal change (ex: summer gardening or hiking), international travel, or life events such as moving homes were a few things reported by our participants. With these activities, our participants may have changed their eating habits, daily physical activity or stress levels. Each of these factors could influence vascular health or SNA independently of MAD therapy. Variability was seen in SBP, DBP, HR and HRV in our participant data, and trends over their individual time points were not necessarily present. The number of days elapsed between time points two and three were, on average, 73.0±43.8 less than the days elapsed between baseline and time point two.

This increased time between baseline and time point two was due to MAD titration. This limitation is helped by using a repeated measures study with a self-controlled design, as opposed to a study using independent controls where lifestyle matching would be nearly impossible. However, this limitation could be further addressed by working with dental providers who use a MAD titration system (like the MATRx plus device once offered by Zephyr technologies), which could facilitate timely device titration and early identification of those who will respond best to MAD therapy. Identifying "responders" to MAD therapy by using a titration system prior to MAD delivery would also improve participant selection by identifying and then excluding "non-responders" from the study prior to testing.

Another limitation of our study was the inability to use objective measures of compliance for MAD wear. This would require seeking referrals from dental providers who deliver MADs with embedded compliance sensors. As recruitment was already challenging, this was not possible for our study.

Finally, networking with MAD providers proved to be challenging during the pandemic. However, our trial did allow us to build initial rapport with several providers. We expect that recruitment will improve as we continue to strengthen our relationship with the dental sleep medicine community in Edmonton, Alberta. Dissemination of preliminary trial findings will build stakeholder confidence in our research and potentially increase cooperation and referrals to future studies. Continued communication with the dental sleep medicine community in Alberta, Edmonton is being actively pursued.

Conclusion

Our feasibility trial identified that the ideal study configuration would involve conducting measurements from a laboratory within a clinical practice under the guidance of a single dental provider with staff who may closely follow device delivery, titration and patient management to ensure tests are scheduled ideally (so as to prevent MAD wear prior to baseline testing and to spare participants from multiple visits between clinical and laboratory appointments).

Ideally, this single provider would use a titration system that allows early identification of responders vs non-responders prior to MAD fabrication. In addition, working with a provider who delivers MADs with compliance sensors would allow more precise identification of compliant vs non-compliant participants.

However, if this ideal is not possible and multiple providers must be used for recruitment, the feasibility of the study may be enhanced through the following measures:

1. Dental providers may be recruited from areas nearest the laboratory. Although this will introduce selection bias, recruitment of city-wide participants proved to be a significant challenge to us. Selection bias is less of a concern as the current goal is data collection to inform resource management for future research.
2. Enhanced communication with referring offices may be achieved through establishing a name-by-name relationship with a front desk staff serving as an additional touch point to the referring dentist, who, in our experience, maybe less available for correspondence. With dentist and participant consent, correspondence with a receptionist could be used to schedule laboratory visits ideally in accordance with clinical visits. This would lessen the difficulty of the research team communicating with patients about when device titration has been completed, which proved to lead to inaccurate scheduling in our trial.

In either of the above scenarios, the feasibility of a study measuring SNA using microneurography and vascular health using FMD may be enhanced through the following measures:

1. Regular practice of equipment setup, troubleshooting and calibration to decrease equipment malfunctions and the length of testing sessions.
2. Working with a cohesive and experienced laboratory team that excels in managing participants' expectations and potential anxieties during testing. Our finding was that a participant who feels like an important component of the team would return for testing even after experiencing discomfort during testing and in the absence of compensation.

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