SLEEP APNEA CAUSED BY ROSTRAL FLUID SHIFT: IDENTIFYING RISK FACTORS AND DEVELOPING A TREATMENT USING CALF MUSCLE ELECTRICAL STIMULATION

by

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A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy Graduate Department of Institute of Biomaterials and Biomedical Engineering University of Toronto

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Abstract

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Rationale: Fluid accumulation in the legs due to a sedentary lifestyle can increase overnight fluid shifting from the legs and into the neck (i.e. rostrally) when lying down to sleep. Rostral fluid shift can increase pressure on the pharyngeal airway and severity of obstructive sleep apnea (OSA). This thesis contains three studies that together investigate risk factors for neck fluid accumulation when lying down, and explores a therapy of calf muscle electrical stimulation (ES) for reducing daytime leg fluid accumulation to treat OSA caused by fluid shift.

Methods: The first study used data previously collected on the time-course of fluid shift from the legs and into the neck while lying supine for 90-minutes. Using demographic, anthropometric and fluid-based data captured before lying down, risk factors for increased neck fluid accumulation while supine were identified. Study two was a sham-controlled trial into the effects of using calf muscle ES while seated for two-and-a-half hours on leg fluid accumulation, consequent rostral fluid shift when supine, as well as OSA and snoring. Study three explored the time-course of seated leg fluid accumulation between sham and active calf muscle ES conditions to identify the optimal time-period for using the device to reduce leg fluid accumulation.

Results: Study one found that male sex and greater leg and neck fluid volumes prior to lying down increased risk for neck fluid accumulation when lying down. Study two demon-
strated that compared to sham ES, active calf muscle ES reduced seated leg fluid accumulation by 46%. Consequently, rostral fluid shift out of the legs and into the neck reduced by 17% and 31%, respectively. Lastly, while OSA severity did not change after ES, calf muscle ES reduced snoring index. Study three did not identify an optimal time-period for using calf muscle ES, but found the effectiveness in reducing leg fluid accumulation increases with time.

**Conclusion:** The thesis shows that calf muscle ES is an effective therapy for reducing leg fluid accumulation and consequent pharyngeal narrowing likely through reduction of overnight neck fluid accumulation. Future work should explore the effects of long-term use of calf muscle ES on OSA severity.
I dedicate my PhD dissertation to my Wife, as well as my Mom and Dad. None of this would have been possible without all of your support and encouragement over these many challenging years.
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Chapter 1

Introduction

Obstructive sleep apnea (OSA) is a form of sleep-disordered breathing caused by collapse or narrowing of the pharynx leading to complete (apnea) or partial (hypopnea) cessations in airflow to the lungs during sleep. OSA is a serious disorder occurring in 10% of the general population [1] and increasing the risk of cardiovascular morbidity and mortality [2] and the frequency of motor-vehicle crashes [3]. The pathophysiology of sleep apnea is multifactorial, contributed to by various factors including body-mass index (BMI), age, race, sex, and physiology and anatomy of the pharynx (e.g. pharyngeal length and diameter, and pharyngeal dilator muscle activity) [4]. Yet, the underlying mechanism of collapse is still not fully understood. For example, a high BMI and neck girth, through increased external pressure on the pharynx, are often cited as significant factors contributing to the pharyngeal collapse [5]. However, BMI and neck girth only account for 4% and 29% of the variability in OSA severity, respectively, as measured by the number of apneas and hypopneas per hour of sleep (apnea-hypopnea index, AHI) [6]. In addition, individuals with fluid-retaining diseases such as renal failure and heart failure are 1.5 and 5 times more likely to have OSA, independent of BMI [7, 8].

Given the discrepancies surrounding the pathophysiology of OSA and the high prevalence of sleep apnea in those with fluid retaining diseases, the role of fluid retention and nocturnal
fluid shift on sleep apnea were explored. It has been demonstrated that increased nocturnal fluid shift from the legs to the neck, known as rostral fluid shift, leads to increased mucosal water content in the neck tissues [9] leading to pharyngeal narrowing [10], increased airway resistance [11] and collapsibility [12] and most importantly increased OSA severity [13]. The discovery of the role of fluid shift on OSA gave rise to several studies investigating therapies that target the reduction of fluid retention and nocturnal fluid shift. Alternative therapies for OSA are in high demand because the standard therapy, known as continuous positive airway pressure, is not well tolerated and associated with 85% non-adherence [14]. Studies investigating alternative therapies demonstrated that wearing compression stockings during the day [15–17], walking [18, 19], diuretics [20], and fluid removal via ultrafiltration [21] all reduced overnight rostral fluid shift in association with 16-48% reduction in sleep apnea severity as measured by AHI.

The modest effect of these therapies motivates the need to identify a phenotype for individuals that are at the greatest risk for OSA defined by the fluid shift mechanism. Targeting this group will increase treatment efficacy and enable fluid targeted therapies to be a viable means of treating OSA. Currently, it has been determined that men are at an increased risk for neck fluid accumulation and sleep apnea due to rostral fluid shift [22, 23]. In addition, age is a risk factor, with men over 40 years of age experiencing more sleep apnea by fluid overload [24]. In Part I of my thesis, my first research objective aims to identify risk factors for increased rostral fluid shift when lying down.

Part II of my thesis explores calf muscle electrical stimulation (ES) as an alternative means of reducing daytime leg fluid accumulation, which addresses the limitations of the therapies explored in prior research. The use of diuretics and ultrafiltration are limited in that their applications are strictly confined to specific clinical populations. For example, it is not practical to apply ultrafiltration to any population outside of renal failure. Compression stockings are limited by their discomfort leading to low patient adherence [25]; and increased risk of thrombosis [26] and exudative skin lesions [27] due to improper fit and use. Physical
activity is a practical intervention that improves prognosis of several diseases, which is why Canadian guidelines recommend adults to accumulate 150 minutes of physical activity weekly to maintain good health [28]. However, once again compliance is an issue since only one in five adults achieve this recommendation [29] due to various reasons including cost, lack of time or motivation, and illness or disability [30]. Calf muscle ES is a method for reducing leg fluid by activating the skeletal muscle pump, moving blood back to the heart and reducing fluid pooling in the legs; similar to the mechanism by which walking reduces leg fluid accumulation [31]. The benefit of calf muscle ES over competing therapies is that it is considered to be relatively comfortable [32–34], and can be developed into a convenient wearable device that can be worn during prolonged sedentary periods. Part II of my thesis therefore explores the effect of calf muscle ES on leg fluid accumulation over a prolonged period sedentary and the consequent effects on rostral fluid shift.
Chapter 2

Literature Review

The purpose of this chapter is to summarize the literature describing how fluid shifts in the body while in upright and supine postures. In addition, the literature review will summarize the factors that contribute to airway collapse to setup the context within which neck fluid accumulation impacts pharyngeal collapse and sleep apnea. Finally, the effects of different interventions on leg fluid accumulation will be summarized to highlight the advantages of calf muscle electrical stimulation (ES) as a therapy to reduce leg fluid accumulation during the day and rostral fluid shift overnight.

2.1 Leg fluid accumulation while upright

Fluid moves between the capillaries and the interstitial spaces according to the balance of hydrostatic and oncotic forces, as per Starlings equation, shown in Equation 2.1.

\[ J_v = K_f (P_c - P_i) - \sigma (\pi_c - \pi_i) \]  

(2.1)

Where \( J_v \) is the net fluid movement between compartments, \( P_c \) is the capillary hydrostatic pressure, \( P_i \) is the interstitial hydrostatic pressure, \( \pi_c \) is the capillary oncotic pressure, and \( \pi_i \) is the interstitial oncotic pressure.
is the interstitial oncotic pressure, $K_f$ is the filtration coefficient, and $\sigma$ is the reflection coefficient. From Equation 2.1, increased hydrostatic pressure within the capillary spaces causes blood plasma to move out into the interstitial spaces through the process of filtration. Conversely, reduced hydrostatic capillary pressure will lead to reabsorption of interstitial fluid from interstitium into the capillaries. Similarly, decreased intra-capillary oncotic pressure leads to filtration of blood plasma into the interstitium; while increased intra-capillary oncotic pressure leads reabsorption of interstitial fluid into the capillaries.

Posture change affects the balance of hydrostatic forces, such that moving from lying down to upright will cause a rapid shift of up to 500 ml of blood into the lower extremities due to gravitational effects [35–39]. This rapid shift in blood takes approximately 1 to 2 minutes and is facilitated by increased filling capacity of the veins in the legs which act as a reservoir for the blood. At the end of this rapid filling, vascular filling is almost completed and the remaining slow increase in volume is explained by interstitial fluid accumulation [36,40]. The slow, gradual accumulation of interstitial fluid is caused by increases in hydrostatic pressure in the capillaries of the legs caused by the shift in vascular fluid into the veins [41–45].

The pressure required for filtration of blood plasma from the capillaries to the interstitial spaces is 15 to 20 cmH$_2$O. Standing and sitting both result in a capillary pressure of 90 to 120 cmH$_2$O [42,46,47] and 48 cmH$_2$O [48], respectively; which substantially exceeds the threshold for fluid filtration. While standing for 30 to 60 minutes, plasma volume (in intravascular space) decreases by 300 to 400 ml, but leg volume increases by 50 to 250 ml, as a result of the accumulation of fluid in the interstitial space [41,42,45,49–51]. These results demonstrate that high hydrostatic pressures in the capillaries of the legs during seated and standing posture causes the formation of a fluid reservoir stored in the leg’s interstitium.

The accumulation of leg fluid while seated occurs at a lower rate compared to standing, likely explained by the increased capillary pressure in the legs while standing, compared to sitting [42,46–48]. Sitting for 15 to 120 minutes causes increased leg volume by 14.8 to 42 ml (1.2 to 3.3%) [52]. However, results vary as in the study by Goddard et al. that reports
only a slight increase in leg volume over 30 minutes seated of approximately 1 ml/h in a
group of 54 healthy women [53]. Examining the data more closely, the study discovered an
edematous subgroup that accumulated fluid at approximately 14 ml/h; while the nonedema-
tous subgroup experienced a reduction in leg fluid (8.9 ml/h) over the seated period. The
reduction in leg fluid while seated in the non-edematous group is not explained by Goddard
and colleagues in the paper. The most significant methodological contrast to comparable
studies is the inclusion of only women participants. It is plausible that women accumulate
leg fluid differently than men. While sex-related differences in leg fluid accumulation while
upright have never been reported, there is evidence that fluid shift out of the legs and into
the upper body while supine is reduced in women, compared to men [54]. Seated leg fluid
accumulation has also been studied over the long term. These studies find sitting for 6 to
10 hours increases leg volume by between 40 and 180 ml (3 to 14%) [55–59]. Overall, the
evidence is clear that prolonged sitting during the day (i.e. a sedentary lifestyle) will lead to
excess fluid accumulation in the leg’s interstitium to a lesser degree than standing, but still
up to 180 ml over a full day seated.

2.2 Rostral fluid shift while supine

Understanding the mechanism by which fluid shifts out of the legs and accumulates in
the neck is important for encouraging informed development of interventions that prevent
rostral fluid shift and its negative effects. When lying down, as much as 500 ml of venous
blood shifts rostrally out of the peripheral vasculature and increases filling of the the right
atrium, primarily via the inferior vena cava [60, 61]. In addition, interstitial fluid that has
accumulated in the legs while upright will re-enter the venous system and also shift back
to the heart. Over a 60 minute supine period, after this initial shift in blood volume, leg
fluid volume continues to decrease by 240 to 360 ml [49], while plasma volume increases by
250 to 400 ml [43, 44, 50, 54]. As more blood shifts back to the heart (i.e. as venous return
increases), pressure in the cardiac chambers increases, leading to increased central venous pressure. As a result, venous pressures are increased at the entry to the right atrium [62], particularly in the superior and inferior branches [62–64]. Increases in the superior vena cava are significant because this will impact pressures of the veins that lie superior to the heart, such as the internal jugular vein. Increased pressures can lead to engorgement of the internal jugular vein, which lies adjacent to the lateral walls of the pharynx and could apply pressure and thereby narrow the pharyngeal lumen. This was simulated in animal studies where inflating a balloon in the peripharyngeal tissues of a rabbit led to pharyngeal narrowing proportional to the space consumed by the balloon’s volume [65]. Indeed, internal jugular vein pressures increase upon transitioning from the upright to supine position, evidenced by studies demonstrating increased cross-sectional area of the internal jugular vein while in the supine position, compared to upright [66].

Increases in hydrostatic pressure in the veins superior to the heart will lead to proportional increases in capillary pressures [46] and drive filtration of blood plasma into the tissue spaces. While the degree to which blood is translocated from the vasculature to the tissue spaces is proportional to the increase in the pressure gradient, the neck is a site of concern because it has implications for the narrowing the pharyngeal airway. More specifically, increased peripharyngeal mucosal edema can lead to swelling of some of the critical structures in the pharynx responsible for pharyngeal airway narrowing and collapse, such as the tongue, soft palate, and the lateral pharyngeal walls. In fact, OSA patients generally have enlarged soft palate, tongue, and lateral pharyngeal walls [67]. In addition, OSA patients have shown increased UA edema, particularly in the lateral pharyngeal walls, in tissue specimens [68] and MRI studies [69], which might contribute to enlargement of these pharyngeal tissues in OSA patients.

Majority of the fluid that shifts from the legs and accumulates in the neck occurs within the first 90 minutes of lying supine [54]. Over this period, it was demonstrated that 160 ml of fluid shifts out of the legs and 17 ml accumulates in the neck, which is associated with a 0.6
cm increase in neck circumference [54]. During an overnight period, in a group of otherwise healthy men with OSA, approximately 130 to 250 ml of fluid shifts out of the legs, associated with a 1 cm increase in neck circumference signaling accumulation of neck fluid [13]. Overall, there is strong evidence that the transition from upright to recumbent, followed by sustained recumbency is associated with a shift of fluid out of the legs that will accumulate in the neck in the form of both intravascular and interstitial fluid. The next section will summarize the evidence describing the impact of these changes on the physiology of the pharyngeal airway.

2.3 Effects of rostral fluid shift

2.3.1 Pathophysiology of pharyngeal airway collapse

Fluid impacts narrows the pharyngeal airway and contributes to collapse mainly through mechanical means. Before elaborating on the role of fluid shift and neck fluid accumulation on airway collapse, this section will briefly summarize the biomechanical and anatomical factors that interact with the effects of neck fluid accumulation and its impact on pharyngeal airway collapse. It is important to point out that there are several other factors that contribute to airway collapse outside of biomechanical and anatomical factors [70], however this is outside the scope of the thesis and are therefore not included in this literature review.

The pharyngeal airway is a non-rigid structure composed of soft tissue and muscles and a substantial portion of it spanning from the hard palate to the larynx is collapsible. As a result there are several anatomical and physiological factors that contribute pharyngeal airway collapse [71], which are illustrated in Figure 2.1. As displayed in Figure 2.1, fluid accumulation in the neck is one of many factors explaining the pathophysiological mechanism for airway narrowing and collapse.

From an anatomic perspective, a narrow pharyngeal airway is more prone to collapse than a larger one independent of obesity, and patients with OSA tend to have a narrower airway [72]. Both soft tissue and skeletal structures enveloping the airway influence narrowing of the
pharyngeal lumen. The pharyngeal airway is surrounded by a bony cage, within which are soft tissue structures (e.g. lateral pharyngeal walls and tongue) that form the walls of the pharynx. These soft tissues tend to be larger in volume in the OSA patient and since they are contained externally by a bony cage, the soft tissues can only displace inward, impinging on the airway lumen and contributing to a narrower airway [69, 73]. Abnormality of the skeletal structure has a similar effect. Most commonly, shortening of the maxillary and mandibular lengths cause the tongue and soft tissues surrounding the airway to shift posteriorly (toward the pharynx) and apply pressure to the pharyngeal airway causing narrowing and predisposition to airway collapse [74, 75]. Lastly, length of the airway plays a role in pharyngeal airway collapse, as this increases the length of the collapsible portion of the pharyngeal airway. Indeed, men with OSA tend to have longer pharyngeal airways, compared to men without OSA [76, 77].

Collapsibility of the pharyngeal airway is also increased in the OSA patient [78]. Pharyngeal collapsibility is a function of external pressure on the pharyngeal airway, the intraluminal pressure, and the compliance of the airway. From a purely mechanical perspective, pharyngeal airway collapse occurs when intraluminal pressure is lower than the extraluminal pressure. Factors impacting extraluminal pressure are the soft tissue and skeletal factors, already described. Intraluminal pressures are affected by pressures downstream from the pharyngeal airway originating in the lungs. As downstream pressures become more negative during inspiration, intraluminal pressure will also become more negative, contributing to airway narrowing or collapse. Furthermore, as the airway becomes more narrow, more negative intrathoracic pressures are required to maintain airflow through the narrowed airway, leading to more negative intraluminal pressures, and further narrowing of the pharyngeal airway [79].

Collapsibility of the pharyngeal airway is quantified by the critical pressure at which the airway collapses (Pcrit). The Pcrit is determined by applying positive airway pressure to an individual, then immediately removing it, or applying some degree of negative pressure. The pressure at which the airway collapses is known as the passive Pcrit. A more collapsible
Figure 2.1: Factors that increase the risk for airway collapse

- **↑ Soft tissue volume**
- **Skeletal structure abnormalities**
- **↑ Neck fluid volume**
- **↑ Pharyngeal length**
- **Narrow pharynx**
- **↑ Extraluminal pressure**
- **Sleep onset**
- **↓ Pharyngeal dilator muscle activity**
- **Negative intraluminal pressure**

Pharyngeal airway collapse
airway is defined by having a more positive Pcrit (i.e. the airway collapses at positive levels of intraluminal pressure). Individuals without OSA have a negative Pcrit in the range of < 10 cmH\(_2\)O, snorers from 5 to 0 cmH\(_2\)O, and OSA have a Pcrit > 0 cmH\(_2\)O \[78\].

Passive Pcrit describes pharyngeal collapsibility as determined by passive factors, such as skeletal and soft tissue factors. However, perhaps more significant is the active collapsibility of the pharyngeal airway, as determined by the activity of several pharyngeal dilator muscles \[80,81\]. These muscles act to oppose extraluminal forces and maintain airway patency. For example, during wakefulness the OSA patients tend to have increased pharyngeal dilator muscle activity to compensate for anatomical compromises and keep the airway patent \[82\]. However, this becomes an issue during sleep when, in both normal individuals and patients with OSA, pharyngeal dilator muscle activity reduces \[83\]. As a result, reflex pharyngeal mechanoreceptor response to negative intraluminal pressure and mucosal sensory sensitivity required to keep the airway open are diminished, increasing risk of pharyngeal collapse \[84,85\]. In the normal population this can lead to small increases in airway resistance. However, in OSA patient with an anatomically compromised airway, this can lead to pharyngeal airway collapse.

### 2.3.2 The role of neck fluid accumulation on pharyngeal airway collapse

Fluid accumulation in the neck can increase extraluminal pressure surrounding the pharyngeal airway and have additive effects to the already compromised pharyngeal airway. Fluid that accumulates in the neck in the form of both intravascular and interstitial fluid can both contribute to increased extraluminal airway pressure. The mechanism by which fluid shifts from the legs and accumulates in the neck was previously described at the beginning of this section. In brief, moving from upright to supine increases venous return and central venous pressure, and leads to increased volume and pressure in the veins of the neck. This can increase capillary pressure and drive filtration of blood plasma into the tissue spaces of the
This concept and its impact on the pharyngeal airway have been demonstrated in several studies that varied the shift of fluid out of the legs and recorded its impact on the pharyngeal airway. Shepard and colleagues were the first to investigate this relationship and they found that the pharyngeal airway dilatation that occurs at the end of expiration was absent when fluid shift out of the legs was enhanced by raising the legs [86]. A sequence of studies followed and simulated rostral fluid shift by applying lower-body positive pressure to the legs, using anti-shock trousers. In these studies, a shift of 160 to 190 ml of fluid out of the legs increased neck circumference by approximately 0.1 cm after 1 and 5 minutes [9–12]. Within the same time frame, the shift in fluid narrowed the pharyngeal airway by 9% [10], increased airway resistance by 100% [11], and increased collapsibility (as determined by a 20% increase in Pcrit) [12].

The role of fluid shift in OSA was subsequently investigated by Redolfi and colleagues who found that AHI and neck circumference increased significantly and proportionally to the overnight shift in leg fluid volume. In addition, overnight change in leg fluid volume and neck circumference were the only independent correlates of AHI, accounting for 68% of the variability of AHI [13]. Severity of OSA was also correlated with the overnight shift in leg fluid volume in patients with hypertension, and men with end-stage renal disease and heart failure [87–89]. Interestingly elevating head of the bed 7.5% was found to reduce sleep apnea by 32% (reduction in AHI from 15 to 10 events/hr of sleep) [90]. While fluid shift was not measured, it is plausible that head-of-bed elevation prevented neck fluid accumulation by reducing the effects of gravity on rostral fluid shift.

The increase in neck circumference when lying down or when lower-body positive pressure is applied demonstrates the external expansion of peripharyngeal tissues. Neck circumference is measured at the level of cricothyroid cartilage where tissues can expand outward. However, pharyngeal tissues and veins of the neck adjacent to the pharynx are bounded by the mandible, maxilla, and cervical spine. As a result, outward expansion is not possible and
instead the tissue expands inward, applying pressure to the pharynx.

The exact mechanism by which increased neck fluid volume causes expansion of the peripharyngeal tissues is not fully understood. However as summarized in Figure 2.2, it is hypothesized that the causative factors are increased volume of the great veins of the neck, formation of edema in the peripharyngeal tissues, and increased pharyngeal blood volume in the arterial spaces. As already described, the cross-sectional area of the jugular vein increases upon transitioning from upright to supine [66, 91, 92]. As a result, in the enclosed region of the peripharyngeal airway, increased jugular vein volume will medially displace the lateral pharyngeal wall and thereby narrow the pharyngeal airway [86]. Indeed, in animal studies, increasing peripharyngeal volume by inflating balloons in this space increased extraluminal pressure on the pharyngeal airway, decreasing pharyngeal airway cross-sectional area and increasing airway resistance [65, 93].

There is also evidence supporting that neck fluid accumulating in the interstitial spaces, also known as pharyngeal tissue edema, contributes to increased peripharyngeal volume. Magnetic resonance imaging (MRI) of the pharyngeal airway demonstrated that applying lower body positive pressure to shift fluid to the neck increased pharyngeal mucosal water content and reduced pharyngeal airway size in patients with OSA, but not in health controls [9]. In addition, in patients with OSA comorbid with end-stage renal disease, mucosal tissue water content of the pharyngeal airway captured from MRI was found to be positively correlated and the AHI [94]. In summary, increased pressure in the capillaries of the neck due to fluid shift may lead to the formation of pharyngeal edema which can narrow the pharyngeal airway and increase severity of OSA. However, the degree of edema formation caused by rostral fluid shift has not been established.

Pharyngeal mucosal blood volume could also contribute to peripharyngeal tissue expansion. This was tested in anesthetized cats by decreasing vascular tone using systemic vasodilator drugs to reduce pharyngeal mucosal blood volume. Vasodilation resulted in increased (more positive) Pcrit and narrowed the pharyngeal airway, which they attributed to
Chapter 2. Literature Review

Figure 2.2: Mechanisms explaining neck fluid accumulation and its role in pharyngeal narrowing.
increased thickness of the pharyngeal mucosa [95]. In humans, increasing vascular tone using local vasoconstrictors did the opposite, decreasing pharyngeal airway resistance [96]. This change in resistance was independent of upstream (nasal) resistance and pharyngeal airway dilator muscle activity, and therefore likely a reflection of reduced thickening pharyngeal mucosa. Altogether, these results suggest that active central neural control of vasomotor tone in the pharyngeal airway soft tissues are important in regulating pharyngeal airway mechanics.

It has been suggested up to this point, that fluid shift caused by gravitational effects is the primary factor by which fluid accumulates in the neck and contributes to pharyngeal airway collapse. Alternatively, it is feasible that increased negative intrathoracic airway pressure caused by sustained respiratory effort while the pharynx is occluded can contribute to neck fluid accumulation. During such an event, the negative pressures in the thoracic cavity cause pressures to drop in the cardiac chambers which might in turn cause the shift of blood out of the legs through this pressure differential, instead of the effects of gravity. However, this has been challenged in a study with heart failure patients, where CPAP prevented apneas but did not affect fluid shift out of the legs, compared to a control night where CPAP was not applied. This phenomenon should be studied further, but current evidence suggests that overnight rostral fluid shift by way of gravity persists despite removal high negative intrathoracic pressures caused by apneas [88].

Factors unrelated to a sedentary lifestyle or rostral fluid shift can also contribute to the accumulation of fluid into the neck. For example, neck fluid can also accumulate through inadequate drainage of venous blood from the head and neck. It has been demonstrated that venous outflow from the head and neck during inspiration while sleeping is reduced in the OSA patient compared to controls [97]. In addition, there are several case reports of coexisting OSA with superior vena cava syndrome, where an obstruction in the superior vena cava limits venous drainage from the head and neck [98, 99]. Meanwhile, treatment of superior vena cava syndrome through stenting has been demonstrated to relieve OSA symptoms [100]. Elevated pressures in the systemic pulmonary arteries and systemic veins
can also prevent venous drainage from the head and neck. For example, individuals with pulmonary arterial hypertension have an 89% prevalence of OSA [101]. In addition, patients with end-stage renal disease show a strong correlation with internal jugular vein volume and AHI [94]. Lastly, right heart dysfunction, even at a subclinical level and in the absence of heart failure and independent of pulmonary hypertension, can also contribute to increased pressure in the systemic veins of the neck [102]. In summary, while it is very likely that poor venous drainage from the head and neck contribute to neck fluid accumulation and compromise airway patency. However, it is important to note that his does not preclude the role of rostral fluid shift from the legs. In fact, poor venous drainage could magnify the effects of neck fluid accumulation on OSA and should therefore be considered when trying to identify those at risk for OSA defined by the fluid shift mechanism.

2.4 Interventions to leg fluid accumulation

Preventing the accumulation of fluid can be practically achieved by reducing the hydrostatic pressure gradient between the intra- and extravascular compartments of the leg. As already described, sedentary living or prolonged sitting increase hydrostatic pressure in the vasculature and drive fluid into the tissue spaces forming a fluid reservoir in the legs. There are several methods for altering this pressure gradient to prevent leg fluid accumulation. One is through application of compression around the legs to increase the hydrostatic pressure of the tissues to counteract the filtration of fluid out of the capillaries [103]. In addition, activation of the skeletal muscle pump will reduce the hydrostatic pressure gradient between intra- and extra-vascular compartments by pumping blood towards the heart and reducing the intravascular pressure of the capillaries, counteracting filtration of fluid out of the capillaries [104]. Peripheral lymphatic transport is also linked to skeletal muscle pump activity, and encourages contractile transport from initial lymphatics (lymph capillaries that specialize in lymph collection) to the larger lymph vessels that contain valves and propel the lymph
forward [105]. At a more global level, fluid in the legs can also be reduced by reducing whole body fluid volume by increasing the excretion of water through ultrafiltration or diuretic pharmaceuticals [20, 21]. However, these are less practical interventions and mainly indicated for a clinical population. The following section summarizes the literature studying the impact of methods that utilize compression and skeletal muscle pumping for prevent leg edema formation.

Compression-based technologies for preventing or treating the formation of leg edema include compression stockings and intermittent pneumatic compression [106]. Compression stockings have been demonstrated to reduce overnight shift in leg fluid volume by 15 to 40% and reduce AHI by 27 to 60% [15–17]. The main challenge with compression stockings as a potential treatment for OSA caused by the fluid shift mechanism is low rates of compliance among users, mainly due to their discomfort [25]. Additionally, with improper fit and use, they can increase the risk of exudative skin lesions and thrombosis [26, 27]. Intermittent pneumatic compression are an alternative to compression stockings and have also demonstrated efficacy in reducing leg edema. However, pneumatic compression devices are large, heavy and therefore less convenient due to their lack of portability. Additionally, they can lead to peroneal nerve injury, excessive heat and sweating under the cuff of the device and are generally considered uncomfortable [107,108].

Voluntary activation of the skeletal muscle pump is typically achieved through physical activity such as walking. Compared to the control condition, walking twice daily for 45 minutes for four weeks [18], and once daily for 2 hours for one week [19] were effective in reducing overnight shift in leg fluid by 20 to 40% and AHI by 30 to 38%. However, physical activity is limited in that it also suffers from poor compliance. In Canada four out of five adults do not participate in the 150 minutes of weekly physical activity recommended by Canadian guidelines to maintain good health [28]. The most common reason for poor compliance with physical activity guidelines are cost, lack of time or motivation and illness or disability [30].
There are also devices available that can facilitate voluntary activation of the calf skeletal muscle pump while seated. The two devices that have been investigated in the context of seated leg fluid accumulation are a seated stepping device (StepIt, StepIt.com LLC, Clearwater Beach, Florida) and a portable cycle ergometer. Over a 6 hour period starting from 9 in the morning, leg volume (computed using circumferences of the ankle and knee captured with a measuring tape) increased by 2.0% after using the StepIt device, which was significantly lower than the increase of 2.6% on the control day [58]. Over a 4 hour period starting after participants lay supine for 30 minutes, leg volume (as measured using bioelectrical impedance) increased by 162 ml (6%) when using the StepIt device, which was significantly lower than the leg volume increase of 216 ml (8%) on the control day [109]. In addition, in a subgroup of individuals with a high Mallampati score (a visual assessment of the visibility of the epiglottis upon mouth opening and tongue protrusion), calf muscle activity reduced the snore duration captured on the same night as the intervention. Lastly, after a 15 minute period of quiet sitting where calf circumference increased by 1.5%, a 20 minute period of seated cycling followed and was able to completely reverse the leg fluid accumulation [35]. These seated exercise devices have the potential to have increased adherence compared to walking or other physical activities. However, they are still limited in that they require the motivation and presence of mind to voluntarily use them on a daily basis.

Skeletal muscle pump activation can be also be achieved involuntarily using electrical stimulation of the muscle motor point or the associated peripheral nerve [110], as well as plantar surface vibration which activates the plantar reflex causing contraction of the calf muscle [53]. The effect of involuntary activation of the skeletal pump has not been investigated in the context sleep apnea or to mediate rostral fluid shift when lying down. Several studies have investigated the effects of calf muscle electrical stimulation on venous flow and velocity. These studies find that activation of the skeletal muscle pump increases venous flow and velocity, which increase further with stimulation amplitude (magnitude of the electrical current) [111–113]. However, while this will likely reduce capillary pressures, these studies
do not provide evidence that leg fluid accumulation can be reduced by activating the skeletal muscle pump. In fact, few studies have investigated the effect of involuntary activation of skeletal muscle pump on leg fluid accumulation over the short and long term.

Over the short term, in a group of 52 women, Goddard and colleagues found two different patterns of leg fluid accumulation among two populations grouped as edematous and non-edematous. In the edematous group, leg fluid increased by 14ml/h during quiet sitting, and skeletal muscle pump stimulation by plantar reflex-based stimulation induced a rapid initial decrease of 3 ml followed by a sustained decrease of 2.7 ml/h. In the non-edematous group, leg fluid reduced by 8.9 ml/h during the 30-minute quiet sitting period and skeletal muscle pump activation accelerated reduction to 36 ml/h in the first 5 minutes, followed by a sustained reduction of 5.7 ml/h [53]. Another group investigated a similar plantar reflex-based stimulation device, but they did not report any outcomes related to leg fluid accumulation or fluid shift. However, they did find that using the plantar reflex-based stimulation device was able to improve subjective measures of sleep quality [114].

Skeletal muscle pump activation using ES has also been investigated while standing for 30 minutes. During quiet standing, foot and ankle volume increased by 3.5% (51 ml), while skeletal muscle pumping resulted in a non-significant increase in foot and ankle volume of 0.8% (12 ml) [51]. In a post-operative setting, 16 hours of electrical stimulation while recumbent resulted in a greater reduction in knee and calf edema compared to a similar isometric exercise routine. Over a two week period, change in knee and calf swelling were lower in the electrical stimulation group (-1.3% and 0.5%, respectively) compared to the voluntary exercise group (4.8% and 3.5%, respectively) [115].

Involuntary activation of the calf muscle via electrical stimulation addresses several of the limitations associated with compression and physical activity. Calf muscle electrical stimulation is generally considered to be comfortable, especially after prolonged use [32–34], providing an advantage over compression-based solutions. In addition, the electrical stimulator can be developed into a convenient wearable platform that could address compliance issues with
both physical activity and compression-based solutions. Furthermore, for individuals with mobility limitations (i.e. elderly, post-stroke, or spinal cord injured), the involuntary aspect of calf muscle electrical stimulation provides a significant advantage over physical activity for reducing leg fluid accumulation.

Overall, the following literature review provides a strong basis for studying the prevention of rostral fluid shift and alleviating OSA through treatments aimed at reducing leg fluid accumulation during the day. While interventions have been developed to treat OSA by targeting fluid retention, their reported effects are modest and the therapies tested have several limitations that may limit their convenience and therefore adherence. As a result, this thesis explores factors that increase the risk of neck fluid accumulation and also tests involuntary calf muscle activation using calf muscle ES as a novel and potentially more convenient therapy to reduce leg fluid accumulation and rostral fluid shift. As described, the research on the effects of calf muscle ES on leg fluid accumulation is limited; and its effects on rostral fluid shift and OSA is non-existent. Therefore, this thesis will provide the proof-of-concept that calf muscle ES can reduce leg fluid accumulation while sedentary and subsequently reduce rostral fluid shift when lying down and alleviate its affects on pharyngeal airway narrowing and OSA.
Chapter 3

Risk Factors for Neck Fluid Accumulation While Supine

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3.1 Introduction

Overnight neck fluid accumulation is a risk factor for upper airway pathologies and respiratory disorders [10–13,116]. The accumulation of neck fluid could cause distension of the neck veins and/or edema formation in the pharyngeal soft tissue. These changes increase pressure on the upper airway and could cause the narrowing of the upper airway [10], increase upper airway resistance [11] and collapsibility [12], and increase the severity of obstructive sleep apnea (OSA) [13]. Understanding the factors that increase the likelihood of fluid accumulation in the neck can help identify patients at risk for fluid related respiratory disorders and also identify avenues for new therapies that could target these factors. The redistribution of fluid from the legs into the upper body while recumbent is an important mechanism through which fluid may accumulate in the neck. It has been demonstrated that the volume of fluid leaving the legs while recumbent is correlated with increased neck circumference (NC) signaling the accumulation of fluid in the neck [13,22,87].
The literature review presented in Chapter 2 provided a detailed mechanistic pathway for fluid accumulation in the legs while upright, its shift to the upper body while supine, and the subsequent effects on the upper airway. In brief, the effects of gravity and Starlings forces cause fluid redistribution during the transition from a standing to a recumbent position. Based on the Starling model, the balance of hydrostatic and oncotic forces controls the fluid movement between the capillaries and the interstitial spaces [117]. When hydrostatic pressure is higher in the capillary spaces compared to the interstitial spaces, e.g., in sitting or standing postures, the resulting pressure gradient promotes the movement of blood plasma out of the capillaries and into the interstitial spaces [47]. Therefore, these postures result in an increased overall leg fluid volume (LFV) between 100 and 300 ml [42, 49, 52]. When lying down, the reverse occurs; hydrostatic pressure in the capillaries of the legs is reduced, allowing interstitial fluid to be reabsorbed back into the venous system [37, 43, 64, 118], which moves rostrally through the vascular system to the upper body due to gravity. The result is reduced fluid volume in the legs and associated increases in fluid volumes of the thorax and neck [37, 43].

Identifying patients that are susceptible to increased neck fluid accumulation after prolonged recumbency can be a useful predictor of individuals at risk for upper airway pathologies due to fluid shift, such as OSA defined by the fluid shift mechanism [119]. However, measuring the rostral shift of fluid from the legs to the neck during prolonged recumbency is inconvenient and time consuming. It either requires data collection for hours or overnight [13], or the use of anti-shock trousers to apply lower body positive pressure to quicken LFV shift and simulate prolonged rostral fluid shift [11], which can be cumbersome. Therefore, it is important to investigate simple measurements that can be performed quickly and conveniently (e.g., in clinic), which in turn could predict the amount of fluid that would accumulate in the neck after prolonged recumbency and potentially identify individuals at risk for OSA caused by the fluid shift mechanism. Throughout this chapter, we refer to these instantaneous, one-time measurements taken before prolonged recumbency as baseline measures.
Previous studies have shown that in heart failure patients, an increased baseline leg edema score is significantly correlated with the overnight change in LFV and the increase in NC [22,88]. However, leg edema score is determined subjectively, requiring the application of pressure to a small area on the lower leg to create an indentation in the tissue. A subjective score from 1+ (less severe) to 4+ (more severe) is given based on the depth of indentation and how long the indentation remains [120]. In addition, these studies only focused on heart failure patients experiencing fluid overload who have more obvious leg swelling caused by edema formation.

The current study explored various objectively measured baseline metrics to improve prediction of neck fluid accumulation in the general population. These metrics included: baseline LFV, NC, neck fluid volume (NFV), and upper-airway cross-sectional area (UAXSA). The purpose of the present study was to develop a model based on baseline measurements of body fluid, as well as demographics and anthropometrics of the participants to predict fluid accumulation in the neck.

3.2 Methods

3.2.1 Participants

The protocol was approved by the Research Ethics Board of the Toronto Rehabilitation Institute, and all participants provided written informed consent prior to participating in the study. Criteria for inclusion were men and women between 18 and 65 years of age with a body mass index (BMI) < 30 kg/m2, and a blood pressure ≤ 140/90 mmHg. Women were included if they were premenopausal and did not have their menstrual period at the time of experiments. The exclusion criteria were a history of hysterectomy, having metal implants, history of cardiovascular, renal, or respiratory diseases, use of prescribed medications for those diseases, or taking any over the counter medication that might influence fluid retention, such as diuretics or non-steroidal anti-inflammatory agents. Participants were instructed to
Chapter 3. Risk Factors for Neck Fluid Accumulation While Supine

abstain from alcohol or caffeine consumption on the day of the study. Participants were recruited from the community by advertisement.

3.2.2 Neck Circumference, upper-Airway cross-sectional area, and airway length measurements

At the beginning of each session, a tape measure was used to measure NC just above the cricothyroid cartilage. A line was drawn at this level to ensure the NC measurement at the end of the experiment was made at the same level, as described in past studies [12, 88]. Upper-airway length (L_{UA}, distance from velum to glottis) and the average UAXSA from velum to glottis were measured using acoustic pharyngometry [121].

3.2.3 Leg and neck fluid volumes measurements

Fluid was measured in the leg and neck using bioelectrical impedance, a non-invasive technique used to estimate the fluid volume of tissues. The bioelectrical impedance method of fluid measurement is well validated and highly reproducible with an accuracy of 0.5% compared to reference measures of total body water, repeatability within 0.3%, and test-retest correlation > 95% [60, 122].

The method is based on Ohms law \( V = IR \), where the resistance of a tissue to electrical current is inversely related to its fluid content and directly related to its length: \( R = \rho L^2 / v \), where \( \rho \) is the resistivity of the fluid, \( L \) is the segments length, \( v \) is the fluid volume, and \( R \) is resistance [123–125]. The resulting equation \( v = \rho L^2 / R \) has been widely used to estimate total body water, where \( L \) is replaced with the subjects height [60, 122]. Past studies have also used bioelectrical impedance to measure the fluid volume of individual body segments. In these studies, segments are assumed to be cylindrical in shape, with \( L \) representing the length of the segment [126–129]. In a previous study, we developed a system for continuous measurement of fluid volumes in various body segments [54]. We used a modified version of
this equation to reflect the tapered shape of the body segments (leg, abdomen, chest, and neck). Therefore, fluid volume was estimated as [130]:

\[ v = \frac{\rho^{2/3}}{3(4\pi)^{1/3}} \left( \frac{L}{C_1 C_2 R} \right)^{2/3} L \left( C_1^2 + C_2^2 + C_1 C_2 \right) \]  \hspace{1cm} (3.1)

Where \( C_1 \) and \( C_2 \) are the top and bottom circumferences of the segment, respectively, \( L \) is the segments length, \( R \) is the segments resistance, and \( \rho \) is blood resistivity, which is estimated as 47 Ωcm [125].

For both the leg and the neck, two surface electrodes were used to inject a low amplitude (400µa), high frequency current (25 kHz and 50 kHz for the leg and neck, respectively) and two surface electrodes were used to measure the voltage (Figure 3.1) to estimate bioelectrical impedance of the segment (\( R \) in Equation 3.1) [54]. In Figure 3.1, electrodes to inject current are denoted as I- and I+ and electrodes to measure voltage are denoted as V- and V+. Voltage measuring electrodes were placed at the ankle and upper thigh of the right leg for the LFV measurement. To measure NFV, voltage-measuring electrodes were placed on the right side of the neck below the right ear and at the base of the neck (Figure 3.1). The current-injecting electrodes were placed one inch away from the voltage-measuring electrodes. To isolate the signal from different body segments for bioelectrical impedance measurements, a different current frequency was used for the leg and neck. The electrodes were secured to the skin using adhesive tape. At the beginning of the study, length and circumference of each segment were measured with a measuring tape at the level of the voltage-measuring electrodes. For NFV, the NC measurement was used for both circumferences \( C_1 \) and \( C_2 \) (Equation 3.1).

### 3.2.4 Protocol

This study was conducted using data from a previous study [54] conducted at the Toronto Rehabilitation Institute. While seated, blood pressure was measured to ensure subjects were normotensive. Next, surface electrodes were applied to the participant, as shown in Figure
Figure 3.1: Positions of the surface electrodes for recording the bioelectrical impedance in the leg and neck. I+ and I- denote surface electrodes used to inject a low amplitude (400 µa), high frequency (25 kHz and 50 kHz for the leg and neck, respectively) current. V+ and V- denote surface electrodes used to measure the voltages across the leg and neck segments. LFV denotes leg fluid volume and NFV denotes neck fluid volume.
3.1, to measure the bioelectrical impedance. All experiments were performed in the same room with the temperature maintained between 22°C and 24°C. Next, participants were asked to stand motionless for 5 minutes. For the following 90 minutes, the subjects then lay awake in the supine position on a bed without a pillow. Bioelectrical impedance of the leg and neck were recorded continuously and simultaneously during both standing and supine positions. Subjects were instructed to remain motionless during the recordings.

3.2.5 Baseline and outcome variables

Baseline variables were selected as quantities that could be easily measured and represent fluid content in the body and/or have been shown to have a strong correlation with increases in NC while supine. The variables included were LFV after standing for 5 minutes (LFV\textsubscript{st5}), NFV after standing for 5 minutes (NFV\textsubscript{st5}), LFV at baseline supine (LFV\textsubscript{sp1}), NFV at baseline supine (NFV\textsubscript{sp1}), immediate change in LFV upon transitioning from standing to supine position (\(\Delta\text{LFV}_P = \text{LFV}_{sp1} - \text{LFV}_{st5}\)), immediate change in NFV upon transitioning from standing to supine position (\(\Delta\text{NFV}_P = \text{NFV}_{sp1} - \text{NFV}_{st5}\)), NC measured at baseline supine (NC\textsubscript{sp1}), upper-airway cross-sectional area at baseline supine (UAXSA\textsubscript{sp1}), and length of the upper-airway (between the velum and the glottis) measured at baseline supine (L\textsubscript{UA}). Also computed was \(\Delta\text{LFV}_{90} = \text{LFV}_{sp90} - \text{LFV}_{sp1}\), where LFV\textsubscript{sp90} is the LFV after 90 minutes supine. Lastly, demographic information including age, sex, height, weight, and BMI were also included in the regression model. Sex was defined as a binary variable with 0 and 1 representing men and women, respectively.

Outcome variables were selected as \(\Delta\text{NC}_{90} = \text{NC}_{sp90} - \text{NC}_{sp1}\), \(\Delta\text{NFV}_{90} = \text{NFV}_{sp90} - \text{NFV}_{sp1}\), and \(\Delta\text{UAXSA}_{90} = \text{UAXSA}_{sp90} - \text{UAXSA}_{sp1}\), where NC\textsubscript{sp90}, NFV\textsubscript{sp90}, and UAXSA\textsubscript{sp90} were the NC, NFV and UAXSA after lying supine for 90 minutes, respectively.
3.2.6 Data analysis

The analysis was performed in three steps. In the first step, individual correlations were analyzed between each of the baseline variables and the outcome variables using Pearson correlations with normally distributed data, and Spearman’s rank correlation with non-normally distributed data. Normality of the data was determined using the Kolmogorov-Smirnov test. A correlation was considered significant with a two-sided $p$-value $< 0.05$.

In the second step of the analysis, a multiple linear regression model was developed to determine the most significant factors that contributed to the changes in outcome variables. Baseline variables were selected for the model using a forward stepwise selection. Using this approach, the model starts with no variables from the variable set, adding a variable if their associated $p$-value is below 0.05, and removing variables if their $p$-value is above 0.10. The variable set included the entire baseline variable set already described, excluding $\Delta LFV_P$ and $\Delta NFV_P$, since they are linear combinations of the baseline leg and neck fluid volume measurements in the supine and standing position. We also excluded weight and height in the same variable set as BMI, given that BMI is a function of weight and height.

The final step of the analysis was to investigate whether the subjects can be classified into two groups of high risk and low risk regarding the effects of fluid accumulation in the neck. To do this, input variables were converted into standardized $z$-scores and a method based on the combination of principal component analysis (PCA) and unsupervised clustering was implemented. PCA is a statistical method that converts a set of correlated variables into a new set of uncorrelated (orthogonal) variables called principal components [131]. The principal components are linear combinations of the original variables weighted by their contribution to explaining the variance in a particular orthogonal dimension. PCA was used to reduce the dimensionality of an input variable set and to identify a subset of input variables that account for most of the variability in the data. The number of components to retain was selected based on the point on inflexion on the Scree plot (Figure 3.2a) which plots the component number versus the Eigenvalue as the dependent variable. The Eigenvalue
is a scalar indicator of the substantive importance of the associated component with larger 
Eigenvalues representing more important components. Components were retained if they 
were to the left of the point of inflexion, but not including the inflexion point itself. We 
applied a similar approach for selecting input variables by plotting the factor weightings of 
each input variable in descending order (Figure 3.2b). A point of inflexion was identified 
and variables to the left of this point were retained as a variable subset for further clustering 
analysis.

An unsupervised K-means clustering was then applied to the selected subset of variables 
to cluster the subjects into two groups (K=2). This clustering method separates the data 
into K groups by establishing a centroid for each group. The position for the centroid is 
initialized and data points are assigned to the closest centroid (the assignment step). The 
centroid is then repositioned to minimize the within-cluster sum of squares (the update 
step). The assignment step and the update step are repeated until convergence (i.e. when 
the assignments no longer change) [132]. Outcome variables were compared between the two 
clusters using the student t-test for normally distributed data and the Kolmogorov-Smirnov 
test for non-normally distributed data; a two-sided p-value of less than 0.05 was considered 
significant. Statistical analysis was performed using SAS 9.3 (SAS Institute Inc., Cary, NC), 
and PCA and clustering were performed in Matlab (MathWorks, Natick, MA).

3.3 Results

Fifty-two candidates consented to participate in the study. One participant declined to 
continue after being instrumented. Eleven participants had movement artifacts in the bio-
electrical impedance signals and their data were excluded. Ten participants did not have 
standing data collected and were excluded from this analysis. After all exclusions, a total 
of 30 participants (13 men and 17 women) were included in the analysis. Baseline fluid 
measures, as well as anthropometric data and variables for men, women, and all subjects
are shown in Table 3.1. After lying supine for 90 minutes, NFV and NC in all subjects increased significantly (p<0.01), while the upper-airway cross-sectional area reduced significantly (p=0.04). Compared to men, women had smaller weight, height, baseline NC and UA length, but had similar age and BMI. In addition, women had lower baseline NFV and LFV at standing and supine. However, both experienced a similar change in LFV over the 90-minute supine period. In terms of outcome variables, men experienced a greater change in NC after 90 minutes supine compared to women (p=0.01). There was also a trend to suggest a greater increase in NFV after 90 minutes supine in men compared to women (p=0.09). However, the differences between women and men in the change in UAXSA after 90 minutes supine was not statistically significant (p=0.14).

Individual correlations between baseline variables and outcome variables are summarized in Table 3.2. Three baseline variables were significantly correlated with the change in NC after 90 minutes supine: sex (r=0.56, p<0.01), and baseline LFV at standing (r=0.48, p=0.01) and supine (r=0.51, p<0.01).

These correlations demonstrate that male sex and increased baseline LFV corresponded to an increase in NC over the 90-minute supine period. Change in NFV after lying supine for 90 minutes was directly and significantly correlated with baseline NFV at standing (r = 0.41, p=0.03) and baseline UAXSA (r = 0.38, p=0.04). It was also borderline significant with NFV at supine (r = 0.36, p=0.05). These same variables were also significantly correlated to the narrowing (∆) in the UAXSA after lying supine (NFV<sub>st5</sub>: r = -0.44, p=0.02, NFV<sub>sp1</sub>: r = -0.46, p=0.01, UAXSA<sub>sp1</sub>: r = -0.48, p<0.01). Lastly, ∆LFV<sub>90</sub> was significantly correlated with baseline LFV at standing (r=0.39, p=0.03) and supine (r=0.44, p=0.01). In terms of the correlation across outcome variables, the change in NC was significantly correlated with the change in NFV after 90 minutes supine (r=0.364, p=0.047). It was not correlated with the change UAXSA after 90 minutes supine (p=0.82). In addition, the correlation between change in NFV and the change in UAXSA after 90 minutes supine was not statistically significant (r=-0.32, p=0.09).
Table 3.1: Baseline and outcome variable data in all subjects, and associated p-values for statistical differences between men and women

<table>
<thead>
<tr>
<th>Variable</th>
<th>All</th>
<th>Men (n=13)</th>
<th>Women (n=17)</th>
<th>P-value between sexes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>39.2±11.7</td>
<td>37.8±13.2</td>
<td>39.17±11.7</td>
<td>0.77</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>23.3±2.9</td>
<td>25.0±4.3</td>
<td>22.8±2.6</td>
<td>0.77</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>69.0±11.8</td>
<td>81.7±15.7</td>
<td>63.0±9.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Height, cm</td>
<td>172.0±10.7</td>
<td>180.7±9.3</td>
<td>165.9±7.5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NCsp1, cm</td>
<td>36.6±3.8</td>
<td>41.0±3.4</td>
<td>34.0±2.0</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>UAXSA_{sp1}, cm²</td>
<td>2.9±1.3</td>
<td>3.0±1.5</td>
<td>2.7±1.1</td>
<td>0.24</td>
</tr>
<tr>
<td>NFV_{st5}, ml</td>
<td>223.9±60.2</td>
<td>253.8±72.5</td>
<td>200.7±26.6</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LFV_{st5}, ml</td>
<td>2196±289</td>
<td>2532±483</td>
<td>2058±256</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ΔNFVₚ, ml</td>
<td>12.1±8.9</td>
<td>11.0±12.5</td>
<td>11.9±7.6</td>
<td>0.7</td>
</tr>
<tr>
<td>ΔLFVₚ, ml</td>
<td>66.7±28.7</td>
<td>59.6±51.5</td>
<td>68.0±21.0</td>
<td>0.42</td>
</tr>
<tr>
<td>NFV_{sp1}, ml</td>
<td>236.0±60.5</td>
<td>264.8±71.9</td>
<td>212.6±28.1</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LFV_{sp1}, ml</td>
<td>2129±279</td>
<td>2473±499</td>
<td>1990±246</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ΔLFV₉₀, ml</td>
<td>143.7±37.3</td>
<td>160.9±56.6</td>
<td>138.9±32.4</td>
<td>0.18</td>
</tr>
<tr>
<td>L_{UA}, cm</td>
<td>8.8±1.5</td>
<td>10.1±1.6</td>
<td>8.1±0.9</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>ΔNC₉₀, cm</td>
<td>0.52±0.36</td>
<td>0.67±0.31</td>
<td>0.35±0.32</td>
<td>0.01</td>
</tr>
<tr>
<td>ΔNFV₉₀, ml</td>
<td>15.7±4.4</td>
<td>16.8±5.2</td>
<td>14.3±3.1</td>
<td>0.08</td>
</tr>
<tr>
<td>ΔUAXSA₉₀, cm²</td>
<td>-0.30±0.72</td>
<td>-0.44±0.71</td>
<td>-0.19±0.72</td>
<td>0.14</td>
</tr>
</tbody>
</table>
Table 3.2: Correlation coefficients for both the individual baseline variables and the multiple linear models developed from stepwise regression

<table>
<thead>
<tr>
<th>Variables</th>
<th>$\Delta$NC90</th>
<th>$\Delta$NFV90</th>
<th>$\Delta$UAXSA90</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>-0.561*</td>
<td>-0.28</td>
<td>0.191</td>
</tr>
<tr>
<td>BMI</td>
<td>0.106</td>
<td>0.144</td>
<td>0.068</td>
</tr>
<tr>
<td>Weight</td>
<td>0.23</td>
<td>0.124</td>
<td>-0.112</td>
</tr>
<tr>
<td>Height</td>
<td>0.241</td>
<td>0.288</td>
<td>-0.206</td>
</tr>
<tr>
<td>Age</td>
<td>0.263</td>
<td>-0.229</td>
<td>0.066</td>
</tr>
<tr>
<td>NC$_{sp1}$</td>
<td>0.314</td>
<td>0.089</td>
<td>-183</td>
</tr>
<tr>
<td>UAXSA$_{sp1}$</td>
<td>-0.022</td>
<td>0.380*</td>
<td>-0.484*</td>
</tr>
<tr>
<td>NFV$_{st5}$</td>
<td>-0.046</td>
<td>0.406*</td>
<td>-0.437*</td>
</tr>
<tr>
<td>LFV$_{st5}$</td>
<td>0.483*</td>
<td>0.281</td>
<td>-0.002</td>
</tr>
<tr>
<td>$\Delta$NFV$_P$</td>
<td>-0.346</td>
<td>-0.167</td>
<td>0.083</td>
</tr>
<tr>
<td>$\Delta$LFV$_P$</td>
<td>-0.091</td>
<td>0.002</td>
<td>-0.176</td>
</tr>
<tr>
<td>NFV$_{sp1}$</td>
<td>-0.106</td>
<td>0.357*</td>
<td>-0.463*</td>
</tr>
<tr>
<td>LFV$_{sp1}$</td>
<td>0.510*</td>
<td>0.292</td>
<td>0.014</td>
</tr>
<tr>
<td>$\Delta$LFV$_{90}$</td>
<td>0.348</td>
<td>0.306</td>
<td>0.069</td>
</tr>
<tr>
<td>L$_{UA}$</td>
<td>0.16</td>
<td>0.121</td>
<td>-0.281</td>
</tr>
<tr>
<td>Model</td>
<td>0.819*</td>
<td>0.406*</td>
<td>0.484*</td>
</tr>
</tbody>
</table>

Asterisks (*) represent a significant correlation (P<0.05)
The linear regression models developed using stepwise feature selection for each outcome variable are summarized in Table 3.3. Models for $\Delta NFV_{90}$ and $\Delta UAXSA_{90}$ only included one variable ($NFV_{sp1}$ and $UAXSA_{sp1}$, respectively). The linear regression model of $\Delta NC_{90}$ showed that sex, $NFV_{sp1}$, $LFV_{st5}$, and $NC_{sp1}$ contributed to the increased NC after lying supine for 90 minutes. Models developed with weight and height replacing BMI in the baseline variable set did not change the models and were not reported. The correlation between the linear regression models and the outcome variables are listed in Table 3.2. Since the models developed for $\Delta NFV_{90}$ and $\Delta UAXSA_{90}$ included only one variable, the correlations were unchanged compared to those from single-variable models. On the other hand, the correlation of the proposed model with $\Delta NC_{90}$ was higher than the correlations of individual variables with $\Delta NC_{90}$.

Table 3.3: Results of the stepwise regression for each outcome variable

<table>
<thead>
<tr>
<th>Dependent Variable</th>
<th>Step</th>
<th>Variable entered</th>
<th>Partial $R^2$</th>
<th>Model $R^2$</th>
<th>P-value in the final model</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Delta NC_{90}$</td>
<td>1</td>
<td>Sex</td>
<td>0.293</td>
<td>0.293</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>$NFV_{sp1}$</td>
<td>0.204</td>
<td>0.497</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>$LFV_{st5}$</td>
<td>0.117</td>
<td>0.614</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>$NC_{sp1}$</td>
<td>0.073</td>
<td>0.687</td>
<td>$&lt;0.01$</td>
</tr>
<tr>
<td>$\Delta NFV_{90}$</td>
<td>1</td>
<td>$NFV_{sp1}$</td>
<td>0.188</td>
<td>0.188</td>
<td>0.02</td>
</tr>
<tr>
<td>$\Delta UAXSA_{90}$</td>
<td>1</td>
<td>$UAXSA_{sp1}$</td>
<td>0.234</td>
<td>0.234</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Only the first principal component from the PCA was retained, since the point of inflexion was positioned at the second component (Figure 3.2a). The first principal component explained 37.1% of the variance in the data set. The point of inflexion in the modified Scree plot of the first principal component (Figure 3.2b) was positioned around BMI. Thus, sex, baseline NC, and baseline NFV/LFV at both standing and supine positions were retained for further analysis. Given the co-linearity of the standing and supine measures of LFV and NFV, only standing LFV and NFV were included in the variable subset. Clustering based on
these variables yielded two distinct groups. The dominant factor that separated the clusters was sex, such that cluster 1 contained only men, while cluster 2 contained only women. To explore the data further, sex was removed from the variable subset and K-means clustering was repeated.

These results are illustrated in Figure 3.3. As shown, clusters 1 and 2 are still separated based mostly on sex, with cluster 2 consisting of only women and cluster 1 consisting of majority men and two women. The clusters show a good separation across all of the input variables NCsp1, NFVst5, and LFVst5, which were significantly different between clusters (see Table 3.4). Differences in the value of the outcome variables of ΔNC90, ΔNFV90, and ΔUAXSA90 between clusters 1 and 2 are shown in Table 3.4. There was a non-significant tendency for the participants of cluster 2 to have a greater increase in ΔNC90, ΔNFV90 and a greater reduction in ΔUAXSA90 compared to cluster 1.

Figure 3.2: (a) Scree plot of the Eigenvalues of each component used to identify the inflexion point used as a threshold for the number of components to retain. The point of inflexion is located at Component 2, which means that only the first (Component) is retained. (b) A modified scree plot of the variables and the weightings of the variables on the first component, similarly used to identify the point of inflexion as a threshold for number of variables to retain. The inflexion point is located at the BMI variable, meaning all variables to the left of BMI are retained.
Figure 3.3: Plots of clusters and centroid locations on the two-dimensional plot of (a) baseline neck fluid volume (NFV) and baseline neck circumference (NC), and (b) baseline leg fluid volume (LFV) and baseline neck fluid volume (NFV). Clustering based on the variable subset that included baselines NC (NC_{sp1}), baseline standing LFV (LFV_{st5}), and NFV (NFV_{st5}) yielded two distinct clusters, with cluster 2 consisting of only women, and cluster 1 consisting of majority men. The input variables NC_{sp1}, NFV_{st5}, and LFV_{st5}, were significantly different between clusters, but not the outcome variables including the change in NC, NFV and upper-airway cross-sectional area after 90 minutes supine (ΔNC_{90}, ΔNFV_{90} and ΔUAXSA_{90}, respectively).

### 3.4 Discussion

The most important finding of the present study was that demographic information and baseline measures of fluid in the body could be used to predict the amount of fluid leaving the legs and the increase in neck circumference after lying down for 90 minutes. For the first time, we showed that baseline standing and supine LFV have a positive and significant correlation with changes in LFV and NC after lying supine for 90 minutes. Prior studies have shown similar findings that in patients with heart failure, subjective clinical measures of leg edema score before sleep is correlated with the overnight change in LFV and increased severity of sleep apnea [22, 88]. It has also been shown that the change in LFV during sleep is the strongest predictor of the change in NC, as well as the consequent increase in the severity of sleep apnea [13, 88]. These studies show strong evidence for the importance of the
Table 3.4: Mean and standard deviation of the variables within each cluster and associated p-values for statistical differences between the clusters.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Cluster 1</th>
<th>Cluster 2</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>NFV$_{st5}$</td>
<td>249.7±73.4</td>
<td>198.1±26.4</td>
<td>0.02</td>
</tr>
<tr>
<td>LFV$_{st5}$</td>
<td>2341±276</td>
<td>2050±225</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>NC$_{sp1}$</td>
<td>39.7±2.6</td>
<td>33.5±1.3</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>∆NC$_{90}$</td>
<td>0.64±0.40</td>
<td>0.40±0.28</td>
<td>0.14</td>
</tr>
<tr>
<td>∆NFV$_{90}$</td>
<td>17.0±5.2</td>
<td>14.4±3.1</td>
<td>0.31</td>
</tr>
<tr>
<td>∆UAXSA$_{90}$</td>
<td>-0.43±0.67</td>
<td>-0.18±0.76</td>
<td>0.15</td>
</tr>
</tbody>
</table>

overnight change in LFV as a predictor of fluid accumulation in the neck and sleep apnea severity. However, they are based on measurements of LFV acquired overnight or during a prolonged period of recumbency. Our results are unique to suggest that a simpler objective measure of LFV taken at baseline could be used as a strong predictor of the amount of fluid that will leave the legs, causing an increase in NC during a prolonged period of recumbency.

Another demographic that predicted the change in NC was sex. Although change in LFV was similar between the sexes, men experienced a greater increase in the NC during the supine period. The effect of sex on rostral fluid shift has been previously shown in overnight studies in patients with heart failure [22]. This study demonstrated that the overnight change in LFV in men was significantly correlated to the increase in NC. For women with the same amount of fluid leaving the legs, an overnight change in LFV did not correlate with the overnight change in NC. As such, men with heart failure experienced an overnight increase in NC seven times greater than women. Recently, Yadollahi et al. studied the dynamics of fluid shift in men and women lying awake in the supine position for 90 minutes to investigate the differences in fluid shift between the two sexes [54]. They found that men and women experienced a similar volume of fluid shift out of their legs, with men accumulating more fluid in their thorax and neck. In addition, there was also a trend suggesting that women accumulate more fluid in their abdomen, compared to men. It has been suggested that since
women have larger gonadal veins and the large venous plexus around the uterus [133,134], the venous pooling in the pelvic region of women is greater than that of men [135]. Therefore, in women, the fluid shifting out of the legs may be accumulating more in the pelvis and reducing fluid redistribution into the neck [22,54].

Our results comply with previous studies showing that sex has a significant effect on the change in NC after 90 minutes. Clustering based on the subset of variables selected from the PCA separated the data mainly based on sex. Even when sex was excluded from the clustering analysis, the data was grouped based on baseline NC and baseline LFV and NFV (significant contributors to the first PCA), which were significantly different between the sexes (Table 3.1). In the cluster analysis presented in Figure 3.3, cluster 1 represents female subjects with lower baseline NC, NFV and LFV, whereas cluster 2 represents male subjects with higher baseline NC, NFV, and LFV. However, the stepwise regression model shows that in addition to sex, LFV_{st5}, NFV_{sp1}, and NC_{sp1} also entered the model. Together, these factors accounted for 40% of variation in ∆NC_{90}, as indicated by the partial R^2 (Table 3.3). The inclusion of these variables, specifically baseline LFV, demonstrates that baseline LFV has an independent effect on ∆NC_{90}, beyond the effects of sex. This complies with previous studies showing that interventions reducing baseline LFV, such as compression stockings [15–17], exercise [136], and diuretics [20], also led to less overnight increases in NC.

The change in NC (ΔNC_{90}) during the supine period was also correlated with the change in NFV (ΔNFV_{90}), demonstrating that the change in NC is indeed a reflection of the change in fluid accumulating in the neck. This finding has been established by the previous work of Yadollahi et al. [54]. Similarly, while not significant there was a trend to suggest that ΔNFV_{90} and ΔUAXSA_{90} were negatively correlated, indicating that more fluid entering the neck during the supine period corresponded to further narrowing of the UAXSA. One likely explanation that the correlation between these variables did not reach significance is the short duration (90 minutes) of the supine period. This did not allow enough time for the airway to narrow as a result of neck fluid accumulation.
Variables that were found to be predictive of both $\Delta NFV_{90}$ and $\Delta UAXSA_{90}$ were baseline UAXSA and baseline NFV (standing and supine). These variables formed positive relationships with $\Delta NFV_{90}$ and negative relationships with $\Delta UAXSA_{90}$. Therefore, increased baseline NFV and UAXSA were related to an increased neck fluid volume and further narrowing of the upper-airway over the 90-minute supine period, both of which signal the accumulation of fluid in the neck. A likely explanation is that a higher baseline NFV and a narrower baseline UAXSA could be symptoms of having a higher overall fluid retention at baseline. Thus, when lying supine, those with a greater baseline fluid retention experience an increased fluid shift, resulting in greater increases in NFV and a narrowing of UAXSA over the 90-minute period.

The results of this study identify characteristics that could possibly predict those at risk for OSA through the fluid shift mechanism. Past studies have shown that an increased overnight fluid shift from the legs and an increased overnight change in NC are both strongly related to an increased OSA severity [13]. This study identifies phenotypic characteristics, specifically the male sex and increased baseline LFV, which are related to both an increased fluid shift from the legs and an increased change in NC when lying supine for a prolonged period. Future work is aimed at exploring the capability of these characteristics and predicting those at risk of OSA through the fluid shift mechanism.

This study is subject to limitation, mainly imposed by the sensitivity of bioelectrical impedance measurements to movement. Bioelectrical impedance measurements are sensitive to movement and body posture, and because our participants could not remain still for more than 90 minutes, the duration of study was limited to just that. As a result, the magnitude of fluid shift might be lower compared to overnight fluid shift. Our study was performed during wakefulness to limit involuntary body movements during sleep. Since the pattern of fluid redistribution out of the legs and into the neck could vary depending on whether or not the subject was awake or sleeping, future work should include studies conducted during sleep. Finally, we did not examine correlations between baseline variables and outcome
variables in men and women separately due to the small number of subjects in each group. In the future we aim to confirm our findings in larger groups of men and women to gain a better understanding of fluid shift between the sexes. In addition, future work should adapt the models developed in our study using data from overnight polysomnography, the gold standard for diagnosing sleep apnea. Models developed with these data can be used to predict outcomes related to respiratory disorders affected by fluid, such as OSA.

Our study is also limited by the study sample, which only included healthy non-obese men and women. Sleep apnea is more prevalent in the obese population [1], as well as those co-morbid with end-stage renal disease [137] and cardiovascular diseases such as hypertension [138] and heart failure [139]. Furthermore, there is evidence to suggest that sleep apnea associated with fluid shift is a significant problem in these comorbid populations [21,87–89]. It is therefore important that future research into phenotypic characteristics that predict OSA associated with fluid shift, include individuals with obesity and the aforementioned comorbidities.

In conclusion, this study demonstrates that a baseline measure of LFV is predictive of the amount of fluid leaving the legs and the change in NC after lying supine for 90 minutes. Our findings also suggest that with comparable changes in LFV over the supine period, men experience greater fluid accumulation in the neck, compared to women. Applications of these findings are in identifying characteristics of individuals at risk of OSA through an overnight rostral fluid shift. There are also applications in the development of novel therapies aimed at reducing baseline LFV to minimize the associated rostral fluid shift and accumulation in the neck during sleep. Such therapies may include reducing sedentary behaviors such as prolonged sitting [52] and wearing compression stockings [15,17]. Future work should explore other interventions such as physical activity [136,140,141] or electrical stimulation of the calf muscle pump [53] to reduce baseline leg edema and neck fluid accumulation in a population at risk for OSA caused by the fluid shift mechanism. In the next chapter, we explore electrical stimulation of the calf muscle pump as a potential therapy for reducing the accumulation of
fluid in the legs while sedentary and the consequent accumulation in the neck while supine.
Chapter 4

The Effect of Calf Muscle Electrical Stimulation While Seated on Rostral Fluid Shift, Snoring and Sleep Apnea

Submitted: Sleep. Dec 2017

4.1 Introduction

Obstructive sleep apnea (OSA) is a common disorder that occurs in 10% of the population [142] and is characterized by intermittent narrowing or collapse of the pharynx during sleep. The pathophysiological mechanism for pharyngeal collapse can occur due to multiple interacting factors [79]. For example, the pharynx can collapse when the normal reduction in pharyngeal dilator muscle tone at sleep onset occurs concurrently with a pharynx that is narrowed due to anatomical factors (e.g. neck fat deposition). However, there is a growing body of evidence that accumulated fluid in the neck tissue surrounding the pharynx is a significant factor contributing pharyngeal narrowing and collapse [119]. Fluid accumulates in the neck by way of fluid shifting out of the legs due to gravity when lying down to sleep, which has been demonstrated to increase the severity of OSA [13].
More fluid that accumulates in the legs during the day contributes to a greater shift of fluid into the neck when lying down [143]. Daytime leg fluid accumulation is increased with more time spent sedentary [13]. This causes blood to pool in the legs, which raises pressure in the capillaries of the legs causing the filtration of fluid out of the vasculature and into the tissue spaces (i.e. interstitial fluid), according to the Starling mechanism [117]. Therapies aimed at reducing fluid retention can treat pharyngeal narrowing and OSA. For example, daily walking, wearing compression stockings during the day, ultrafiltration, and diuretics, reduce rostral fluid shift in association with a 16-38% reduction in OSA severity, as assessed by the apnea-hypopnea index (AHI) [16,18,20,21].

Among the treatments investigated, compression stockings and exercise are most practical for the general population, however both are associated with low adherence for other indications [25,28,144]. Another method for reducing leg fluid is involuntary activation of the calf muscle via electrical stimulation (ES) [53,145]. As a device-based therapeutic approach, calf muscle ES can be especially useful for individuals that are sedentary due to aging or mobility impairment. Calf muscle contractions via ES reduces leg fluid by activating the skeletal muscle pump, moving blood back to the heart, reducing hydrostatic pressure in the capillaries of the legs, and counteracting the filtration of fluid out of the capillaries into the interstitium [146,147]. Indeed, calf muscle ES has been shown to reduce leg fluid accumulation while seated [53,145]. However, to the best of our knowledge the effect of calf muscle ES on rostral fluid shift and OSA have never been investigated.

The primary objective of this study was to examine the effect of calf muscle ES on leg fluid accumulation while seated and its impact on rostral fluid shift when lying supine, and subsequent snoring and OSA. We hypothesize that calf muscle ES will reduce the rostral shift of fluid out of the legs and into the neck, reduce snoring and alleviate OSA.
4.2 Methods

4.2.1 Participants

The research protocol was approved by the Research Ethics Board of the Toronto Rehabilitation Institute University Health Network. Participants were recruited from the community through advertisement and provided written informed consent prior to participation. Criteria for study inclusion were adults with a body mass index (BMI) \(< 30 \text{ kg/m}^2\), blood pressure \(<140/90 \text{ mmHg}\) and a positive screening for sleep apnea with an AHI \(\geq 10\), which took place less than two weeks prior to the first study day (see Appendix A). Participants were excluded if they had a history of treated sleep apnea, cardiovascular, renal, neurological, or respiratory disorders, and were taking prescribed medication for these conditions. Obese participants were excluded to avoid the ceiling effect of neck fat deposition on airway collapse, which might confound the effects of the intervention.

4.2.2 Fluid measurement

Leg fluid volume (LFV) and neck fluid volume (NFV) were measured using the method of bioelectrical impedance, described previously [54] and in Appendix A. Neck circumference (NC) was measured at the level of cricothyroid cartilage, and calf circumference was measured around the largest part of the calf. Circumferences were measured at the beginning and end of both sitting and supine periods. A line was drawn around the neck and calf during the first measurement to ensure repeated measurements were made at the same level.

4.2.3 Monitoring of activity and diet

Activity and diet can both impact body fluid retention [31,148] and were therefore monitored for 5 and 3 days leading up to the study day, respectively (see Appendix A). Activity variables of interest extracted from the activity log were time spent sitting, time spent sedentary, and time spent active. Diet variables extracted from the 3-day dietary record were calories, water
and sodium.

### 4.2.4 Electrical stimulation protocol

Calf muscle ES was applied to both calf muscles at a duty cycle of 2 seconds on, followed by 2 seconds off (see Appendix A). The left and right gastrocnemii were stimulated out of sync, such that when the right gastrocnemius was contracted the left was relaxed and vice versa. Stimulation amplitude was set to the maximally tolerated stimulation amplitude (typically between 20 and 40 mA).

### 4.2.5 Study protocol

The study was a randomized, single-blind, double crossover protocol. Participants were blinded in that they were not told which study condition they were randomized too, and in both conditions (sham and active ES) they could feel the electrical current passing through their lower leg. However, it was possible for participants to deduce the study condition if they realized their calf was only contracting when receiving active ES. The investigator could not be blinded as he was responsible for administering the calf muscle electrical stimulation. As illustrated in Figure 4.1, participants initially completed an activity log prior to the study day. On the study day, participants arrived at 11:00 AM and were instrumented with bioelectrical impedance surface electrodes and calf muscle ES electrodes. Next, circumferences of the neck and leg were measured. Participants then lay still on a bed in the supine position without a pillow for 30 minutes. Next, participants sat for two and a half hours while active or sham ES was applied. Finally, participants again lay still on a bed in the supine position without a pillow for the final 60 minutes to evaluate the magnitude of rostral fluid shift. On the night of the study day, participants used a portable sleep monitoring device to measure their AHI and snoring index (described below). Following the first study day and night, participants returned to the laboratory one week later to participate in the other study condition.
4.2.6 Data analysis

4.2.7 Fluid measurements

To characterize fluid accumulation in the leg while seated, the change in LFV ($\Delta LFV_{sit}$) and calf circumference ($\Delta Calf_{sit}$) over the seated period were computed (Figure 4.1). The immediate shift of fluid from the legs to the neck ($\Delta LFV_{imm}$ and $\Delta NFV_{imm}$, respectively) upon lying supine was calculated from fluid volumes measured from the end of sitting to the beginning of the supine period. Lastly, to characterize fluid shift over the 60 minute supine period, the change in LFV ($\Delta LFV_{sup}$), calf circumference ($\Delta Calf_{sup}$), NFV ($\Delta NFV_{sup}$), and NC ($\Delta NC_{sup}$) were measured over the supine period.

4.2.8 Apnea-hypopnea index and snoring index

AHI and snoring index (number of snoring episodes per hour of sleep) were measured on the nights of the study days using a validated portable sleep monitoring device (BresoDx™, BresoTec, Toronto, Canada) [149–153]. The device records breathing sounds on a memory card, which is post-processed and analyzed to compute the overall AHI, supine AHI, non-supine AHI, and snoring index. A detailed description of the device is available in Appendix A.

4.2.9 Statistical analysis

Differences in activity levels and diet outcomes, as well baseline blood pressures, heart rate, LFV, calf circumference, NC and NFV at the start of sitting between the active and sham ES study conditions were evaluated using the paired t-test or Wilcoxon signed-rank test. Changes in fluid measurements over the seated and supine period were assessed using the paired t-test or Wilcoxon signed-rank test. To evaluate differences in fluid measurements over the seated and supine periods between active and sham ES study conditions, a repeated-measures ANOVA with time and study condition as factors was performed. A significant
Figure 4.1: Study protocol describing that an diet and activity log were completed for three and five days leading up to the study day, respectively. On the study day, participants initially lay supine for 30 minutes, sat for two-and-a-half hours (with active or sham ES), and then lie supine for 60 minutes. Leg fluid volume and calf circumference were measured at the start of the seated period (LFV\textsubscript{sit1}, Calf\textsubscript{sit1}) and the end of the seated period (LFV\textsubscript{sit2}, Calf\textsubscript{sit2}). Leg and neck fluid volume, neck circumference and calf circumference were measured at the beginning of the supine period (LFV\textsubscript{sup1}, NFV\textsubscript{sup1}, NC\textsubscript{sup1}, Calf\textsubscript{sup1}) and end of the supine period (LFV\textsubscript{sit1}, Calf\textsubscript{sit1}) (LFV\textsubscript{sup2}, NFV\textsubscript{sup2}, NC\textsubscript{sup2}, Calf\textsubscript{sup2}). Participant’s sleep was monitored on the same night of the study day.

An interaction effect (time×condition) indicated a difference between the active and sham ES study conditions. A two-sided P-value < 0.05 was considered significant for the statistical tests conducted. Statistical analyses were conducted using R open source statistical software version 3.2.1 (http://www.r-project.org). Data are presented as mean ± SEM.

4.3 Results

4.3.1 Participants

One hundred and thirty-seven participants were pre-screened for eligibility and 50 participants moved on to be screened for sleep apnea. From this group, 17 participants were found to have an AHI ≥ 10 and were willing to participate in the full study. One participant was
lost to follow-up for a final sample size of 16. Figure 4.2 shows the recruitment flow diagram of the study.

Mean age of the participants was 51.3 ± 7.5 years. All participants were non-obese men with a mean BMI of 26.3 ± 2.7 kg/m². Participants were all normotensive with mean systolic blood pressure of 117 ± 11 mm Hg, diastolic blood pressure of 80 ± 8 mm Hg and heart rate were of 68 ± 10 bpm. Mean AHI was 19.0 ± 3.2 events/hr. Sleep apnea severity was mild (AHI ≥ 10, but < 15 events/hr) in 9 participants, moderate (AHI ≥ 15, but < 30 events/hr) in four participants and severe (AHI ≥ 30 events/hr) in three participants.

Baseline characteristics, shown in Table 4.1 were comparable between the two study conditions. Baseline LFV, NFV, and NC measured at the start of sitting were similar between the active and sham ES conditions (P>0.10). However, calf circumference measured at the beginning of sitting was slightly smaller in the sham ES condition, compared to the active ES condition (P=0.03). Blood pressures and heart rate were similar between the active and
sham ES conditions (all P>0.10). Time spent sitting, time spent sedentary and time spent active in the days leading up to study days were similar between the active and sham ES condition (all P>0.50). In addition, average water and caloric intake were similar between the active and sham ES conditions (all P>0.10). However, average sodium intake was greater in the active ES condition, compared to the sham ES condition (P=0.02).

Table 4.1: Baseline characteristics of participants in the sham and active electrical stimulation conditions. P-values represent differences between conditions.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham ES</th>
<th>Active ES</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leg fluid volume (ml)</td>
<td>1333.3 ± 54.3</td>
<td>1394.5 ± 48.5</td>
<td>0.59</td>
</tr>
<tr>
<td>Calf circumference (cm)</td>
<td>39.1 ± 0.7</td>
<td>39.5 ± 0.7</td>
<td>0.03</td>
</tr>
<tr>
<td>Neck fluid volume (ml)</td>
<td>487.2 ± 45.8</td>
<td>483.0 ± 26.6</td>
<td>0.88</td>
</tr>
<tr>
<td>Neck circumference (cm)</td>
<td>41.1 ± 0.7</td>
<td>41.6 ± 0.9</td>
<td>0.11</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113.6 ± 3.2</td>
<td>114.6 ± 2.6</td>
<td>0.60</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>77.1 ± 2.8</td>
<td>79.1 ± 1.7</td>
<td>0.32</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>66.3 ± 2.2</td>
<td>66.3 ± 2.8</td>
<td>0.37</td>
</tr>
<tr>
<td>Percent Time Sitting (%)</td>
<td>36.6 ± 4.2</td>
<td>37.6 ± 12.4</td>
<td>0.63</td>
</tr>
<tr>
<td>Percent Time Sedentary (%)</td>
<td>87.4 ± 14.1</td>
<td>85.7 ± 12.2</td>
<td>0.55</td>
</tr>
<tr>
<td>Percent Time Active (%)</td>
<td>12.5 ± 4.5</td>
<td>14.3 ± 5.8</td>
<td>0.40</td>
</tr>
<tr>
<td>Sodium intake (ml)</td>
<td>2056 ± 172</td>
<td>2696 ± 192</td>
<td>0.02</td>
</tr>
<tr>
<td>Water intake (g)</td>
<td>2817 ± 398</td>
<td>2554 ± 372</td>
<td>0.81</td>
</tr>
<tr>
<td>Calories (kcal)</td>
<td>2069 ± 205</td>
<td>2073 ± 217</td>
<td>0.75</td>
</tr>
</tbody>
</table>

4.3.2 Seated leg fluid accumulation

Over the two-and-a-half hour seated period, LFV increased significantly in both the active (P<0.001) and sham ES conditions (P<0.001). However, the increase in LFV while seated was significantly lower when using active ES (51.7 ± 7.2 ml) compared to sham ES (95.5 ± 8.5 ml, P<0.001, Figure 4.3a). While calf circumference increased significantly over the
4.3.3 Fluid shift while supine

The immediate shift of LFV upon lying supine was statistically significant in both the active ES (101.4 ± 7.5 ml, P<0.001) and sham ES conditions (132.7 ± 12.5 ml, P<0.001), with significantly less fluid shifting out of the legs after using active ES (P=0.02, Figure 4.4a). The shift in LFV over 60 the minute supine period was also significant in both the active ES (62.5 ± 5.3 ml, P<0.001) and sham ES (68.1 ± 5.0 ml, P<0.001) conditions, but the LFV shift was not different between the study conditions (P>0.10, Figure 4.4a). Similarly, over the supine period calf circumference decreased significantly in both the active ES (0.41 ± 0.1
Figure 4.4: Change in leg fluid volume (ΔLFV) immediately upon lying down and over the supine period (a) and change calf circumference (ΔCalf) over the supine period (b). The immediate shift of fluid out of the legs was reduced after using active ES, compared to the sham ES. However, there was no difference between study conditions in LFV shift and change in calf circumference over the supine period.

cm, P<0.001) and sham ES (0.49 ± 0.1 cm, P<0.001) conditions, but these changes were not different between the study conditions (P>0.10, Figure 4.4b).

The immediate accumulation of fluid into the neck was also significant in both the active ES (44.6 ± 8.4 ml, P<0.001) and sham ES conditions (56.6 ± 12.4 ml, P<0.001), but less fluid accumulated in the neck after using active ES (P<0.01, Figure 4.5a). Similarly, over the supine period the accumulation of neck fluid in both the active and sham ES conditions was significant (both P<0.001). However, after using active ES, neck fluid accumulation was halved (19.6 ± 2.5 ml), compared to the sham ES condition (38.3 ± 5.3 ml, P<0.001, Figure 4.5a). NC also increased significantly over the supine period in both the active ES (P<0.05) and sham ES conditions (P<0.001). However, after using active ES, the increase in NC was about three times smaller (0.34 ± 0.1 cm), compared to sham ES (1.2 ± 0.2 cm, P<0.001, Figure 4.5b).
Figure 4.5: Change in leg fluid volume ($\Delta$NFV) immediately upon lying down and over the supine period (a) and change neck circumference ($\Delta$NC) over the supine period (b). Neck fluid accumulation was reduced immediately upon lying down and over the supine period after using active ES, compared to the sham ES condition. Similarly, over the supine period NC increased significantly less after using active ES, compared to the sham ES condition.
Δ = -46.7±18.8 events/hr
(15% reduction)
P = 0.03

Figure 4.6: Difference in the snoring index between sham and active sham ES condition. Square points and error bars represent the mean±SD.

4.3.4 Apnea-hypopnea Index and snoring index

Calf muscle contraction by active ES did not reduce overall AHI (active ES: 18.3 ± 3.6 and sham ES: 20.2 ± 3.8 events/hr, P>0.10), supine AHI (active ES: 29.5 ± 6.5 and sham ES: 31.4 ± 5.6 events/hr, P>0.10) or non-supine AHI (active ES: 16.1 ± 3.3 and sham ES: 18.4 ± 3.7 events/hr, P>0.10). However, there was a significant 15% reduction in the snoring index after using active ES compared to using sham ES (266.4 ± 52.7 vs. 313.1 ± 60.9 episodes/hr, P=0.03), as illustrated in Figure 4.6.

4.4 Discussion

The present study demonstrates that reducing leg fluid while sedentary with repeated activation of the calf muscles by ES reduces the accumulation of fluid in the legs by 46% and rostral shift of fluid from the legs by 17% and into the neck by 31% when lying down. In addition,
a single session of active ES reduced snoring index by 15%, suggestive of reduced pharyngeal narrowing during sleep. Interestingly, sodium intake was higher in the days leading up to the active ES study day, which can increase body fluid retention and diminish the effect of interventions on reducing body fluid. Yet, calf muscle ES was still effective at reducing rostral fluid shift and its associated effects on snoring. These findings support a proof-of-concept that calf muscle ES has potential as a therapeutic device capable of reducing daytime leg fluid accumulation, rostral fluid shift, and its effects on the pharyngeal airway.

A study similar to the present study investigated the effects of calf muscle activation using a seated stepping device on leg fluid accumulation over a four-hour seated period [109]. Compared to four hours of stationary sitting, seated stepping reduced fluid accumulation in both legs by 54 ml (25%). This reduction is approximately 40% lower than the present study, where calf muscle ES reduced leg fluid accumulation in both legs by an estimated 88 ml (44 ml in one leg). Calf muscle ES was likely more effective in the present study due to the consistent regular intervals that contractions were elicited by ES (every two seconds). In the prior study, participants stepped at a self-selected pace which averaged to about 20 contractions per minute on each side; two-thirds the rate of contraction of the present study. Another study exploring calf muscle pump activation on rostral fluid shift and OSA investigated an exercise intervention consisting of 40 minutes of daily walking for four weeks [18]. Compared to a control group, four weeks of daily walking reduced the overnight change in NC by approximately 0.3 cm and reduced AHI by 38%. In the present study, change in NC when lying down was reduced after using active ES, however OSA severity was unchanged compared to sham ES. It is possible that the increased duration of the walking intervention (four weeks) compared to calf muscle ES (one day) contributed to its improved efficacy. In fact, four weeks of daily calf muscle pump stimulation improved subjective measures of sleep quality, and moderately reduced daytime sleepiness, however associated fluid data was not reported [114]. Furthermore, while AHI was unchanged in the present study, a single session of calf muscle ES produced a modest reduction in the snoring index, potentially reflecting
a reduction in overnight pharyngeal narrowing. Future studies should explore the effects of daily calf muscle ES for a four week period on overnight rostral fluid shift and OSA.

While walking is an effective method for treating OSA caused by the fluid shift mechanism, it has several disadvantages that limit its application. First, unlike calf muscle ES, walking is less accessible for individuals with mobility limitations, which might be the cause of their sedentary lifestyle. Second, compliance with physical activity is low with only one in five adults achieving the recommended 150 minutes of weekly physical activity [29] due to factors including cost, lack of time or motivation, and illness or disability [30]. Compression stockings are another competing therapy to calf muscle ES that have demonstrated efficacy in reducing OSA in association with reduced overnight fluid shift [16,17]. However, compression stockings are limited by their discomfort and difficulty to apply and remove, also leading to low patient adherence [25,144]. Compression stockings also increase risk of thrombosis [26] and exudative skin lesions [26] due to improper fit and use. Conversely, calf muscle ES under the protocol described in the present study has been demonstrated to be comfortable over the short- and long-term [33, 154]. In addition, calf muscle ES can be developed into a convenient wearable platform that can apply calf muscle ES during sedentary periods when leg fluid is accumulating at a rapid rate. In this form, calf muscle ES is a potential therapy for OSA caused by the fluid shift mechanism that can address the issues of compliance to therapies currently available.

The pattern by which rostral fluid shift is reduced by calf muscle ES in the present study adds new insights into the mechanism by which fluid accumulates in the neck. Past studies report a reduction in the gradual overnight shift in LFV after the intervention, which contributes to the reduction in overnight neck fluid accumulation [18,20,21]. In the present study, calf muscle ES did not impact the gradual shift in LFV over the 60 minute supine period. Instead, the immediate shift of fluid upon transitioning from sitting to supine was reduced after using active ES. This corresponded to reduced neck fluid accumulation immediately upon lying down, and gradually over the 60 minute period supine.
The immediate shift of fluid out of the legs corresponds to the immediate shift of fluid into the neck and can be explained by shifts in venous blood. Fluid that shifts rapidly upon lying down represents the shift of venous blood out of the legs and back to the heart. In the sham ES condition, compared to the active ES condition, upon lying down more blood shifts back to the heart (i.e. venous return increases), likely increasing pressures in the cardiac chambers, and leading to increased central venous pressure. As a result, venous pressures are increased at the entry to the right atrium [62], particularly in the the superior and inferior branches of the vena cava [62–64]. Pressure and volume increases in the superior vena cava are significant because this will impact pressures and volumes of the veins that lie superior to the heart, such as the internal jugular vein. Indeed, upon lying supine volume and cross-sectional area of the internal jugular veins increases [66]. As such, the the increase in the immediate shift of fluid into the neck upon lying down after sham ES is likely controlled by venous return, cardiac pressures, and hydrostatic pressures in the veins of the neck.

Increases in hydrostatic pressure in the veins superior to the heart will also lead to proportional increases in capillary pressures [46] and drive filtration of blood plasma into the tissue spaces. In the present study, the gradual increase in neck fluid over the 60 minute supine period was greater in the sham ES condition compared to the the active ES condition. Previous studies have reported that the slower, gradual accumulation of fluid is representative of interstitial fluid accumulation [40,155,156]. It is possible that the increased shift in venous blood after using sham ES, resulted in the proportional increase in venous pressure in the veins that are superior to the heart, such as the neck veins, which resulted in greater filtration of blood plasma into the tissue spaces. Indeed, shifting blood rostrally into the neck using compression pants while lying supine has been demonstrated to increase neck circumference [11], signaling fluid accumulation in the neck, and increase pharyngeal mucosal water content in individuals with OSA [9].

Among the past studies that explored fluid shift and OSA [13,16,18,21], none measured the rapid shift of intravascular fluid upon lying down. Given the potential role of vascular
fluid shift on the slower, more gradual accumulation of fluid in the neck over a longer period supine, it should be considered as a risk factor for overnight neck fluid accumulation and associated OSA. In addition, reducing the volume of vascular fluid that will shift when lying down might be a therapeutic target to consider when testing interventions to treat OSA caused by the fluid shift mechanism.

The main limitation of this study is associated with the sensitivity of the bioelectrical impedance to movement. As a result, the supine period to monitor rostral fluid shift was limited to 60 minutes. The study is also limited to detecting fluid shifts in the neck and the leg. In addition, the method of bioelectrical impedance employed mainly detects extracellular fluid, and cannot distinguish between vascular and interstitial fluid. As a result, when less fluid accumulates in the neck after calf muscle ES, it is impossible to evaluate how the fluid is redistributed in the body to produce this change in neck fluid volume.

The study was also limited to testing the effect of a single session of calf muscle ES on rostral fluid shift, OSA and snoring. It is important that future studies evaluate clinical effectiveness of the device over the long term. In general, clinical effects are dependent on therapeutic efficacy, adherence to the treatment, and sustainability of the treatment. An efficacious therapy in the context of sleep apnea will reduce the AHI by at least 50%, or to an AHI less than 10 events per hour of sleep. In addition, adherence to calf muscle ES should be considered because a treatment could be highly efficacious, however if participants are unable to adhere to the treatment, it will have a diminished effect on treating the disease or disorder. For example, continuous positive airway pressure has extremely high efficacy, eliminating sleep apnea in most patients in the ideal laboratory setting. However, continuous positive airway pressure therapy has a non-adherence as high as 85% [14], substantially diminishing its clinical effect [157]. Lastly, sustainability of the treatment should be considered. While the therapy might work over a period of several weeks, it is important to investigate effectiveness over a longer duration to understand if there are physiological changes that reduce or increase the treatment effect. It is therefore important that future studies into the clinical effectiveness
of calf muscle ES, not only consider efficacy, but also adherence to therapy and sustainability of the treatment over the long term.

In conclusion, this study successfully demonstrates that repeated activations of the calf muscle by ES while seated for 150 minutes reduces the rostral shift of fluid out of the legs and into the neck upon lying supine. Snoring index was also reduced after using calf muscle ES, potentially indicating reduced overnight pharyngeal narrowing through reduced overnight neck fluid accumulation. The present study provides the proof-of-concept that calf muscle ES is a viable intervention that can be developed into a wearable device to reduce daytime leg fluid accumulation and rostral fluid shift. Future studies should explore the utility of long-term calf muscle ES as a potential treatment for OSA caused by the fluid shift mechanism.
Chapter 5

Temporal Pattern of Leg Fluid Accumulation While Seated With and Without Electrical Stimulation of the Calf Muscle


5.1 Introduction

Fluid accumulation in the legs is a known consequence of sedentariness and is associated with edema formation. Development of leg edema is associated with complications including increased risk of leg ulcers, painful swelling, reduced elasticity of the veins and arteries of the legs, and thromboembolic events [158, 159]. More fluid in the legs is also associated with a greater shift of fluid toward the upper-body when moving from upright to supine posture [143], which can increase risks for supine hypertension, acute decompensation in heart failure [160], pulmonary edema [161], nocturnal asthma [116], and increased OSA
severity [13]. This is particularly problematic in settings that facilitate sedentariness such as the office [55] or long-haul flights [162], and in high risk populations such as the spinal cord injured [163] and individuals with fluid-retaining diseases [159]. Reducing leg fluid accumulation can serve to prevent the genesis or worsening of these diseases and disorders in high risk populations and settings.

During upright sitting or standing, fluid accumulates in the legs by first rapidly filling the capacitance vessels of the lower body [155,164,165]. According to the Starling equation, fluid filters from vasculature into the tissue spaces when capillary pressure is increased and/or tissue pressure is decreased [117]. Fluid continues to shift from the upper to the lower body by displacing fluid that filters into the interstitial spaces of the leg, driven by the enhanced capillary transmural pressure [156, 166, 167]. Conversely, fluid is reabsorbed into the vasculature when tissue pressure is increased and/or capillary pressure is reduced.

Compression stockings are a commonly prescribed and effective therapy for reducing leg fluid via increased tissue pressure. However, they are limited by their discomfort and difficulty to apply and remove, contributing to their low adherence [25]. In addition, improper fit is a common problem, which increases the risk of developing exudative skin lesions [27] and deep vein thrombosis [26]. Alternatively, activation of the skeletal pump in the legs reduces leg fluid accumulation by reducing capillary pressure in the legs and increasing tissue pressure [35]. Walking is one method of activating the skeletal pump; however it is limited to able-bodied individuals and less feasible for the elderly or those with mobility limitations. Therefore in the present study, we experiment with activation of the skeletal pump using electrical stimulation (ES) of the calf muscles to prevent leg fluid accumulation while seated.

The overall purpose of this study was to investigate the efficacy of calf muscle ES in preventing leg fluid accumulation while seated for two-and-half hours. The main objectives of this study were first, to determine if ES can prevent the accumulation of leg fluid over the seated period; and second, to identify the minimum time period required for active ES to reduce seated leg fluid volume. A secondary objective was exploratory in nature and aimed
Chapter 5. Calf Muscle ES on Leg Fluid while Seated

5.2 Methods

5.2.1 Participants

The protocol was approved by the Research Ethics Board of Toronto Rehabilitation Institute and participants provided written informed consent prior to participation. The study was performed in accordance with the approved guidelines and regulations. Criteria for study enrolment were adult men and women with a body mass index < 30 kg/m², and blood pressure < 140/90 mmHg. The study was part of a larger study investigating the efficacy of calf muscle ES on fluid shift and sleep apnea severity, so all participants had a positive screening for OSA with an apnea-hypopnea index ≥ 10, as determined by a portable sleep monitoring device [152, 153]. Participants abstained from alcohol and coffee consumption for 24-hours. Participants were excluded if they reported a history of cardiovascular, renal, neurological, or respiratory disorders; taking prescribed medication for these disorders; or taking over-the-counter medication that might influence fluid retention. Participants were recruited from the community through advertisement.

5.2.2 Measurements

Fluid volume was measured in the dominant leg continuously while seated using a bioelectrical impedance measurement system. As previously described in Chapter 4, this method is a non-invasive technique used to estimate fluid volumes of the tissues. The method has been well validated and is highly reproducible with an accuracy within 0.5% compared to reference measures of total body water, repeatability within 0.3%, and test-retest correlation >95% [60, 122]. The method is based on Ohms law which states that the resistance of tissues to electrical current is directly proportional to its length: \( R = \rho L^2 / v \), where \( \rho \) is the resistivity.
of the fluid, \( L \) is the segments length, \( v \) is the fluid volume and \( R \) is the measured resistance [123–125]. This formula has since been adapted to measure impedance of tissues in individual body segments (leg, abdomen, thorax, and neck) and calculated according to Equation 3.1 (Chapter 3) [54,130].

Surface electrodes were placed on the skin at the top and bottom of the lower leg (ankle and knee) as illustrated in Figure 5.1 using hypoallergenic adhesive tape. At the ankle, a voltage measuring (V+) electrode was placed just below the lateral malleolus. At the knee the voltage measuring (V−) electrode was placed between the fibula head and tibial tubercle. Current injecting electrodes labeled I+ and I− were positioned 1-inch outside the voltage measuring electrodes and injected a small amplitude (400 \( \mu A \)), high frequency (25 kHz) current into the leg. Voltage drop across the leg measured by V+ and V− electrodes were used to calculate resistance used in Equation 3.1 (Chapter 3).

Using a measuring tape, segment length was measured as the distance between the measurement electrodes. Circumferences of the ankle and knee were measured at the level of the measurement electrodes at the start and end of sitting. A line was drawn around the ankle, and knee to ensure measurements were consistent when repeated. Using the same method, calf measurements were also made around the largest part of the calf at the start and end of sitting, for use as an alternative measure of fluid accumulation.

### 5.2.3 Electrical stimulation protocol

Calf muscle ES was performed using a programmable 4-channel neuromuscular stimulator (Compex Motion, Switzerland), as previously described in Chapter 4. Electrical current was delivered transcutaneously to the gastrocnemius muscle groups of both legs using 5 cm by 9 cm StimTrode neurostimulation electrodes (Axelgaard Manufacturing, California, USA) positioned on the calf over the approximate position of the motor points [168]. The stimulation protocol used an asymmetrical biphasic waveform with 40 Hz frequency, 300 \( \mu s \) pulse duration, at the maximally tolerated stimulation amplitude (typically between 20
Figure 5.1: Position of the bioelectrical impedance electrodes on the lower leg. I+ and I− represent electrodes applying current, while V+ and V− are the electrodes measuring the voltage across the lower leg.

and 40 mA). The duty cycle was 2 seconds on, followed by 2 seconds off. The left and right gastrocnemii were stimulated out of sync, such that when the left gastrocnemius was contracted the right gastrocnemius was relaxed and vice versa. A sham electrical stimulation protocol was also utilized as a control. The sham ES protocol was identical to the active ES protocol in terms of the current waveform used and electrode type and position. However, the main difference was the amplitude of the electrical current which was set much lower in the sham ES protocol, at a level that elicited the sensation of electrical current travelling through the lower leg, without causing calf muscle contraction.

5.2.4 Modeling temporal fluid data

Temporal fluid data in the active and sham ES conditions consisted of two distinct portions: an initial portion and a latter portion which differed by their apparent slopes. In addition, the initial portions appeared to increase according to an exponential decay function, whereas the latter portions appeared linear. Similarly, the difference in fluid volume between the
active and sham ES conditions also consisted of two distinct portions. Each portion was modeled with both a linear and exponential decay function, described in Equations 5.1 and 5.2; where \( LFV \) is the leg fluid volume (ml), \( m \) is the slope of the line (ml/min), \( b \) is the y-intercept (ml), \( t \) is time (minutes), \( \tau \) is the time constant (minutes), and \( LFV_{\text{end}} \) is the expected final value of the leg fluid volume (ml). The model with the best fit was selected to describe each portion of data.

\[
LFV(t) = mt + b \tag{5.1}
\]

\[
LFV(t) = LFV_{\text{end}} \left(1 - e^{(-t/\tau)} \right) \tag{5.2}
\]

### 5.2.5 Change in leg fluid volume per contraction

The change in leg fluid volume per contraction was estimated using the change in resistance (\( \Delta R \)) per contraction. Illustrated in Figure 5.2, this metric was computed by subtracting the mean resistance during each contraction (active) from the resistance when contraction ended (inactive). This was performed for every contraction over the 150 minute seated period, from which mean \( \Delta R \) per contraction was computed, as described in equation 5.2.5, where \( N \) is the total number of contractions. Given that contractions occurred every two seconds, each participant experienced approximately 4500 contractions in a single study session.

\[
\Delta R = \frac{1}{N} \sum \left[ \text{active} - \text{inactive} \right]_n \tag{5.3}
\]

### 5.2.6 Study protocol

The study was a randomized, single-blind double crossover protocol. Data collections were completed over two days set one week apart in a quiet, temperature-controlled room following
Figure 5.2: Representative data showing the active and inactive portions of two contractions. As illustrated, impedance increases with contraction (active) representing fluid ejection from the legs, and then drops when there is no contraction (inactive).

abstention from alcohol and caffeine. Experiments began in the morning between 10 and 11AM for all participants. On the first morning, participants were randomly assigned to sham or active ES. On each morning, surface electrodes were placed on the legs to collect bioelectrical impedance data, as illustrated in Figure 5.1. Participants initially lay supine for 30 minutes to ensure all participants began sitting with a similar level of leg fluid. Next, participants sat for a two-and-a-half hour period with either active or sham ES being applied to their calf muscle. Participants sat with their legs still, but were free to move their upper body.

5.2.7 Data acquisition and analysis

Bioelectrical impedance data was collected continuously at 1.25 kHz using Biopac data acquisition system (MP150, California, USA) and imported into Matlab (2014a, Massachusetts, USA) for pre-processing. Signals were manually adjusted for motion artifacts based on documented events of movement or seat adjustment occurring during data collection. Signals were then averaged every minute to reduce variability in the signal due to noise or other artifacts.

Bioelectrical impedance signals were converted to fluid volumes using Equation 3.1 (Chapter 3). To account for changing leg circumferences over the seated period, circumferences
measured before and after the seated period were used to interpolate all values in between. Difference in leg fluid accumulation between the active and sham ES study conditions were computed by subtracting the fluid data from each participant in the two study conditions. The effect size of the difference in leg fluid was calculated by dividing the mean difference at each time point by the standard deviation at the same time point.

Statistical differences in baseline values of leg fluid volume, and calf circumference between study conditions were analyzed using the paired t-test (or Wilcoxon signed-rank test for non-normally distributed data). A repeated measures ANOVA was used to compare changes in leg fluid volume, and calf circumference from the start to the end of sitting, between study conditions. The ANOVA included two factors: timing (start and end) and study condition (sham ES or active ES). The interaction effect tested differences in the change in leg fluid or calf circumference from the start to the end of sitting between active and sham ES conditions. Lastly, the paired t-test (or Wilcoxon signed-rank test for non-normally distributed data) was used to test if the change in leg fluid or calf circumference from start to end of sitting within each study condition was statistically significant.

Data distributions were assessed using the Shapiro-Wilk test of normality and considered non-normally distributed if the null hypothesis was rejected at a significance level of $p<0.05$. Correlations were performed using Pearson correlations with normally distributed data and Spearman's rank correlation with non-normally distributed data. A $p$-value $<0.05$ was considered statistically significant. All data are reported as the mean ± SEM.

## 5.3 Results

As part of a larger study examining the effect of calf muscle ES on fluid shift and sleep apnea, one hundred and thirty-two participants were pre-screened for eligibility and 45 participants moved on to be screened for sleep apnea. From this group, 15 participants were found to have mild to severe OSA (apnea-hypopnea index $= 21.1 \pm 4.0$) and were willing to participate
in the full study. One participant was found to have varicose veins and therefore excluded, and another was lost to follow-up for a final sample size of 13. The participants included are a sub-sample of those reported in chapter 4, for which two additional participants were recruited following the completion of the present study. Weight, height, and blood pressure were measured at a screening visit to confirm participant eligibility. Although sex was not an exclusion criteria, all participants were non-obese men with a mean age of 51 ± 2 years and body mass index (BMI) of 26.5 ± 0.7 kg/m2. Participants were all normotensive with a mean systolic blood pressure, diastolic blood pressure, mean arterial pressure, and heart rate of 117.5 ± 3.2 mmHg, 80.4 ± 2.6 mmHg, 93.0 ± 2.5 mmHg, and 68.3 ± 2.9 beats/min.

Baseline leg fluid volume measured at the start of sitting, did not differ between sham (1306.3 ± 63.9 ml) and active (1370.8 ± 56.9 ml) ES conditions (P>0.10). Similarly, baseline calf circumferences measured at the start of sitting were also similar between the sham (38.9 ± 0.8 cm) and active (39.1 ± 0.8 cm) ES conditions (P>0.10). All participants were free from symptoms of any serious diseases or disorders.

Leg fluid volume increased significantly over the seated period in both the sham ES condition (∆=91.5 ± 8.9 ml, P<0.001) and in the active ES condition (∆= 51.9 ± 8.8 ml, P<0.001). However, the interaction effect demonstrated that leg fluid volume increased significantly more in sham ES condition compared to the active ES condition (P<0.001). Calf circumference increased significantly over the seated period in the sham ES condition (∆=0.79 ± 0.1 cm, P<0.05), but was unchanged in the active ES condition (∆=0.10 ± 0.1 cm, P>0.10). The interaction effect was significant, demonstrating the increase in calf circumference in the sham ES condition was significantly greater than the change during active ES condition (P<0.001).

The temporal patterns of leg fluid accumulation in the active and sham ES conditions are displayed in Figure 5.3. The temporal change in the difference in leg fluid accumulation between active and sham ES conditions is illustrated in Figure 5.4. Model equations and the corresponding portions of temporal data they represent are also described in Figure 5.3.
and Figure 5.4. In the sham ES condition, leg fluid initially accumulated according to an exponential decay function (Figure 5.3, equation A), with a point of inflection at 25 minutes. After 25 minutes, leg fluid increased linearly, with a slope of 0.53 ml/min (Figure 5.3, equation B). In the active ES condition, leg fluid increased according to two linear functions, with a point of inflection at 33 minutes. Up to the 33 minute time point, fluid increased linearly with a slope of 0.60 ml/min (Figure 5.3, equation C). After 33 minutes, fluid also increased linearly but with a smaller slope of 0.30 ml/min (Figure 5.3, equation D).

As illustrated in Figure 5.4, the temporal pattern of difference in leg fluid accumulation between active and sham ES conditions continues to increase as sitting time progresses. The mean difference in leg fluid accumulation at the end of the 150 minutes period between active and sham ES conditions was 40.0 ± 9.7 ml. The rate of increase of differences between sham and active ES conditions occurs linearly throughout the seated period in two distinct portions separated at the 23 minute time point and differentiated by their slopes (Figure 5.4, equations E and F). As illustrated, the slope of the line in the initial portion (0.52 ml/min) is greater than the slope of the line in the latter period (0.23 ml/min).

The difference in leg fluid accumulation between active and sham ES also demonstrated a rise in the variability among subjects (Figure 5.4) at the 20 minute time point. This was best illustrated with the effect size, shown in Figure 5.5, which describes the magnitude of the difference normalized to the variability. As shown, there was a distinct peak in the effect size at 20 minutes when mean difference in leg fluid accumulation was relatively high and the variability was low. This was followed by a sharp drop in the effect size due to the rise in variability. The effect size gradually increased again as differences between active and sham ES continued to increase, while variability remained relatively stable (Figure 5.5).

The correlation between the ∆R per contraction during active ES and final leg fluid volume difference between the active and sham ES conditions is illustrated in Figure 5.6. As shown in Figure 5.6-a, there was no relationship between ∆R per contraction and the difference in leg fluid accumulation across the entire seated period. After data were separated
Figure 5.3: Mean change in leg fluid volume over the 150 minutes seated period in the active (red) and sham (blue) ES study conditions with standard error bars. Temporal pattern of fluid accumulation was different between the active and sham ES study conditions, with fluid accumulating more rapidly in sham ES study condition. The temporal patterns of fluid accumulation were also different between the initial and latter portions of sitting. The models representing the initial and latter portions of sitting in both the active and sham ES conditions are described by equations A through D, listed below.

A: \( LFV(t) = 34.0 \left(1 - e^{-0.03t}\right) \)
B: \( LFV(t) = 0.53t + 26.8 \)
C: \( LFV(t) = 0.60t + 1.1 \)
D: \( LFV(t) = 0.30t + 20.7 \)
Figure 5.4: Mean difference in leg fluid accumulation between the active and sham ES conditions over the 150 minute seated period with standard error bars. The temporal pattern of differences in leg fluid accumulation between study conditions shows steady increase as sitting time progresses, and differences in the rate of increase between the initial and latter portions of sitting. The models representing the initial and latter portions of the difference in fluid volume are described by equations E and F, listed below.

E: \( LFV(t) = 0.52t + 1.0 \)
F: \( LFV(t) = 0.23t + 9.2 \)
Figure 5.5: Effect size (Cohen's $d$) of volume difference over the 150 minutes seated period. Effect size is computed as the mean difference divided by the standard deviation of differences. Overall, effect size rises throughout the entire period seated, with a distinct peak at 20 minutes driven by the sharp rise in the difference in leg fluid accumulation and low variability.
into two data sets based on the transition point at 20 minutes, the data set from the initial 20 minutes showed no relationship between $\Delta R$ per contraction and final volume difference (Figure 5.6-b). However, in the latter 130 minutes, there was a significant positive correlation between volume difference and change in resistance per contraction (Figure 5.6-c, $P = 0.046$).

### 5.4 Discussion

The present study reports several key findings: first, electrical stimulation of the calf muscles reduced the accumulation of fluid in the legs by 43% over a two-and-a-half hour (150 min) seated period. Second, during sham ES, leg fluid accumulated according to an exponential decay during the first 25 minutes of sitting, then linearly in the latter period of sitting. Third, variability in the difference in leg fluid accumulation between active and sham ES conditions increased substantially after 20 minutes seated. Lastly, over the final 130 minutes seated, $\Delta R$ per ES contraction was associated with the difference in leg fluid volume between the active and the sham ES conditions.

The present study reports that calf muscle ES reduced leg fluid accumulation by 40 ml and reduced calf circumference by 0.7 cm. The clinical context for these results can be derived from prior studies investigating interventions that reduced leg fluid prior to sleeping and effectively reduced sleep apnea by 27% to 36% [16,18,21]. Comparing fluid volume data with prior studies is not possible because they measured fluid volume of the whole leg (from hip to ankle), while the present study measured fluid volume in the lower leg (from knee to ankle). In terms of calf circumference, wearing compression stockings for one week reduced evening (pre-sleep) calf circumference by 0.5 cm [16], one day of ultrafiltration reduced evening calf circumference by 1.0 cm [21], and four weeks of walking exercise caused no change in evening calf circumference [18]. Calf muscle ES performed comparably at reducing calf swelling by 0.7 cm, despite only a single session of use. This merits future study of long-term (such as 4 weeks) use of calf muscle ES to reduce leg fluid accumulation and fluid shift from the legs to
Figure 5.6: Scatterplots illustrating correlations between final volume differences and the change in resistance per contraction ($\Delta R$ per contraction) for: a) all study data b) the first 20 minutes seated and c) the latter 130 minutes seated. Correlations are assessed by either Pearson’s correlation coefficient or Spearman’s rho depending on normality of the data distribution as assessed by the Shapiro-Wilk test of normality.
Few studies have investigated the immediate effect of calf muscle contraction on leg fluid while upright [36, 53]. To our knowledge the findings of the present study are the first to demonstrate the immediate capability of calf muscle contraction via electrical stimulation to reduce leg fluid accumulation while seated, compared to a sham control. The experiment by Goddard et al. compared 30 minutes of quiet sitting followed by 30 minutes of calf muscle activation using a plantar reflex-based stimulation device [53]. Results differ from the present experiment, as they report no accumulation of leg fluid volume over the 30-minute quiet sitting period, and a sustained reduction in leg fluid over the 30-minute period with leg muscle contractions. Differences in the pattern of leg fluid accumulation can likely be attributed to the methodological differences between the studies. Specifically, leg fluid was measured using air plethysmography, only women were studied, and participants sat for 15 minutes prior to beginning leg fluid measurements which means majority of vascular fluid accumulation was not recorded. Furthermore, since each seated period started with 30 minutes of quiet sitting, calf muscle pumping was tested after 45 minutes of quiet sitting. Therefore leg fluid volume was higher when muscle contractions began. As a result, their study investigates the removal of fluid already accumulated in leg, rather than prevention of leg fluid accumulation which was the purpose of the present study.

Stick et al. measured changes in leg fluid volume using a mercury strain gauge around the largest circumference of the calf while participants were recumbent for 30 minutes, then upright for 10 minutes, then quiet sitting for 15 minutes and then sitting while using a cycle ergometer for 20 minutes [36]. They found that calf circumference increased substantially while upright and seated. During cycling, the study demonstrated a strong effect, reducing calf circumference by approximately the same proportion that it increased during quiet sitting (i.e. complete reversal of leg fluid accumulation). In the present study, measures of calf circumference were also suggestive that active ES had a strong effect in resisting accumulation of fluid in the legs. Active ES of the calf muscle resulted in no significant change in calf
circumference over the entire seated period, while sham ES resulted in a 0.79 cm increase in calf circumference. Comparison in efficacy between cycling and calf muscle ES should be done with caution given the methodological differences between the studies. In particular, Stick et al. evaluated the reduction of leg fluid accumulation during calf muscle exercise after 10 minutes of standing and 15 minutes of quiet sitting. Therefore as previously explained, Stick et al. report the removal of fluid already accumulated, rather than prevention of leg fluid accumulation that was addressed in the current study.

During the first 25 minutes of quiet sitting, fluid accumulated according to an exponential decay function and at a more rapid rate than the latter portion of quiet sitting (Figure 5.3). Past studies have reported that the initial rapid accumulation of fluid was suggestive of rapid filling of the capacitance vessels in the lower body [164, 165]. However, rapid vascular filling while upright was complete after approximately 5 minutes [40, 155, 156], much less than our reported 25 minutes. One explanation for the discrepancy in vascular filling time is differences in body position. In the present study, participants were sitting in a chair, while in the past studies participants were standing at varying degrees head-up tilt. Regardless of the discrepancy in the time point where rapid vascular filling was complete, it is clear that leg fluid accumulated according to two different patterns separated at the 25 minute time point. Following the rapid vascular filling, further increase in leg fluid was considered to be driven by enhanced transmural pressure leading to fluid filtration into the interstitial space [156, 166, 167]. Therefore, the latter portion of sitting was likely a reflection of the accumulation of interstitial fluid.

The difference in fluid accumulation between the active and sham ES condition described the reduction in leg fluid accumulation due to active ES while seated. As illustrated in Figure 5.4, the difference in leg fluid accumulation between the sham and active ES conditions increased with time. However, the difference in leg fluid between the study conditions is not an accurate measure of the effectiveness of active ES because it does not take into account the variability across the participants. A better measure for this is the effect size, which is
the mean difference normalized to the variability across the participants. A local peak in the
effect size occurred at the 20 minute time point, illustrating that effectiveness of active ES
at reducing leg fluid accumulation steadily increased up to the 20 minute time point (Figure
5.5). The sharp drop at 20 minutes is the result of the increased variability and is indicative
of active ES being less effective at reducing leg fluid in some of the participants. After the
sharp drop, variability remained high; however the effect size continued to rise in proportion
to the continued increase in the mean difference in leg fluid accumulation. Altogether, the
results suggest that there was a strong effect of ES in the first 20 minutes, but the longer
the participant used ES, the more effective it was at reducing leg fluid while seated, despite
high variability.

One plausible explanation for the rise in variability after the first 20 minutes is the type
of fluid being removed. In the first 20 minutes, active ES was likely preventing vascular
filling; while in the latter 130 minutes active ES had variable effectiveness in preventing fluid
filtration into the interstitium. From this, we hypothesized that differences in contraction
strength across the subjects might explain the variability in effectiveness of active ES in
preventing interstitial fluid accumulation. As illustrated in Figure 5.6, increasing volume of
leg fluid ejected per contraction (R per contraction) was associated with a greater difference
in fluid accumulation between active and sham ES in the latter 130 minutes seated. These
results demonstrate that consistently strong contractions causing high fluid ejection from the
legs is required during the period using ES to prevent accumulation of interstitial leg fluid.

The greatest strength of an electrical stimulator for activating the skeletal pump and
reducing leg fluid is its versatility. An electrical stimulation system can be developed into
a compact device and applied in a wide variety of settings and in individuals at risk for
leg fluid accumulation and its negative consequences. Groups at risk for high levels of leg
fluid accumulation include sedentary office workers who experience leg swelling due to long
periods sitting [55]; flight attendants or frequent flight travellers susceptible to leg swelling
due to a long immobilized period [162]; individuals with fluid retaining diseases such as renal
failure and heart failure [159]; and the spinal cord injured [163]. Individuals experiencing venous pooling and leg fluid accumulation are at increased risk for diseases such as deep vein thrombosis as well as the formation of leg edema, which is associated with complications including increased risk of leg ulcers, painful swelling, reduced elasticity of the veins and arteries of the legs, and thromboembolic events [158,159]. Prevention of leg fluid accumulation in these at risk groups can treat disorders that worsen with increased fluid shifting into the the upper-body such as supine hypertension, acute decompensation in heart failure [160], pulmonary edema [161], nocturnal asthma [116], and increased OSA severity [13]. In fact, with regards to sleep apnea in particular, the reduction of the leg fluid accumulation via walking and compression stockings were capable of reducing the overnight fluid shift into the upper-body and the severity of the sleep apnea [18] [16,17,19,136]. In this context, calf muscle electrical stimulation has the potential to treat a variety of diseases and disorders through the prevention of daytime leg fluid accumulation.

The main limitation of this study was that vascular and interstitial fluid were not directly measured and inferences made on the transition from vascular to interstitial fluid accumulation are based on the rates of change in leg fluid described by their mathematical models. To confirm these findings, more objective measures should be considered. Indicator dilution methods are an objective measure of fluid compartments, however it is invasive, requiring blood samples for each time measurement [169]. Ultrasound is a non-invasive method of estimating blood volume in major veins and arteries that could be performed in future studies to detect time-series changes in blood volume [170]. Our study was also limited in its lack of women participants. While the study was open to women, fewer women responded to the study advertisement and those that did were not eligible for various reasons. The main challenge with enrolling women to participate was the requirement for a sleep apnea diagnosis, which is a more common disorder in men [1]. To the best of our knowledge, there are no studies investigating differences between the sexes in leg fluid accumulation while upright. However, we have shown in prior studies that fluid shift due to gravity when lying down
differed between the sexes. In short, baseline LFV was greater in men compared to women, yet there was no significant difference between the sexes in fluid shift out of the legs when lying down for 90 minutes [54, 143]. In addition, while the volume of fluid leaving the legs was the same, less fluid accumulates in the thorax and neck of women compared to men, possibly due to fluid sequestration in the abdomen in women [54]. Since the same amount of fluid shifted out of the lower body in men and women, it is likely that when assuming the upright position the same amount of fluid will shift into the lower body. With respect to between-sex differences in the effectiveness of calf muscles activation in reducing leg fluid accumulation, to the best of our knowledge this has never been investigated. So while it is unlikely that leg fluid accumulates differently in men compared to women, the effects of calf muscle ES between the sexes cannot be speculated upon, but would be an interesting topic for future study.

In conclusion, calf muscle ES was effective at reducing the accumulation of fluid in the legs while seated, compared to a sham control. There was no clear time point after which calf muscle ES stopped being effective. While effect size reached a local peak at 20 minutes and then dropped substantially, after continued use the effect size increased substantially to an absolute peak at 130 minutes. Therefore, the longer an individual used calf muscle ES, the more effective it was at preventing leg fluid accumulation. Efficacy of calf muscle ES was consistent in preventing vascular filling in the first 20 minutes seated, but variable in preventing interstitial fluid formation in the latter 130 minutes seated. The variability in preventing interstitial fluid formation was found to be attributed to the amount of fluid ejected from the leg per contraction, where more fluid leaving the leg per contraction was associated with greater prevention of interstitial leg fluid formation. This highlights the importance of maintaining a strong contraction throughout the sedentary period using calf muscle ES. Once validated in a larger sample size, calf muscle ES has potential as a device for preventing leg fluid accumulation and associated health consequences in at-risk groups and settings.
Chapter 6

Conclusions and Future Work

The present thesis dissertation discovered that baseline measures, in addition to demographics, can be used to predict an individual’s risk for neck fluid accumulation which may increase risk for OSA defined by the fluid shift mechanism. In particular, LFV measured prior to lying down is predictive for the amount of fluid leaving the legs and accumulating in the neck when lying down, independent of sex, BMI, and age. This research made way for the main study in my PhD investigating calf muscle electrical stimulation (ES) on rostral fluid shift. It was demonstrated that activating the calf muscles of both legs every two seconds using ES over a 150 minute seated period reduced fluid accumulation in both legs by 88 ml (46% reduction), compared to a sham ES condition. In turn, upon lying down this resulted in a reduced shift of fluid out of both legs by 63 ml (17% reduction) and a reduction of 31 ml (31%) in neck fluid accumulation and 72% reduction in NC. Given that the 150 minute period of calf muscle ES is not practical for everyday use, we then examined the temporal fluid accumulation data over the 150 minute period to identify the time point at which effectiveness of calf muscle ES diminishes. Interestingly, over the entire period seated the difference in the accumulation of leg fluid between the active and sham ES conditions continued to increase linearly with time. As such, there was no clear time point that represented the beginning of a decline in effectiveness. However, it was apparent that while overall calf muscle ES was effective
throughout the seated period, it was highly variable across the subjects after the 20 minute time point. Correlation analysis revealed that during the period of variability (the latter 130 minutes), the magnitude of difference in fluid accumulation between active and sham ES was correlated with the contraction strength of active ES (as measured by the amount of fluid leaving the legs per contraction). It is therefore important that any ES system that will activate the calf muscle for more than 20 minutes must maintain a strong contraction to ensure consistent efficacy across all users.

The greatest benefit of an electrical stimulator is that it can be developed into a compact wearable device. This feature provides calf muscle ES with several distinct advantages over comparable interventions such as walking, wearing compression stockings, or seated cycling/stepping. First, as electrode design continues to be improved, the electrical stimulator will be more comfortable and easy to apply, unlike compression stockings which are uncomfortable and difficult to apply and remove, contributing to their non-adherence [144]. Unlike walking or exercise, rather than allocating a period of time for the activity, calf muscle ES therapy is more convenient and can be used at any time such as during work hours while seated for a long period. Lastly, compared to seated cycle/stepping, the ES device involuntarily activates the calf muscle at regular intervals, whereas voluntary activation of the calf muscle using seated cycling/stepping devices requires motivation and the presence of mind to use the device.

My PhD research into the development of a calf muscle electrical stimulator to reduce daytime leg fluid accumulation is partially funded by the industry sponsor, BresoTec (https://bresotec.com/). A start-up company funded by MaRS innovation, BresoTecs ambition is to develop an innovative, new generation of sleep management solutions. Together with BresoTec, the long term goal for calf muscle ES is to develop a convenient, wearable therapeutic device. The details of this device cannot be discussed in this dissertation, so as to not disclose any intellectual property pertinent to the patent. We are currently in the process of applying for a patent for a wearable calf muscle electrical stimulation device to reduce
daytime leg fluid accumulation and alleviate OSA defined by the fluid shift mechanism.

The findings from Chapter 3, coupled with past research, identify that individuals with the greatest risk for neck fluid accumulation and OSA by fluid shift are men [22, 23] over the age of 40 years [24], who spend majority of their day sitting [13], with a high LFV [143]. Furthermore, a paper which I co-authored also showed that larger neck circumference and bioelectrical impedance phase angle were both associated with larger pharyngeal tissue content and increase risk for development or worsening of OSA caused by fluid loading [171]. From these results it is difficult to provide a firm cutoff that identifies risk for excessive neck fluid accumulation that might lead to OSA. However, as a rough guide a volume of leg fluid that would be considered high can be captured from the 75th percentile of leg fluid accumulation. From the data collected in Chapter 3, this range is 2.5 to 2.8 L of fluid in both legs. This range is associated with a NC increase of 0.55 to 0.60 cm.

Continuing from these findings, future work should identify a threshold pre-sleep LFV that identifies increased risk for OSA defined by the fluid shift mechanism. Over the past several years, many studies have been conducted in Dr. Bradleys sleep laboratory at the Toronto Rehab Institute to investigate the role of overnight fluid shift on OSA [13, 172] and the efficacy of interventions to treat OSA by targeting leg fluid [15, 16, 18]. Pooling results from these studies will provide a dataset of 141 patients that underwent a full night polysomnography with simultaneous measurement of leg fluid volumes. From these data a definitive LFV threshold can be established for its association with OSA risk. Once a threshold LFV has been established, additional demographics, as well as other relevant fluid and non-fluid related measures, can be used to develop a model that will identify a subtype of OSA defined by the fluid shift mechanism. As illustrated in Figure 6.1, plotting AHI with the change in LFV (and/or change in NC) one can visually identify four distinct groups of patients: those with OSA defined by the fluid shift mechanism (group I), patients that experience fluid shift that are protected from OSA (group II), individuals without sleep apnea and low overnight fluid shift (group III), and patients with OSA in spite of low overnight
fluid shift volumes (group IV). Using the demographic data and relevant fluid and non-fluid related measures as independent variables, a classification model (e.g. logistic regression) can then be developed to predict patients in group I from groups II, III, and IV. Previously this was not possible because a rule of thumb for classification is one sample (i.e. patient) for each independent variable. With 141 patients, a model with up to 14 predictor variables can be developed.

The relationship between baseline LFV and the rostral shift of fluid from the legs to the neck, adds to evidence supporting the hypothesis that reducing LFV prior to sleeping can reduce overnight rostral fluid shift and alleviate sleep apnea. This served as the impetus for the main study of my PhD, presented in Chapter 4, which provides the proof-of-concept that calf muscle ES can reduce the accumulation of leg fluid during a sedentary period. This subsequently resulted in a reduction of fluid shifting out of the legs, preventing neck fluid accumulation by more than 30%. In addition to the strong effect of calf muscle ES on reducing rostral fluid shift, a single session of calf muscle ES reduced snoring index, suggesting reduced pharyngeal narrowing by limiting overnight rostral fluid shift. These findings support future study of the effect of calf muscle ES over a longer period (e.g. four weeks) on overnight rostral fluid shift and OSA.

One of the challenges that a long term study of calf muscle ES will face is the modest effects it is likely to have on alleviating sleep apnea, as prior studies report [15–21]. Thigh high and knee high compression stockings (for 1 week and two weeks, respectively) and twice daily 45 minutes of walking for one week only modestly reduced OSA severity in the general population by 24%, 27%, and 30%, respectively [15–17]. In addition, once daily 45 minutes of walking for four weeks reduced OSA severity by 36% in patients with coronary artery disease [18]. Before endeavoring into a long term study on the effects of calf muscle ES on OSA, it is imperative to identify the ideal population that will benefit most from therapies aimed at treating OSA by reducing LFV. I previously described a potential analysis using pooled data from data previously collected, aimed at identifying patients at risk of OSA.
Figure 6.1: Hypothetical data illustrating the relationship between the overnight change in LFV and AHI. The horizontal line represents cutoff AHI for sleep apnea diagnosis and the vertical line is an arbitrary LFV threshold. These lines form the boundaries for the different patient endotypes which are: patients with OSA defined by fluid shift (Group I), individuals that experience fluid shift but protected from OSA (Group II), patients without OSA and low overnight fluid shift (Group III), and those with OSA in spite of low overnight fluid shift (Group IV).
by the fluid shift mechanism. However, focusing on the intervention studies and separating patients based on the effectiveness of the intervention (i.e. greater than 50% reduction in OSA and reduction of OSA to below 10 events/hr) models can be developed to identify patients that will benefit most from fluid-targeted therapies. Using a similar methodology described above, demographics as well as relevant fluid and non-fluid related metrics can be used as independent variables that will predict patients that will benefit most from therapies aimed at treating OSA by reducing leg fluid volume. Altogether, the models developed from both of these potential studies will provide a useful tool for predicting patients with OSA defined by the fluid shift mechanism and further identifying those that will benefit from therapies targeting leg fluid accumulation.

The advantage of calf muscle ES over other forms of calf muscle pumping, such as physical activity, is that it can be used by individuals with mobility impairment such as post-stroke patients, and individuals with a spinal cord injury. These are important populations because both have an increased prevalence of OSA, compared to the general population. Sleep disordered breathing is present in up to 72% of ischemic and hemorrhagic stroke and transient ischemic attack patients, and was primarily obstructive in nature [173]. The high prevalence of sleep disordered breathing is mainly attributed to mechanisms linking sleep-disordered breathing with the occurrence of a stroke. However, it has been hypothesized that stroke may also aggravate or cause sleep-disordered breathing. This is supported by findings that sleep disordered breathing improves past the acute phase of stroke [174]. The prevalence of sleep disordered breathing after spinal cord injury has only been investigated in small cohorts. In a systematic review of these cohorts, the prevalence of sleep disordered breathing after spinal cord injury is 2-5 times greater than the general population, with prevalence ranging from 27 to 62% [175]. In spinal cord injured, the pathogenesis of OSA is unknown, but has been attributed to increased neck circumference, increased BMI, neurological dysfunction affecting intercostal and abdominal muscles required to breathe, and the use of medications like baclofen and benzodiazepines that are known to increase the frequency and severity of
sleep disordered breathing events [176]. Given the reduced levels of physical activity in those with stroke and spinal cord injury, there are significant opportunities for future work to study the hypothesis that increased prevalence of OSA in these populations is defined by the fluid shift mechanism. If OSA is defined by the fluid shift mechanisms in these populations or some subgroup therein, calf muscle ES would be a useful therapy for these patients to prevent OSA caused by the fluid shift mechanism.

Another group of at risk for fluid related OSA is postoperative patients. Surgery has been demonstrated to lead to the development or worsening of sleep disordered breathing postoperatively [177]. Postoperative OSA, even in mild forms, can lead to increases in postoperative adverse events, including sudden cardiac death and cardiovascular complications [178]. The cause of postoperative sleep-disordered breathing is unknown; however, large quantities of fluid infused during surgery have been implicated [179]. A recent study infused approximately 2L of saline during daytime sleep in a group of men, simulating the conditions of the saline overloaded postoperative patient, which resulted in the development or worsening of OSA in a subset of these participants [24]. Building on these results, during my PhD I also wrote a paper (Appendix B) investigating the difference in autonomic nervous system response in the group that experienced an increase in AHI in response to saline infusion (AHI+), compared to those in whom AHI did not increase (AHI). Using measures of HRV, we found that cardiac vagal modulation at the onset of sleep prior to saline infusion was similar between the AHI+ and AHI groups. However, after saline infusion, cardiac vagal modulation was reduced in the AHI+ group. These findings highlight the possibility that intravenous fluid infusion during surgery can contribute to development or worsening of OSA and autonomic cardiovascular dysregulation, which may play a role in the development of postoperative cardiovascular complications.

Finally, autonomic dysfunction can also be explored in the context of risk factors for OSA defined by the fluid shift mechanism. The autonomic nervous system is a relevant avenue for fluid related risk factors given its role in regulating blood volume, volume shifts
with posture change, and blood pressure. For example, upon lying supine, the shift of fluid toward the heart increases venous return to the right atrium. Baroreceptors in the carotid body are loaded resulting in the inhibition of sympathetic and enhanced vagal activation. This leads to reduced heart rate, to reduce cardiac output and aid in blood pressure control. In addition, peripheral vascular resistance is reduced to also control for blood pressure and venous compliance is increased to store excess blood volume in the compliant venous system \[180\]. These mechanisms are in place to primarily control for blood pressure; however it also impacts the redistribution of blood volume in the neck and thorax. For example, if the magnitude of sympathetic withdrawal and vagal enhancement was reduced in response to rostral fluid shift upon lying down, heart rate would be higher compared to the normal autonomic response. This may lead to increased cardiac afterload and a rise in central venous pressure, which could increase blood volume and pressure in the large veins in the neck and contribute to increased fluid pressure on the airway.

Given the complexity of the autonomic nervous system, it is difficult to define a specific role that it might play in OSA defined by the fluid shift mechanism. The mechanism described earlier is just one potential mechanism by which the autonomic nervous system impacts fluid redistribution and its potential role in the development of OSA defined by the fluid shift mechanism. There are likely other avenues by which autonomic nervous system can impact fluid distribution, for example through impacts on renal function, which should also be considered and explored. Given its role in controlling the blood volume distribution, the autonomic nervous system should be explored as a potential predictor for OSA defined by the fluid shift mechanism. Detecting differences in autonomic nervous system response to physiological challenges such as posture change via tilt table or the valsava maneuver can be used to develop additional risk factors for predicting OSA defined by the fluid shift mechanism. Direct assessment of muscle sympathetic nerve activity using microneurography is the standard means for measuring central sympathetic outflow \[181\]. Unfortunately, the procedure is invasive and challenging and therefore not practical for widespread use as a
simple measurement technique conducted in a clinical setting. Alternatively, a more practical method for quantifying autonomic nervous system response to physiological challenge is by analyzing the autonomic modulation of the heart using measures of heart rate variability (HRV) [182]. Using this method, a researcher can use an electrocardiogram signal, as short as five minutes, to compute several features related to the vagal and sympathetic modulation of the heart. These features can then be used to predict individuals at risk for OSA defined by the fluid shift mechanism.

In conclusion, the present PhD dissertation explores the hypothesis that the reduction of leg fluid while upright will reduce the rostral shift of fluid from the legs and into the neck and alleviate OSA. Through mining data previously collected, we first identified that the volume of fluid in the legs prior to lying down is one of the main risk factors for rostral fluid shift and accumulation in the neck when lying down, which can potentially increase OSA severity defined by the fluid shift mechanism. In response to this finding, we established the proof-of-concept that activation of the calf muscles using ES can be an effective therapy to reduce daytime leg fluid accumulation and the rostral shift of fluid out of the legs and into the neck and reduce snoring index, likely by alleviating pharyngeal narrowing caused by the fluid shift mechanism. Future work should identify additional risk factors that describe a subgroup of patients that develop OSA defined by the fluid shift mechanism. Focusing treatment on this subgroup will maximize the efficacy of therapies targeting the reduction of daytime leg fluid accumulation and move them into the mainstream of therapies to treat OSA.
Bibliography


Appendix A

Supplementary Methods for Chapter 4

A.1 Methods

A.1.1 Participant screening

Participants were screened for sleep apnea using a portable sleep monitoring device previously described by Alshaer et al. [149–153]. If participants had positive screening for sleep apnea, they were provided with information about the disorder and told to speak to their family doctor about their positive screening.

A.1.2 Fluid Measurement with Bioelectrical Impedance

Fluid volume measurements in the leg and neck using the method of bioelectrical impedance [54] were performed using the Biopac data acquisition system (MP150, California, USA). Using surface electrodes, a small current (400 µA) was applied to the leg and neck at frequencies of 25 and 100 kHz, respectively. The voltage drop across the segment is measured with another pair of surface electrodes, from which impedance of the segment is determined. Fluid volume of the segments was then calculated using Equation A.1, which assumes the
segments as truncated cones [54].

\[ v = \frac{\rho^{2/3}}{3(4\pi)^{1/3}} \left( \frac{L}{C_1 C_2 R} \right)^{2/3} L \left( C_1^2 + C_2^2 + C_1 C_2 \right) \]  \hspace{1cm} (A.1)

Where \( L \) is the length of the segment, \( C_1 \) and \( C_2 \) are the circumferences of the top and bottom of the segment, \( R \) is the segment’s resistance, and \( \rho \) is the resistivity of the blood which is estimated as 98 \( \Omega \text{cm} \) for the leg and neck [125].

Voltage measuring electrodes were positioned at the top and bottom of the segments and adhered to the skin using hypoallergenic adhesive tape. To measure LFV, voltage measuring electrodes were positioned at the level of the ankle (just below the lateral malleolus) and the knee (between fibula head and tibial tubercle). To measure NFV, voltage measuring electrodes were placed at the base of the neck and just below the ear on the right side. Current injecting electrodes were positioned 1-inch from the voltage measuring electrodes, outside of the segment. Segment length was measured as the distance between the voltage measuring electrodes using a tape measure. Circumferences \( (C_1 \) and \( C_2 \) of leg were measured using a tape measure at the level of the voltage measuring electrodes. Neck circumference measured at the level of the cricothyroid cartilage was used for both \( C_1 \) and \( C_2 \).

A.1.3 Monitoring activity and diet

Participants monitored their activity for five days leading up to the study day using a paper diary which required participants to record every half hour whether they were sleeping, lying, sitting, standing, walking at a usual pace, walking briskly, or participating in vigorous physical activity. Time spent sedentary is described by the total time spent sitting, lying, and standing. Time spent active was described by the total time spent walking at usual pace, walking briskly and performing physical activity. Diet was also monitored for three days leading up to the study day using a paper diary that required participants to record the names and amounts of food and drink consumed at every meal or snack. The nutrient
content of foods and beverages were quantified with a specialized software package for dietary analysis (ESHA Food Processor SQL Version 10.14.1, ESHA Research Inc., Salem, OR).

A.1.4 Electrical stimulation protocol

A programmable 4-channel neuromuscular electrical stimulator (Compex Motion, SA, Switzerland) was used to deliver transcutaneous electrical stimulation to the gastrocnemius muscle group in the left and right leg using 9 cm by 5 cm StimTrode electrodes (Axelgaard Manufacturing, California, USA). Stimulation was applied using an asymmetrical biphasic waveform with 40 Hz frequency, and 300 s pulse duration.

A.1.5 Apnea-hypopnea index and snoring index

AHI and snoring index were measured via acoustic analysis of breathing using a self-contained, battery-operated, wireless device. The device consists of an open face frame with an embedded microphone at a fixed distance (approximately 3 cm) in front of the participants mouth, and an electronics compartment that contains a pre-amplifier, microprocessor, analog-to-digital converter, and a microSD card. Digitized breathing sounds are recorded on the microSD for up to 8 hours at a sampling frequency of 16 kHz. Apneas, hypopneas and snoring episodes are detected from the breathing sound data using highly accurate algorithms that have been validated against polysomnography and in the unattended home setting [149–153].
Appendix B

Heart Rate Variability Response of Individuals With and Without Saline Induced Obstructive Sleep Apnea

Under review: J Clin Sleep Med. Sep 2017

B.1 Introduction

A commonly observed phenomenon in middle aged or elderly adults undergoing surgery involving general anesthesia and intravenous volume loading is the development of obstructive sleep apnea (OSA), which continues into the postoperative recovery period [177, 179, 183]. Postoperative OSA, even in mild forms, can lead to increases in postoperative adverse events, including sudden cardiac death and cardiovascular complication [178, 184]. In this context, postoperative OSA may add to postoperative risk by altering cardiac autonomic regulation. There is growing evidence that volume overload during surgery can contribute to the postoperative OSA [179]. The purpose of the present study was to investigate if the onset or worsening of OSA by volume loading will alter cardiac autonomic regulation.

Examination of heart rate variability (HRV), particularly the low frequency range (0.04-
Appendix B. HRV Response to Saline Induced Sleep Apnea

0.15 Hz), has been used to provide a non-invasive estimate of cardiac sympathetic activity, although both sympathetic and parasympathetic inputs may influence HRV at these frequencies [185]. Regarding cardiac parasympathetic activity, it is well established that quantification of HRV in the higher frequency range (0.15 to 0.4 Hz) is a reliable index of cardiac parasympathetic (vagal) modulation [186]. Additionally, HRV is an important index of cardiovascular health, as reduced high frequency HRV is predictive of morbidity and mortality in the general population and patients with cardiovascular disease [187–189].

We previously demonstrated that experimental volume loading by infusion of physiologic saline during sleep can induce or worsen OSA in some non-obese men, but not in others [24]. Using measures of HRV, we tested the hypothesis that among those subjects in whom acute fluid infusion increased their OSA severity as assessed by apnea-hypopnea index (AHI), sympathetic and parasympathetic modulation of HRV would be increased and decreased, respectively, compared to those in whom the AHI did not increase.

B.2 Methods

This study is a retrospective analysis of a previous study to investigate the effects of saline-induced fluid overloading during sleep on OSA severity [24]. The protocol was approved by the Research Ethics Board of Toronto Rehabilitation Institute and participants provided written informed consent prior to participation. The study was performed in accordance with the approved guidelines and regulations.

B.2.1 Study participants

Twenty-one non-obese (body-mass index, BMI < 30 kg/m\(^2\)) and normotensive (blood pressure < 140/90 mmHg) men aged 20-70 years were recruited by advertisement. Seventeen of the participants were included in the original study [24] and four were newly recruited. Only men were included because previous studies have shown that men are more susceptible than
women to worsening of OSA due to fluid redistribution during sleep [22,23]. Individuals were excluded if they were previously diagnosed with OSA, slept for less than one hour during the protocol, or had central dominant sleep apnea (more than 50% of apneas and hypopneas central). Participants were also excluded if they had tonsillar hypertrophy, a history of cardiovascular, renal, neurological, or respiratory disorders, were taking prescribed medication for these disorders, were taking over-the-counter medication that might influence fluid volume, or if their AHI in the control arm of the study was $\geq 30$ events/hr.

### B.2.2 Study procedures

**Polysomnography (PSG)**

PSGs were conducted during the daytime to accommodate scheduling of the nurse required to insert the IV line into the participant. All sleep studies began at approximately 12 noon. To facilitate daytime sleep, participants restricted their sleep to less than 4 hours the night before and refrained from caffeinated beverages and alcohol for at least 12 hours prior to experiments. Subjects slept supine for the study period to control for potential effects of changes in body position on the AHI and other variables. These sleeping conditions, in addition to intravenous volume loading, closely simulated the postoperative period where patients are sleep-deprived prior to surgery and are nursed during recovery in the supine position [183].

Standard techniques and criteria were used to score the sleep stages and arousals [190]. Thoracoabdominal motion was monitored by respiratory inductance plethysmography, airflow by nasal pressure cannulae; and arterial oxyhemoglobin saturation ($\text{SaO}_2$) by pulse oximetry. Apneas were defined as more than 90% reduction from baseline in airflow or thoracoabdominal motion, lasting more than 10 seconds. Hypopneas were defined as more than 30% reduction from baseline in airflow lasting more than 10 seconds, associated with a minimum 3% desaturation or an arousal from sleep [190]. Apneas were defined as central or obstructive as previously described [171]. Severity of sleep apnea was assessed by the AHI.
Appendix B. HRV Response to Saline Induced Sleep Apnea

Obstructive AHI (OAHI) was defined as the number of obstructive apnea or hypopnea events per hour of sleep. The electrocardiogram (ECG) was recorded using a lead-I configuration at 256 Hz.

Blood pressure, heart rate, and respiratory measures

An automated sphygmomanometer (BPM 300, BpTru, BC, Canada) was used to measure systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR) prior to sleep and immediately after the participant awoke. Mean HR was also measured pre- and post-saline infusion using 5-minute ECG segments. Respiratory rate and an index of tidal volume were measured using the summation of thoracic and abdominal movement collected from respiratory inductance plethysmography. Respiratory measures were calculated during the same period of data from which the segments were selected for analyzing HRV.

B.2.3 Protocol

This was a randomized, single-blind, double crossover study [24]. During each study arm, a 20 gauge intravenous catheter was inserted into the left forearm vein to facilitate infusion of physiologic saline (0.9% NaCl in water). In the control arm, saline was infused at the minimum rate needed to keep the vein open. In the intervention arm, saline was initially infused at the minimum rate until the participant achieved at least 5 minutes of stage 2 non-REM (NREM) sleep, at which point a bolus of 22 ml/kg body weight saline was infused over 30 minutes. Complete details of the saline infusion protocol have been previously described [24]. Participants were blind to the study arm, but the investigator was aware of the allocation as he/she was required to start and stop the saline infusion. The sleep technician who scored the PSG was blind to the experimental condition. All participants slept supine on a single foam pillow. One week after the first session, participants crossed-over to the other study arm.

Participants were classified as AHI+ if their AHI or OAHI increased by at least 100%
from the control visit and was $\geq 10$ during the intervention arm [171]. Otherwise, they were classified as AHI$-$. 

**B.2.4 Data analysis**

**Heart rate variability measures**

To compute measurements pre- and post-saline infusion, a single 5-minute ECG segment was selected at the onset of stage 2 NREM sleep before saline was infused, and another single 5-minute segment was selected after saline infusion was complete (Figure B.1). The ECG segment selected post-saline infusion was selected from the first period of stage 2 NREM sleep following saline infusion, which typically occurred during the first sleep cycle. However, some participants experienced REM sleep before the completion of saline infusion. In those cases (2/8 from AHI+ group and 3/13 from AHI$-$ group), ECG segments were selected from the second sleep cycle. ECG signals were only analyzed during stage 2 NREM sleep to control for the possible effects of sleep stages on HRV [191]. ECG data during and 1-minute after an apnea or hypopnea were excluded to avoid the immediate autonomic reflex impact of such events on HRV [192]. R-peaks were identified using a semi-automated peak finding procedure in Matlab software to estimate RR intervals (2014a, MathWorks, Natick, MA).

Preprocessing of the RR interval time series and computation of time and frequency domain measures of HRV were performed using Kubios HRV software [193] in accordance with standard guidelines [182]. Artifact correction was performed to remove RR intervals that differed abnormally from the local mean RR interval. The threshold for removing an RR interval was 0.35 sec, such that any RR intervals greater or less than the local mean by 0.35 sec were removed. After pre-processing, the following time domain measures of HRV were computed: mean of the RR interval (mean RR), standard deviation of the RR interval (SDNN), and root mean square of successive differences (RMSSD). Spectral analysis was also performed using Kubios HRV software. A requirement of spectral analysis is that the RR intervals be equidistantly sampled along the time axis. However, each RR interval datum
Figure B.1: Illustration of the time of ECG captures relative to saline infusion and the entire sleep periods. As shown, 5-minute ECG segments were captured as close to the beginning (pre) and after (post) saline infusion.

occurs whenever an R-wave is detected which means the RR intervals are inherently non-equidistant. A common approach for solving this is to use interpolation methods to convert non-equidistantly sampled time series to equidistantly sampled time series. In particular, we performed a 4 Hz cubic spline interpolation on the RR interval time series. Next, a discrete Fourier transform of the RR time series based on Welch’s method was performed. Spectral measures computed were low frequency power (LF: 0.04–0.15 Hz), high frequency power (HF: 0.15 to 0.4 Hz), total spectral power, and the ratio of LF to HF (LF:HF) power.

B.2.5 Statistical analysis

Subject characteristics for both AHI− and AHI+ groups were examined by the independent t-test or Mann-Whitney test. Spectral data were log-transformed to achieve a normal distribution of the data. A two-way ANOVA was used to compare changes in variables during experiments between the AHI− and AHI+ groups. Data were analyzed pre- and post-saline infusion to compare HRV and ventilatory measures; pre- and post-sleep to compare blood
pressure measurements; and at control and intervention study arms to compare sleep apnea outcomes. The interaction effect evaluated differences in the change in variables from either pre- to post-saline infusion; pre- to post-sleep; and control to the intervention study arm between AHI− and AHI+ groups. If the interaction effect was significant, we tested the differences between groups in outcome variables at pre/post-saline infusion, pre/post-sleep, and control/intervention using the independent t-test or Mann-Whitney test. Relations between HRV measures and AHI, as well as any other variables that differed between the groups, were examined using Pearson correlations or Spearmans rank correlation. A p-value of <0.05 was considered statistically significant. Statistical analyses were conducted using R open source statistical software version 3.2.1 (http://www.r-project.org). Data are presented as mean ± SD.

B.3 Results

Twenty-one men were included in the analysis, of which 13 were in the AHI− group and 8 were in the AHI+ group. Baseline characteristics of height, weight, and BMI were similar between the AHI− and AHI+ groups (all P>0.05, Table B.1). However, there was a trend to suggest the AHI+ group was older by 10 years (P=0.08).

Table B.1: Baseline characteristics of the subjects.

<table>
<thead>
<tr>
<th></th>
<th>AHI− (n=13)</th>
<th>AHI+ (n=8)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>33.1±12.5</td>
<td>42.6±9.0</td>
<td>0.08</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>178.3±4.9</td>
<td>174.2±7.3</td>
<td>0.14</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>74.7±12.4</td>
<td>79.9±9.0</td>
<td>0.32</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.6±3.5</td>
<td>26.0±3.0</td>
<td>0.12</td>
</tr>
</tbody>
</table>

BMI: body-mass index

Illustrated in Figure B.2, the AHI was similar in the control arm between the AHI+ and AHI− groups (12.8±7.9 vs. 9.6±8.2 events/hr, respectively; P>0.10), but following saline
Figure B.2: Change in apnea-hypopnea index (AHI) from control and intervention in both the AHI− and AHI+ groups. Top horizontal lines represent results of the interaction effect; the middle and lower horizontal bars represent results of the comparisons of pre- and post-infusion values between the groups, respectively.
infusion (intervention) the AHI was higher in the AHI+ group, compared to the AHI− group the (36.8±23.3 vs. 7.7±6.5 events/hr, respectively; P<0.01). As shown in Table B.2, OAH and arousal index were also similar in the control arm between the AHI+ and AHI− groups (P>0.10), but following saline infusion, OAH was higher in the AHI+ group, compared to the AHI− group (P<0.01). Additionally, in both the AHI− and AHI+ groups, sleep efficiency and percentage of time spent in stage 3 NREM sleep were lower in the intervention arm, compared to the control arm (P<0.01 and P<0.05, respectively). Minimum oxygen saturation, total sleep time, percentage of time spent in stages 1 and 2 NREM sleep and REM sleep were not different between the groups or study arms (P>0.10).

As shown in Table B.3, the interaction effect for SBP was statistically significant such that SBP measured pre-sleep was similar between the AHI− and AHI+ groups (P>0.10), but post-sleep SBP was higher in the AHI+ than in the AHI− group (P<0.05). DBP showed a similar trend but the interaction effect was not significant (P=0.08, Table B.3). The interaction effect for HR was not statistically significant (P>0.10). Additionally, for both respiratory rate and tidal volume, the interaction effect between groups was not significant (both P>0.10, Table B.3).

With respect to HRV measures, there was no significant interaction effect between the groups for mean RR interval (P>0.10, Table B.3). There was a significant interaction effect for SDNN (P=0.01) and RMSSD (P=0.01), as shown in Table B.3. At pre-saline infusion, SDNN and RMSSD were similar between the groups (all P>0.10). Post-saline infusion SDNN was lower in the AHI+ group (P<0.05) compared to the AHI− group, and RMSSD demonstrated a similar trend (P=0.07).

In the frequency domain, there was a significant interaction effect for HF power (P<0.01, Figure B.3A). At pre-saline infusion HF power was similar between the groups (P=0.63); but post-saline infusion HF power was lower in the AHI+ group (P=0.03). For LF power, there was a significant interaction effect (P<0.01, Figure B.3B); however between the groups LF power was similar pre-saline infusion (P=0.22) and post-saline infusion (P=0.24). There was
Table B.2: Sleep characteristics on the control and intervention day in the AHI− and AHI+ groups

<table>
<thead>
<tr>
<th></th>
<th>AHI−</th>
<th>AHI+</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Intervention</td>
<td>Control</td>
</tr>
<tr>
<td>OAHI, events/hr sleep</td>
<td>6.2±6.8</td>
<td>4.9±5.6</td>
<td>9.7±5.6</td>
</tr>
<tr>
<td>Arousal index, events/hr sleep</td>
<td>15.7±9.5</td>
<td>16.0±13.7</td>
<td>19.4±12.3</td>
</tr>
<tr>
<td>Total sleep time, min</td>
<td>159.1±43.6</td>
<td>136.7±39.4</td>
<td>136.7±41.1</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>81.7±12.6</td>
<td>68.5±16.8</td>
<td>72.3±20.6</td>
</tr>
<tr>
<td>Minimum SaO2, %</td>
<td>93.8±2.0</td>
<td>93.2±2.8</td>
<td>91.2±2.9</td>
</tr>
<tr>
<td>Stage 1 NREM sleep, %</td>
<td>15.8±10.1</td>
<td>19.6±16.1</td>
<td>14.7±10.7</td>
</tr>
<tr>
<td>Stage 2 NREM sleep, %</td>
<td>52.4±13.0</td>
<td>57.7±11.5</td>
<td>59.2±19.2</td>
</tr>
<tr>
<td>Stage 3 NREM sleep, %</td>
<td>22.7±19.1</td>
<td>13.2±13.2</td>
<td>18.1±17.8</td>
</tr>
<tr>
<td>REM sleep, %</td>
<td>14.5±8.2</td>
<td>9.5±9.2</td>
<td>8.0±7.8</td>
</tr>
</tbody>
</table>

OAHI: Obstructive apnea-hypopnea index
P-values represent the interaction effect; asterisks (*) represents statistically significant differences in intervention arm measurements between groups
Table B.3: Blood pressure and heart rate measures captured pre- and post-sleep, as well as respiratory and HRV measures computed from data captured pre- and post-saline infusion.

<table>
<thead>
<tr>
<th></th>
<th>AHI−</th>
<th></th>
<th>AHI+</th>
<th></th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pre-sleep</td>
<td>Post-sleep</td>
<td>Pre-sleep</td>
<td>Post-sleep</td>
<td></td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>109.2±11.3</td>
<td>110.9±8.8</td>
<td>112.3±12.7</td>
<td>124.6±14.4*</td>
<td>0.01</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>72.8±9.2</td>
<td>74.3±7.6</td>
<td>76.6±13.1</td>
<td>85.8±9.5</td>
<td>0.08</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>70.5±12.4</td>
<td>63.3±7.6</td>
<td>80.6±13.1</td>
<td>73.0±4.9</td>
<td>0.91</td>
</tr>
<tr>
<td>Respiratory Rate (breaths/min)</td>
<td>15.1±2.7</td>
<td>14.2±2.2</td>
<td>14.9±3.2</td>
<td>14.1±2.5</td>
<td>0.85</td>
</tr>
<tr>
<td>Tidal volume (arbitrary units)</td>
<td>0.61±0.3</td>
<td>0.62±0.2</td>
<td>0.74±0.3</td>
<td>0.80±0.3</td>
<td>0.44</td>
</tr>
<tr>
<td>Mean RR (ms)</td>
<td>1036.9±139.0</td>
<td>990.6±154.4</td>
<td>965.9±150.2</td>
<td>902.1±143.6</td>
<td>0.57</td>
</tr>
<tr>
<td>SDNN (ms)</td>
<td>62.4±18.7</td>
<td>83.3±30.5</td>
<td>67.4±20.6</td>
<td>60.4±17.9*</td>
<td>0.01</td>
</tr>
<tr>
<td>RMSSD (ms)</td>
<td>57.3±26.7</td>
<td>68.5±35.8</td>
<td>58.7±28.7</td>
<td>43.4±24.3</td>
<td>0.01</td>
</tr>
</tbody>
</table>

SBP: Systolic blood pressure, DBP: diastolic blood pressure
P-values represent the interaction effect; asterisks (*) represents statistically significant differences of post-sleep/saline infusion measures between groups
also a significant interaction effect for total spectral power (P=0.02, Figure B.3C). At pre-saline infusion there was no significant difference in total power between the groups (P=0.64). However, at post-saline infusion there was a trend to suggest total power was lower in the AHI+ group, compared to the AHI− group (P=0.07). Lastly, for the LF:HF power ratio the interaction effect was not statistically significant (P=0.28, Figure B.3D).

The AHI in the saline infusion arm of the study did not correlate with the change in mean RR interval (r=0.06, P=0.80), SDNN (r= 0.29, P=0.21), and total power (r= 0.23, P=0.32), but did correlate with HF power (r= 0.46, P=0.04) and trends were observed for RMSSD (r= 0.41, P=0.06), LF power (r= 0.38, P=0.09), and LF:HF power (r=0.38, P=0.09). In addition, arousal index following saline infusion did not correlate with mean RR interval (r= 0.33, P=0.14), SDNN (r=0.10, P=0.66), RMSSD (r=0.19, P=0.40), LF power (r=0.23, P=0.32), and total power (r=0.08, P=0.72), but did correlate with LF:HF power (r=0.47, P=0.03 and trends were observed for HF power (r= 0.38, P=0.09).

Given the trend that age was higher in the AHI+ group, correlation analysis was also conducted between age and HRV metrics. However, age did not correlate with the change in mean RR interval (r= 0.26, P=0.25), SDNN (r= 0.19, P=0.41), RMSSD (r= 0.37, P=0.10), LF power (r= 0.32, P=0.16), HF power (r= 0.35, P=0.12), total power (r= 0.14, P=0.55), and LF:HF power (r=0.29, P=0.21). While not significant, BMI was also higher in the AHI+ group and therefore a correlation analysis was conducted. However, BMI did not correlate with the change in mean RR interval (r=0.04, P=0.85), SDNN (r= 0.12, P=0.61), RMSSD (r= 0.28, P=0.21), LF power (r= 0.23, P=0.31), HF power (r= 0.27, P=0.24), total power (r= 0.16, P=0.50), and LF:HF power (r=0.11, P=0.64).

B.4 Discussion

The present analysis demonstrates that an acute increase in AHI in response to volume loading by saline infusion, simulating the development of postoperative OSA, alters cardiac
Figure B.3: Boxplots illustrating changes in high frequency (HF) power (A), low frequency (LF) power (B), total power (C) and the ratio of the low frequency to high frequency power (D) from pre- to post-saline infusion in the AHI− and AHI+ groups. Mean value is displayed as the horizontal line within the box; limits of the box represent the standard deviation from the mean; and the limits of the vertical line represent the maximum and minimum values. Top horizontal lines represent results of the interaction effect; the middle and lower horizontal bars represent results of the planned comparisons of pre- and post-infusion values between the groups, respectively.
autonomic regulation in the form of reduced time (SDNN) and spectral (HF power) measures of HRV. These indices reflect reduced cardiac vagal modulation in response to saline infusion in the AHI+ group. Our study conditions therefore demonstrate an immediate impact of OSA on cardiac vagal modulation of heart rate.

These findings are of clinical importance given the effectiveness of HRV in risk stratification for cardiovascular events and mortality. For example, decreased HRV and vagal withdrawal are associated with an increased risk for cardiovascular events in a general population [187] and increased mortality in post-myocardial infarction patients [188, 189]. Few studies have utilized the ECG signals from PSG recordings to develop risk stratification using overnight HRV [194]. However, decreased HRV from overnight PSG, consistent with decreased vagal control, was associated with increased 5-year mortality in the elderly [195].

Prior studies employing HRV analysis have demonstrated that OSA is associated with reduced cardiac vagal modulation during sleep [192, 196–200] and wakefulness [201, 202]. However, in contrast to these studies, the present investigation controlled for baseline AHI and then used saline infusion to increase the AHI in a subset of participants. This permitted specific examination of the effects of OSA on cardiac vagal modulation. The results are consistent with evidence that acute reversal of OSA by CPAP increased HF power and decreased the LF:HF power ratio [200]. Taken together, the present results coupled with the results of previous studies establish that acute increases in severity of OSA during sleep lead to reductions in cardiac vagal modulation that can be corrected upon removal of the stimulus by CPAP.

An alternative mechanism by which saline-induced OSA reduced HRV is through increased atrial stretch caused by the infusion of a saline bolus, which can affect HRV independently of changes to cardiac vagal modulation [203]. Horner et al. showed that after both vagal section and β-blockade, stretching the sinoatrial node of pigs hearts decreased SDRR and HF power. These results demonstrate that increased atrial stretch in response to a saline bolus might directly reduce SDRR and HF power, independent of changes to vagal
outflow [203]. However, in the present study the reduction in vagal heart rate modulation cannot be attributed to stretch alone, because it was specific to those who developed OSA after saline loading.

Our study was limited by the use of HRV to measure autonomic responses, which restricts accuracy and consistency of HRV for assessing sympathetic activity [204–206]. More specifically, surges in sympathetic activity to the ventricle and to resistance vessels may occur without parallel increases in low frequency oscillations in sinoatrial discharge. Without accurate information on the change in sympathetic activity, it was difficult to explain the rise in systolic blood pressure that occurred in the AHI+ group, but not the AHI− group. The likely mechanism is the intermittent apnea-related hypoxia and arousals from sleep, which lead to surges in sympathetic nerve activity causing a rise in blood pressure [207]. However, this was not shown in the data since LF power and the LF:HF power ratio were not different from pre- to post-saline infusion between the groups. Nonetheless, given the limitations of HRV for assessing sympathetic activity, it is possible that the AHI+ group still experienced an increase in sympathetic vasoconstrictor tone in response to worsening of OSA, despite no differences in low frequency oscillations of cardiac rhythm between the groups.

The study is also limited by the sample of participants included in the trial. In particular, there was a trend to suggest that the AHI+ group was older than the AHI− group by 10 years. This is not surprising since our group recently demonstrated that older men (mean age: 46 yrs) are more susceptible to OSA by fluid overloading, compared to younger men (mean age: 30 yrs) [24]. However while aging may partially explain increased saline-induced OSA in the AHI+ group, it does not preclude the effect of apneas on HRV observed in the present study. Furthermore, if age was a primary contributor to between-groups differences in HRV, measures of HRV captured pre-saline infusion should be different between-groups, which was not the case. Instead it was only after saline infusion that HRV was reduced in the AHI+ group. This is more suggestive of OSA as the primary contributor to the difference in HRV response between the groups. Furthermore, age was not correlated to changes in any
of the HRV measures from pre- to post-saline infusion.

The study sample was also restricted to non-obese men, which limits generalizability of the findings. Only men were included because past work from our group showed that in patients with renal failure and heart failure, strong correlations between overnight shift in leg fluid volume and OSA severity were observed in men, but not women [22, 23]. Obesity was an exclusion criterion because it might confound the effects of fluid overload on OSA. The study was also limited in its generalizability by requiring participants to sleep deprive themselves to facilitate daytime sleep. Sleep deprivation can have depressive effects on the upper airway dilator muscles [208], and may therefore amplify OSA severity in the studied group.

Interestingly, the study conditions of sleep deprivation, supine sleep and volume overload closely simulate the conditions of patients during the post-operative recovery period. As a result, the findings of the present study may be applicable to patients who undergo surgery and develop OSA [177], possibly due to perioperative intravenous volume loading [179, 183]. Risk for adverse events, including sudden cardiac death and cardiovascular complications, is increased with the development of OSA in the postoperative period [178], possibly by altering cardiac autonomic regulation.

In conclusion, the present study demonstrates that individuals with saline-induced OSA experience decreased HRV, consistent with cardiac vagal withdrawal, during sleep. The use of infused saline to induce OSA closely simulates the postoperative period where patients are volume loaded and can experience the development or worsening of OSA. In this context, alterations in cardiac autonomic regulation caused by OSA may add to the postoperative risk for cardiovascular events. The possibility that intravenous fluid infusion during surgery can contribute to development or worsening of OSA, cardiac autonomic dysregulation and cardiovascular events could be tested in future studies.