EFFECT OF INTRAVENOUS SALINE INFUSION AND VENOUS COMPRESSION STOCKINGS ON UPPER AIRWAY SIZE AND OBSTRUCTION

By

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A thesis submitted in conformity with the requirements for the Degree of Master of Science,

Institute of Medical Science, University of Toronto

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ABSTRACT

Obstructive sleep apnea (OSA) severity is strongly associated with the degree of overnight peripharyngeal fluid accumulation. We hypothesized that intravenous fluid loading would cause upper airway (UA) narrowing or increase the frequency of apneas and hypopneas per hour of sleep (apnea-hypopnea index; AHI). We employed a controlled, randomized double-crossover experiment in 9 healthy men aged 23-46 years. In the control, subjects were administered approximately 80 ml of normal saline intravenously during sleep. In the intervention, subjects were administered approximately 1850 ml of saline during sleep while wearing compression stockings to localize fluid rostrally. The intervention induced nuchal fluid accumulation, resulting in an increase in neck circumference (+0.1 cm during control, +0.6 cm during intervention, P< 0.01 ) and a decrease in UA cross-sectional area (-0.08 cm$^2$ during control, -0.43 cm$^2$ during intervention, P = 0.023). Although the intervention did not increase the AHI (control AHI = 19.5, intervention AHI = 30.3, P = 0.249), the AHI during the intervention correlated with age (r = 0.8, P < 0.01). Thus, intravenous saline loading during sleep can narrow the UA, which in older men may induce or worsen OSA. Further studies are needed to test this hypothesis.
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LIST OF ABBREVIATIONS

AASM: American Academy of Sleep Medicine
AHI: apnea-hypopnea index
ARP: acoustic reflection pharyngometry
BMI: body mass index
BP: blood pressure
BQ: Berlin Questionnaire
CIH: chronic intermittent hypoxia
CPAP: continuous positive airway pressure
CS: venous compression stockings
ECG: electrocardiogram
EDS: excessive daytime sleepiness
EEG: electroencephalogram
EMG: electromyogram
EOG: electrooculogram
ESRD: end-stage renal disease
ESS: Epworth Sleepiness Scale
GG: genioglossus muscle
HF: heart failure
HR: heart rate
IV: intravenous
LBPP: lower body positive pressure
LFV: leg fluid volume
LV: left ventricle

MAST: medical anti-shock trousers

NC: neck circumference

NREM: non-rapid eye movement

OSA: obstructive sleep apnea

P_{a}\text{O}_2: arterial oxygen partial pressure

PCO_{2}: carbon dioxide partial pressure

P_{\text{crit}}: critical closing pressure

PSG: polysomnogram

REM: rapid eye movement

R_{UA}: upper airway resistance

RV: right ventricle

S_{a}\text{O}_2: arterial hemoglobin oxygen saturation

TP: tensor palatini muscle

UA: upper airway

UA-XSA: upper airway cross-sectional area

V_T: tidal volume
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1. GENERAL AIMS

There is mounting evidence that accumulation of fluid in the nuchal/peripharyngeal tissues during sleep may predispose to obstructive sleep apnea (OSA), a condition characterized by repetitive collapse of the upper airway (UA) during sleep. Induction of nuchal fluid accumulation in healthy awake subjects induces UA narrowing and increases UA collapsibility and resistance to airflow (1-4). To date, no reported study has sought to induce UA obstruction during sleep in healthy subjects via causing nuchal/peripharyngeal fluid accumulation. Thus, the objective of this thesis was to design and implement a novel method to determine whether intravenous fluid infusion during sleep could induce nuchal and/or peripharyngeal fluid accumulation and thereby cause UA narrowing and/or obstruction.
2. LITERATURE REVIEW

2.1. OBSTRUCTIVE SLEEP APNEA

2.1.1 Definitions

*Upper Airway*

The term “upper airway” is used in the present discussion to refer generally to the pharynx, extending from the nasal turbinates to the glottis. **Figure 1** shows a midsagittal view of the head and neck with various subdivisions of the pharynx. The nasopharynx extends from the nasal turbinates to the hard palate. The retropalatal pharynx extends from the hard palate to the caudal margin of the soft palate. The retroglossal segment of the oropharynx extends from the caudal margin of the soft palate to the epiglottis. The hypopharynx extends from the epiglottis to the glottis. The collapsible segment of the pharynx includes the retropalatal pharynx, the retroglossal segment and the hypopharynx. The oropharyngeal junction, also known as the velum, is the narrow point at which the oral cavity meets the retroglossal segment.
Figure 1: Midsagittal section of the head and neck showing the various subdivisions of the pharynx.
Spectrum of Upper Airway Obstruction Occurring During Sleep

During the transition from wakefulness to sleep, firing of the UA dilator muscles decreases, reducing UA calibre and increasing UA resistance to airflow ($R_{UA}$) (5). Thus, physiological narrowing of the UA is a feature of normal sleep. However, narrowing of the UA at sleep onset can become pathological if it increases $R_{UA}$ to such an extent that it causes partial or complete cessation of airflow (hypopnea and apnea, respectively).

An obstructive apnea is defined as either a $> 90\%$ reduction, or complete cessation, of airflow for $\geq 10$ seconds due to complete collapse of the UA, despite continuing respiratory efforts and movements of the rib cage and abdomen. Similarly, obstructive hypopnea is typically defined as a reduction in tidal volume ($V_T$) to $\leq 50\%$ of baseline for $\geq 10$ seconds due to partial collapse of the UA, despite preserved respiratory efforts and out-of-phase movements of the rib cage and abdomen. In its most recent scoring manual (6), the American Academy of Sleep Medicine (AASM) requires hypopneas to be either accompanied by some degree of arterial oxygen desaturation, typically $\geq 3\%$ or $\geq 4\%$, or terminated by an arousal, but this definition is not applied universally. Among some sleep laboratories, oxygen desaturation or arousals are not part of the definition of hypopneas (7). Although apneas and hypopneas are associated with varying degrees of UA obstruction they have similar adverse consequences (8).

Snoring is defined as a breathing noise produced by vibration of the soft palate, pharyngeal walls, epiglottis and tongue during sleep (9). It is produced by mild narrowing of the pharynx, creating turbulent airflow that vibrates UA tissues. Although non-apneic snoring is typically
associated with an increase in inspiratory effort (10), and may be associated with airflow limitation, it is not associated with a significant decrease in $V_T$ or minute ventilation.

**Obstructive Sleep Apnea**

OSA is characterized by recurrent apneas and/or hypopneas during sleep, and is almost universally accompanied by habitual snoring.

The physiological severity of OSA is expressed as the number of apneas and hypopneas per hour of sleep (apnea-hypopnea index; AHI). The AASM classifies an AHI $< 5$ as normal, an AHI of 5 to $< 15$ as mild OSA, and AHI of 15 to $\leq 30$ as moderate OSA and an AHI of $> 30$ as severe OSA. However, because this classification is arbitrary and is not based on outcome data, it is not accepted universally.

**Obstructive Sleep Apnea Syndrome**

Obstructive sleep apnea syndrome (OSAS) is the presence of polysomnographically-confirmed OSA accompanied by symptoms of sleep apnea (11). For example, the AASM defines OSAS as an AHI $\geq 5$ accompanied by either excessive daytime sleepiness (EDS) or 2 or more of the following: choking or gasping during sleep, recurrent awakenings from sleep, unrefreshing sleep, daytime fatigue or impaired concentration (8).
2.1.2 Epidemiology

Prevalence of OSA in the General Population

In 1993, a landmark study by Young et al. reported the prevalence of OSA within a general, middle-aged American population (12). In the study, over 600 men and women aged 30-60 years, selected randomly from an employee payroll database in Wisconsin, underwent full polysomnography (PSG). From these data, the prevalence of OSA (AHI cutoff $\geq 15$) was reported as 9% in men and 4% in women, i.e. more common within the general adult population than another common condition, asthma (13, 14). This finding has been supported by a more recent study of over 1700 Pennsylvanian subjects aged 20-99 years that reported male and female OSA prevalences of 7% (15) and 2% (16), respectively, and by a study of 400 Spanish subjects aged 30-70 years that reported male and female prevalences of 14% and 7%, respectively (all use AHI cutoff $\geq 15$) (17). When OSA was defined by an AHI $\geq 5$, the above studies reported male prevalences of 17-26% and female prevalences of 9-28% (12, 15-17). In the study by Young et al., the male and female prevalences for symptomatic OSA with an AHI $\geq 5$ (i.e. OSAS) were 4% and 2%, respectively (12).

Despite this high prevalence in the general population it is estimated that 80-90% of those with OSA are undiagnosed (18, 19). Clinicians may be unfamiliar with the clinical presentation of OSA and in some cases may not associate a complaint of excessive daytime sleepiness (EDS) with OSA, particularly if they do not inquire about associated symptoms of OSA such as loud snoring, restless sleep, nocturnal choking or morning headaches. The potential consequences of not recognizing and treating OSA include a greater risk for motor
vehicle and occupational accidents, and for cardiovascular morbidity and mortality than subjects without OSA (20-25).

*Risk Factors for Obstructive Sleep Apnea in the General Population*

A number of factors are associated with increased risk of OSA. The prevalence and severity of OSA increase with increasing body weight, assessed by body mass index (BMI; body weight in kilograms divided by the square of height in metres). Thus, overweight (BMI $\geq 25$ kg/m$^2$) and obese (BMI $\geq 30$ kg/m$^2$) individuals are at increased risk of having OSA than normal weight individuals (12, 26, 27). Other risk factors include male sex, advanced age, increased NC, macroglossia, tonsillar/adenoïdal hypertrophy and craniofacial abnormalities such as retrognathia and micrognathia that reduce the size of the bony envelope surrounding the UA, therefore narrowing it (12, 26, 28-32). Although these risk factors are indeed important, they account for only a small proportion of the variability in OSA severity. For example, increases in BMI and NC, traditionally considered to be the among the most clinically-relevant anatomic predictors of OSA, together account for only one-third of total AHI variability (30-33). Thus, it is highly likely that other factors play a role in the pathogenesis of UA obstruction during sleep and increase the risk of OSA.

*Prevalence of OSA in Fluid Overload States: Renal Failure and Heart Failure*

OSA is exceedingly common among patients with renal failure (i.e. end-stage renal disease; ESRD). Several studies of patients with ESRD have reported prevalences of OSA from 50-80% (34-38). Despite this, OSA appears to be grossly underdiagnosed in this population,
perhaps due to attribution of fatigue and EDS to the underlying renal disease rather than to OSA (36, 37).

Like ESRD, heart failure (HF) is associated with a higher prevalence of OSA than in the general population. Epidemiological studies, using AHI cutoffs of ≥10 and ≥15, have reported that 12-53% of HF patients have OSA (39-42). In contrast to patients with OSA but without HF, who often complain of EDS, HF patients with OSA generally do not complain of EDS, regardless of OSA severity (43, 44). The reason for this has not been determined, but it may account, in part, for the very low rate of sleep apnea diagnosis in the HF population. For example, Javaheri et al. recently reported that only 2% of patients newly diagnosed with HF subsequently underwent PSG (45).

The substantially elevated OSA prevalence among both ESRD and HF populations is not explained adequately by traditional risk factors. For instance, OSA patients with either ESRD or HF typically have lower BMIs than otherwise-healthy OSA patients (40, 44, 46, 47). A factor common to both ESRD and HF is systemic fluid retention (48). Thus, it is possible that fluid retention plays a role in the pathogenesis of OSA in these patients, as will be discussed in a later section (2.2.5).

**Obstructive Sleep Apnea and Risk of Cardiovascular Disease**

For reasons that will be discussed in Section 2.1.4, OSA is associated with elevated risk for a number of cardiovascular diseases, as well as for cardiovascular and all-cause mortality. The largest source of epidemiological data on cardiovascular disease in sleep apnea is the Sleep
Heart Health Study (SHHS), conducted in a diverse American population of approximately 6000 participants aged ≥ 40 years. The SHHS group reported increased adjusted odds ratios for prevalent HF, stroke and coronary artery disease of 2.38, 1.58 and 1.27, respectively, for subjects in the upper versus lower AHI quartile (49). The same group found that, for men with mild-moderate OSA (AHI 5-25), each 1 point increase in AHI was associated with a 6% increase in incident stroke risk (50). This is supported by large-scale cross-sectional and longitudinal analyses of over 2500 subjects conducted by Arzt et al., who reported an adjusted odds ratio of 4.43 for stroke prevalence and an unadjusted odds ratio for stroke incidence of 4.31 in subjects with an AHI ≥ 20 compared to those with an AHI < 5(51).

Additionally, the SHHS group has reported adjusted odds ratios for prevalent nocturnal cardiac arrhythmias including atrial fibrillation and complex ventricular ectopy of 1.74 - 4.02 in subjects with OSA compared to those without it (52). Patients with severe untreated OSA (AHI ≥ 30) were found to have increased odds ratio for prevalent hypertension of 1.37 compared to subjects with an AHI < 1.5 (53). Interestingly, a longitudinal study of nearly 2000 men and 2500 women found that OSA is a significant predictor of both incident coronary heart disease and of incident HF in men but not women (54). The adjusted hazard ratio for all-cause mortality in severe OSA (AHI ≥ 30) compared to no OSA (AHI < 5) has been reported as 1.46 by the SHHS and 3.8 by the Wisconsin Sleep Cohort, the latter group reporting an adjusted hazard ratio for cardiovascular mortality of 5.2 in severe OSA (55, 56).

In a study of over 1600 Spanish men, Marin et al. reported adjusted odds ratios of incident cardiovascular morbidity and mortality of 3.17 and 2.87, respectively, in severe untreated OSA (AHI > 30) (57).
2.1.3 Pathogenesis

Partial or complete UA collapse occurs when the normal physiological withdrawal of UA dilator muscle activity at the transition from wakefulness to sleep is superimposed upon an anatomically narrowed or collapsible UA, giving rise to obstructive hypopneas and apneas (58, 59).

Upper Airway Anatomy in Obstructive Sleep Apnea

Whereas the UA lumen of normal subjects is typically elliptical, with the long axis in the lateral dimension, in OSA patients it is typically either circular or elliptical with the long axis in the anterior-posterior plane (Figure 2) (58). This suggests that impingement of the lateral UA wall is a typical feature in OSA. According to Poiseuille’s law (48), under conditions of constant, laminar flow, a unit reduction in the radius of a tube increases flow resistance to the 4th power. Assuming this applies to the UA of patients with OSA, in order to maintain airflow in the face of this increase in resistance, more negative (subatmospheric) intraluminal pressures must be generated during inspiration by the inspiratory muscles. Unfortunately, during sleep, this more-negative intraluminal pressure increases the tendency of the UA to collapse during inspiration.
Several studies using various imaging techniques have demonstrated that the UA lumen is narrower in patients with OSA than in subjects without OSA. For example, MRI and CT studies, conducted mainly in obese subjects, have demonstrated that UA cross-sectional area (UA-XSA) is reduced in subjects with OSA (60-63). Studies conducted with acoustic reflection pharyngometry (ARP) to determine UA luminal dimensions revealed that, compared to body weight- and age-matched controls, UA-XSA is smaller in OSA patients, and that the UA-XSA of non-obese, non-apneic subjects who snored was reduced to the same degree as in OSA patients who snored (64, 65). This suggests that anatomic UA narrowing is common to non-apneic snorers and snorers with OSA in whom it can promote the partial UA obstruction and turbulent airflow that characterizes snoring and the more complete degree of narrowing that causes partial or complete airflow cessation.

**Figure 2**: Representative illustrations of cross sections of the collapsible segment of the pharynx in subjects without (left) and with (right) OSA. Note the relative lateral impingement and reduction in cross-sectional area in subjects with OSA.
The specific site of initial collapse within the UA may vary between subjects, the most common sites being the retropalatal and retroglossal regions, followed less commonly by the hypoglossal region (58). A study of 17 patients with OSA (AHI ≥ 5) found that the predominant site of UA collapse was repeatable within 14 subjects(66).

Enlarged adenotonsillar tissues can obstruct the UA during sleep, and adenotonsillar hypertrophy is the most common cause of OSA in children (67, 68). Although nasal obstruction, such as that caused by nasal septum deviation or rhinitis, increases R_{UA} and thus may predispose to UA collapse, the contribution of nasal obstruction to the pathogenesis of OSA has yet to be delineated (58). Dysgnathias such as retrognathia and micrognathia cause posterior displacement of tongue and soft palate, thereby narrowing the UA and predisposing to OSA (69, 70).

*Upper Airway Compliance and Passive Collapsibility in Snoring and Obstructive Sleep Apnea*

Critical closing pressure (Pcrit) is defined as the intraluminal pressure at which the UA collapses completely. Passive Pcrit is the Pcrit in the absence of pharyngeal dilator muscle activity. Thus, passive Pcrit is a measure of both extraluminal tissue pressure as well as of the intrinsic, mechanical properties of the UA, such as compliance, which together are components of the overall collapsibility of the UA (71, 72). A number of studies have demonstrated an increased UA compliance/collapsibility in subjects with OSA (73-76). Indeed, a feature of some OSA patients, but not of non-apneic snorers or non-snorers, is a positive (supra-atmospheric) passive Pcrit (58, 71). In the absence of UA dilator muscle
activity, the UA of such patients would collapse, suggesting that peripharyngeal tissue pressure must be relatively high in such individuals (28, 77, 78). Taranto Montemurro and colleagues (71) demonstrated in sleeping subjects a graded increase in passive $P_{\text{crit}}$, from non-snoring subjects, to snoring subjects without OSA to snoring subjects with OSA. Thus, increased UA collapsibility contributes to the pathogenesis of OSA.

Respiratory Cycle Timing of Upper Airway Collapse During Sleep

The human UA has been modeled as a collapsible elastic tube or Starling resistor (79), in which generation of negative intraluminal pressure during inspiration narrows the UA and increases $R_{\text{UA}}$. However, during sleep, UA collapse can occur either at end-expiration or the onset of inspiration (58, 80). Although these two phases of the respiratory cycle are temporally adjacent, the mechanisms of UA collapse in either are conceptually distinct. Collapse during end-expiration, which is thought to occur in the majority of obstructive events (80), suggests a passive state of increased UA collapsibility and thus reflects the supra-atmospheric passive $P_{\text{crit}}$ of many apneics. Here, UA collapsibility becomes so great during sleep that the generation of negative intraluminal pressure is unnecessary for UA collapse. Thus, when the UA exhibits end-expiratory collapse, it cannot be said to behave like a Starling resistor. Conversely, UA collapse during inspiratory onset, when generation of negative intraluminal pressure is highest, reflects a (sub)atmospheric passive $P_{\text{crit}}$. In these instances, negative intraluminal pressure is presumed to drive UA collapse, and thus the UA at these times behaves like a Starling resistor. Studies demonstrate that spontaneous end-expiratory UA collapse is associated with factors such as peripharyngeal adiposity that
increase extraluminal tissue pressure, whereas spontaneous inspiratory narrowing/collapse is more likely to occur in non-obese control animals (32, 81).

Normal Upper Airway Dilator Muscle Activity During Wakefulness and Sleep

In order to draw air into the lung, negative intrathoracic pressure must be generated by respiratory pump muscles during inspiration. This negative pressure is transmitted throughout the respiratory tract, including the UA lumen where it tends to draw the UA walls inward. In most non-human mammals, the UA is supported by the surrounding hyoid bone, which articulates with the styloid processes of the skull (80). This creates a rigid structure that is relatively resistant to collapse when exposed to negative intraluminal pressure. In humans, however, the hyoid bone lacks skeletal articulation and is therefore “free floating”. The mobile hyoid bone in humans is thought to be an adaptation to permit greater mobility of the oropharyngeal structures and allow speech production (82). Thus, the human UA is less rigid and more collapsible when exposed to negative intrathoracic pressure than that of non-human mammals. This tendency to collapse in the face of negative intraluminal pressure is counteracted by the contraction of the UA dilator muscles (59). Contraction of these dilator muscles plays a more important role in maintaining UA patency in humans than in other mammals.

The 2 most-studied UA dilator muscles in humans, owing mainly to their easy accessibility, are the genioglossus (GG), which protrudes the tongue and dilates the oropharyngeal lumen (83), and the tensor palatini (TP), which tenses and raises the soft palate (84). Whereas activity of the TP is mainly tonic, that of the GG is mainly phasic during inspiration (58).
The GG is innervated by the medial branch of the hypoglossal nerve (cranial nerve XII). The hypoglossal motor nucleus, in turn, receives input from the respiratory central pattern generator, from the nuclei such as the locus coeruleus that mediate in the drive to breathe during wakefulness, as well as from the nucleus of the solitary tract, which mediates the reflex response to sudden decreases in UA intraluminal pressure detected by pharyngeal mechanoreceptors (84). Accordingly, during wakefulness, GG activity is increased by hypercapnia, hypoxia and the sudden application of negative pressure to the UA (58, 85-87).

The TP, innervated by the mandibular branch of the trigeminal nerve (cranial nerve V), appears to receive the majority of its input from wakefulness-maintaining neurons and is unresponsive to changes in the partial pressure of carbon dioxide (PCO$_2$) (84), hence its tonic activation during wakefulness. Although the TP is activated by sudden experimental application of negative pressure to the UA (88), it does not, in contrast with the GG, respond to physiological variations in UA intraluminal pressure (89).

The transition from wakefulness to sleep is associated with a number of important physiological changes. During this transition, the wakefulness drive to breathe is lost and central drive is diminished (90). This is associated with transient decreases in GG and TP activity, making UA intraluminal pressure more negative (5). However, GG activity is typically restored to levels similar to wakefulness by the 5\textsuperscript{th} post-transitional breath, probably due to the increase in PCO$_2$ at sleep onset (5, 59). In contrast, TP activity remains depressed and continues to decrease as sleep stabilizes, probably because the main respiratory input to the TP is the wakefulness drive to breathe. The result is that, in subjects without OSA, UA
patency is restored and $R_{UA}$ is only slightly elevated during stable NREM sleep compared to wakefulness (91).

Although the transitional decrease in GG activity is partially mediated by the decreases in central drive and UA intraluminal pressure, this decrease may also occur even after both central respiratory drive and negative pressure reflex have been minimized, suggesting that these do not exclusively mediate GG activity during wakefulness. In a study by Lo et al. (92), non-apneic subjects were placed on positive-pressure ventilation during wakefulness such that negative UA intraluminal pressure was eliminated, GG muscle activity was minimized and central drive was attenuated as evidenced by a cessation in spontaneous ventilation. The ventilated subjects were then allowed to sleep while muscle activities of the GG and TP were measured. Despite sustained attenuation of central drive and abolition of UA intraluminal negative pressure during both wakefulness and sleep, activity in both the GG and TP decreased during the transition from wakefulness to sleep. This reduction in GG activity was sustained during stable NREM sleep and decreased further during rapid eye movement (REM) sleep. The TP exhibited a decrement in muscle activity at sleep onset that was maintained at similar levels throughout stable NREM and REM sleep. Thus, the decrement of UA dilator muscle activity during sleep is not just secondary to withdrawal of chemical respiratory drive or to the increase in $R_{UA}$, but is also to withdrawal of the wakefulness drive to breathe (92).

In some non-apneic subjects, GG activity is almost absent in NREM sleep and can only be elicited by the application of inspiratory resistive loading (89). Despite this, these subjects
can sleep without experiencing obstructive events, suggesting that active muscle contraction is less important than the passive properties of the airway (i.e. calibre and/or compliance) in maintaining UA patency.

Although baseline phasic activity of the GG is typically maintained at wakeful levels during stable NREM sleep under normal conditions, particularly if the UA is relatively narrow/compliant, the reflex responsiveness of both the GG (93, 94) and the TP (85) to sudden application of negative intraluminal pressure is attenuated during NREM sleep. Furthermore, compared to NREM sleep, phasic GG activity is reduced overall by 50% during REM sleep (95), and the GG may experience complete atonia for prolonged periods during this time (96). This is explained by the fact that profound attenuation of skeletal muscle activity (with the exception of the diaphragm) is a characteristic feature of REM sleep (48).

The physiological effects of sleep on baseline and reflexive levels of UA dilator muscle activity help to explain why OSA is a condition that occurs exclusively during sleep (59, 80). They also explain why UA obstruction tends to occur more frequently in REM sleep than in NREM sleep (97).

*Upper Airway Dilator Muscle Activity in Patients with Obstructive Sleep Apnea*

During wakefulness, patients with OSA display levels of UA dilator muscle activity that are approximately 2 to 3 times greater than those of control subjects, presumably in response to higher baseline $R_{UA}$ caused by UA narrowing (98-100). Upon the transition from wakefulness to sleep, however, OSA patients lose this augmentation of UA dilator activity,
such that the net decrease in GG and TP activity at sleep onset is greater than in non-apneic controls (100). However, it is not clear whether this plays a major role in predisposing to UA collapse.

Influence of Extraluminal Factors on Upper Airway Collapsibility

Increased pressure in the tissues surrounding the UA can increase UA transmural pressure and reduce UA-XSA. This concept helps to explain the well-defined associations between obesity, enlarged neck circumference (NC) and OSA. In obese patients, increased amounts of nuchal and peripharyngeal adipose tissue can increase tissue pressure around the UA and reduce UA-XSA. Thus, it is not obesity per se that predisposes to UA collapse, but rather, the accompanying peripharyngeal adiposity (78, 101). Experimental evidence for such a mechanism was provided by Koenig and Thach (102), who conducted mass loading experiments on the UAs of anesthetized and post-mortem supine rabbits. To mimic the necks of obese humans, Koenig and Thatch placed thin, lard-filled bags (10-100g) on the anterior surfaces of the rabbits’ necks, such that NC was increased by 20-50%. Endoscopic examinations of the UA lumen, as well as measurements of $R_{UA}$ and UA collapsibility, revealed that mass loading produced narrowing of the UA in association with increases in $R_{UA}$ and UA collapsibility. However, a bag of lard placed on the anterior surface of the neck is not necessarily an accurate model of human peripharyngeal adiposity, as it does not directly mimic adiposity in tissues lateral to the UA.

Further support for the role of extraluminal tissue pressure in producing UA narrowing comes from a more recent study. In this study (103), a prosthetic orbital tissue expander,
consisting of a compliant silicone balloon with a maximum volume of 3 ml, was inserted subcutaneously beside the tissues surrounding the anterolateral pharyngeal wall in anesthetized rabbits. Submucosal extraluminal tissue pressures were monitored throughout the anterolateral surface, as well as exclusively in the anterior surface, of the pharynx. Graded saline expansion of the prosthesis from 0 to 1.5 ml progressively decreased maximum UA-XSA by approximately 20% and increased $R_{UA}$ by approximately 20%. Interestingly, prosthesis expansion produced more than a 2-fold increase in anterolateral tissue pressure, yet did not significantly alter anterior tissue pressure, suggesting greater compressibility of the UA in the lateral-medial axis than in the longitudinal axis. This study is therefore in agreement with previous studies showing thickening and medial displacement of the lateral pharyngeal walls are important anatomical correlates of OSA (28, 104).

**Role of Peripharyngeal Fluid Accumulation in the Pathogenesis of Obstructive Sleep Apnea**

As will be discussed in Section 2.2 of this literature review, overnight accumulation of fluid in the nuchal/peripharyngeal tissues has recently been demonstrated to play a causative role in the pathogenesis of OSA (105). Fluid sequestered in the capacitance veins of the legs during the daytime due to gravity may translocate to the upper body when subjects assume a recumbent position for sleep. If a significant volume of fluid accumulates in the neck, this may increase extraluminal tissue pressure of the pharynx, predisposing to UA collapse in a manner similar to peripharyngeal adiposity. Indeed, the degree of overnight translocation of fluid from the legs to the neck strongly correlates with degree of OSA severity in sedentary men (105), non-obese men (46), men with heart failure (HF) (47), and subjects with refractory hypertension (106). Moreover, in sedentary men, attenuation of this overnight
Rostral fluid shift by prevention of daytime fluid accumulation in the legs reduces the severity of OSA, suggesting that overnight nuchal/peripharyngeal fluid accumulation can contribute to the pathogenesis of OSA (105).

**Lung Volume Dependence of Upper Airway Cross-Sectional Area**

During inspiration and expiration, UA-XSA increases and decreases, respectively (58). One factor contributing to this lung volume dependence of UA calibre is the caudal displacement of the trachea during inspiration (107). This places traction on the UA, lengthening it, and thereby making it more rigid and increasing its calibre (32, 58). Compared to body weight-matched, non-apneic control subjects, the dependence of UA-XSA on lung volume is greater in both obese (108) and non-obese apneics (65). Moreover, and the degree of lung volume dependence of UA-XSA correlates with OSA severity (109). In obese men with OSA, tonic activity of the diaphragm decreases at sleep onset, leading to a significant decrease in end-expiratory lung volume at sleep onset compared to non-obese, non-apneic controls (110). Thus, a reduction in lung volume at sleep onset may contribute to the increased propensity for UA narrowing/collapse in OSA patients during sleep.

**Age-Related Increase in Upper Airway Collapsibility**

OSA prevalence is considerably higher in middle-aged and elderly adults compared to young adults, and its prevalence increases with age (12, 26, 111, 112). It has been demonstrated that advanced age is associated with increased UA collapsibility in healthy, non-obese men. Worsnop and colleagues (113) examined the activity of UA dilator muscles during wakefulness-to-sleep and sleep-to-wakefulness transitions in 2 groups of healthy, non-obese
men: 9 men aged 20-25 years and 9 men aged 42-67 years. Compared to younger men, older men had greater decreases in UA dilator muscle activity during the wakefulness-to-sleep transition as well as greater increases during the sleep-to-wakefulness transition. Fogel and colleagues (114) later conducted similar studies examining UA dilator muscle activity and $R_{UA}$ during wake-sleep transitioning with and without the application of positive airway pressure in younger (18-25 years) and older (45-65 years) healthy, non-obese men. Compared to younger men, older men had a higher waking $R_{UA}$, which was accompanied by a higher level of UA dilator muscle activity. During the transition from wakefulness to sleep, older men had a greater increase in $R_{UA}$ compared to younger men, which was accompanied by a greater attenuation in UA dilator muscle activity. Interestingly, among older men, application of continuous positive airway pressure (CPAP) during the wake-sleep transition decreased levels of both UA dilator muscle activity and $R_{UA}$ down to those observed in younger men without CPAP. Since dilator muscle activity and $R_{UA}$ are increased in older men, and CPAP-mediated reduction in $R_{UA}$ is associated with a commensurate reduction in dilator muscle activity, the age-related increase in UA collapsibility during wake-sleep transitioning cannot be attributed to a decrease in neuromuscular responsiveness to negative intraluminal pressure. Thus, this observed relative increase in collapsibility likely suggests that normal ageing is associated with anatomic narrowing of the UA, increased UA tissue compliance and/or increased extraluminal tissue pressure (114). Such mechanisms are plausible explanations for at least some of the increase in OSA prevalence with age independent of body weight (26).
Influence of Sex on Upper Airway Anatomy and Physiology

Although UA-XSA, normalized for body size, is similar between men and women, men have a more collapsible UA than women (115, 116). A number of factors have been suggested to explain this increased collapsibility and the subsequent increase in male OSA prevalence, including differences in peripharyngeal adiposity and sex hormone release. However, none of these constitute a conclusive explanation (40, 117). As will be discussed in Section 2.2, these sex differences might be explained at least partially by differences in peripharyngeal fluid dynamics (4).

Reversal of Collapse

OSA is characterized by temporary cessations of airflow during sleep due to UA obstruction, rather than a single, fatal episode of UA obstruction. Accordingly, mechanisms exist to terminate UA obstruction during sleep in order to prevent fatal asphyxiation.

Because of attenuation or cessation of alveolar ventilation during UA obstruction, subjects become acutely hypoxic and hypercapnic. These stimuli both increase central respiratory drive, which in turn increases the activity of respiratory pump muscles in a struggle to inspire against the collapsed UA. These ineffectual inspiratory efforts increase negative intraluminal UA pressure, which contributes further to UA collapse. In addition, inspiratory efforts against a collapsed UA produce large, negative swings in intrathoracic pressure (39). Eventually, the combined effects of hypercapnia, hypoxia and the inspiratory efforts to breathe lead to a transient arousal from sleep (118), accompanied by an abrupt and marked increase in respiratory drive. Although it is not entirely clear as to what degree this increase
reflects a reflex response to either cortical arousal itself or to the related chemical and mechanical stimulation of apnea, the increased respiratory drive causes powerful activation of GG motor units, temporarily resolving obstruction, allowing restoration of airflow (119-121).

Neuromuscular reversal of UA obstruction is therefore associated with transient cortical arousal as a key defense against fatal asphyxiation. Some researchers, however, contend that cortical arousal is not a necessary component of obstructive event resolution (122, 123). However, this is a controversial concept and constitutes a minority view. The great majority of obstructive apneas and hypopneas are terminated by an arousal, and abolition of OSA by, for example, CPAP, invariably reduces the frequency of arousals, indicating that such arousals are caused by obstructive events (124, 125).
2.1.4 Pathophysiological Effects

*Excessive Daytime Sleepiness*

Recurrent arousal from sleep associated with respiratory disturbances can fragment sleep markedly, reducing sleep time and quality. Accordingly, EDS is an important, but not universal symptom of OSA symptom, particularly in patients without stroke or HF (44, 126-130). EDS is differentiated from fatigue, in that *sleepiness* refers to a subject’s difficulties in appropriately maintaining wakefulness throughout the day, whereas *fatigue* refers to a subject’s subjective deficits in physical and mental energy that often are not associated with sleepiness, although patients may use the terms interchangeably (131). The societal costs of EDS are high, as the presence of OSA increases likelihood for motor vehicle and occupational accidents and decreased occupational performance (23-25).

*Cardiovascular Sequelae*

OSA produces a number of adverse effects on the cardiovascular system that, over years, may contribute to the development of cardiovascular disease. The adverse effects can be divided into chemical, mechanical, autonomic and oxidative/inflammatory disturbances and their consequences.

*Chemical Effects*

The waxing and waning of alveolar ventilation caused by apnea and hypopnea produces a pattern of chronic intermittent hypoxia (CIH), which, in turn, contributes to the mechanical, autonomic and oxidative/inflammatory disturbances of OSA.
Mechanical Effects

CIH induces hypoxic pulmonary vasoconstriction that increases pulmonary artery pressure and thereby increases right ventricular (RV) afterload. Ineffectual inspiratory efforts against the occluded UA exaggerate normal inspiratory negative intrathoracic pressure swings (132). By increasing the difference between intra-ventricular and intrathoracic pressure, exposure of the left ventricle (LV) to these negative pressure swings increases LV transmural pressure and thus afterload. Additionally, exposure of the thoracic aorta to negative intrathoracic swings may promote aortic expansion (133) and/or dissection (134). Negative intrathoracic pressure also increases the pressure gradient for venous return from the peripheral vessels outside the thorax to the right heart, thus increasing preload of the RV (132). Subsequent distension of the RV, coupled with the increase in RV afterload from hypoxic pulmonary vasoconstriction, can induce tricuspid regurgitation (135), which may cause peripheral edema. Moreover, the increases in RV preload and afterload can cause a leftward shift of the intraventricular septum during diastole (132). This impedes LV filling, thereby reducing LV end-diastolic volume, stroke volume and cardiac output (136, 137).

Autonomic Effects

Each of hypoxia, hypercapnia, reduced cardiac output and arousal from sleep increase sympathetic nervous firing rate at the end of each apnea (39). This, in turn, causes recurrent elevations in BP and heart rate (HR) that increase further the mechanical load on the LV (39, 138, 139). The combination of reduced myocardial oxygen delivery due to apnea-related hypoxia and increased myocardial oxygen demand can lead to myocardial ischemia in the presence of flow-limiting coronary arterial lesions (140, 141). Furthermore, chronic exposure
of the myocardium to high levels of norepinephrine can stimulate myocyte hypertrophy, apoptosis and necrosis (142-145). Thus, OSA exerts several adverse effects on ventricular structure and function that can ultimately contribute to the development of HF (22).

Increased sympathetic activation caused by hypoxia in the systemic circulation during sleep may induce persistent systemic arterial vasoconstriction and diurnal hypertension, perhaps due to augmentation of peripheral chemoreflex sensitivity or upregulation of central sympathetic outflow (146-150). Indeed, approximately 40% of OSA patients are hypertensive (151).

Arousals from sleep cause recurrent withdrawal of efferent parasympathetic (i.e. vagal) outflow to the heart, leading to surges in HR at the termination of apnea (139). The combination of right and left atrial stretch due to negative intrathoracic pressure, CIH, sympathetic activation and vagal withdrawal can trigger atrial fibrillation (152, 153). Ventricular arrhythmias may also occur in response to increased RV and LV afterload, sympathetic activation, vagal withdrawal and myocardial ischemia. Indeed, a large-scale observational study found patients with OSA to be at significantly greater risk for ventricular tachycardia and ventricular ectopy than appropriately-matched controls (52). Similarly, in an observational study of 283 patients with HF, those with untreated sleep apnea (n = 113) were at greater risk for malignant ventricular arrhythmia than those with no/mild sleep apnea breathing (n = 170) (154). Ventricular arrhythmias appear to be triggered by apnea and hypopnea, as arrhythmias are approximately 18 times more likely to occur following such events than during normal breathing in subjects with sleep apnea (155). Additional causative
evidence has been generated by a randomized controlled trial involving 18 HF patients, demonstrating that treatment of OSA by CPAP for 1 month reduced the frequency of ventricular premature beats during sleep by 58% (156).

Oxidative/Inflammatory Effects

The CIH of OSA can promote oxidative stress and a pro-atherosclerotic systemic inflammatory response similar to that induced by ischemia/reperfusion injury (157). Markers of systemic inflammation, including matrix metalloproteinase-9, C-reactive protein, interleukin-6, interleukin-18 and tumour necrosis factor-α, are elevated in the serum of OSA patients compared to weight-matched controls (158, 159). Increased carotid intima-media thickness (CIMT) and decreased pulse transit time, both indices of early atherosclerosis, are present in patients with OSA and correlate with the AHI and degree of apnea-related hypoxia (159, 160). Similarly, in patients with stable coronary artery disease, the AHI is shown to correlate with coronary atherosclerotic plaque volume, as assessed by 3-dimensional intravascular ultrasound (161). Treatment of OSA by CPAP reduces carotid intima-media thickness and increases pulse transit time (162).

Additionally, oxidative stress can promote hypertension in OSA patients through impairment of nitric oxide-dependent vasodilation via damage to the vascular endothelium (157, 163). In turn, hypertension also appears to promote carotid atherosclerosis: Drager et al. (164) found that carotid intima-media thickness is increased independently by either OSA or hypertension and that the coexistence of both conditions is associated with an additive increase in carotid intima-media thickness.
Regardless of its cause, the increase in carotid atherosclerosis with OSA may be reflected as an increase in stroke risk, as the presence of carotid atherosclerosis correlates well with the presence of intracranial atherosclerosis (165). Indeed, moderate-to-severe OSA is associated with a 3-4 fold increased risk of stroke, and the prevalence of OSA is 4-6 fold greater in stroke patients compared to the general population (166, 167).
2.1.5 Screening and Diagnosis

Clinical Presentation

In addition to the risk factors mentioned in the previous section, there are a number of symptoms commonly associated with OSA. Patients often present to clinic with complaints of EDS, recurrent awakenings from sleep, unrefreshing sleep, morning headaches, daytime fatigue and/or impaired concentration (8, 125, 131). Additionally, patients (or their bed partners) may report habitual snoring, choking, gasping or apneas during sleep (8, 125).

Assessment of Oropharyngeal Crowding

Oropharyngeal crowding can be associated with OSA and can be assessed using the Mallampati Scale. The Mallampati Scale was originally devised to predict the degree of difficulty in endotracheal intubation by visual assessment of oropharyngeal structures (168). The Mallampati Scale ranges from 1 to 4; a score of 1 indicates full visibility of the tonsillar pillars, uvula, fauces and soft palate, 2 indicates visibility of the uvula, fauces and soft palate, 3 indicates visibility of the soft palate and the base of the uvula and 4 indicates no visibility of any of these structures, with only the hard palate visible (169). Mallampati Scale score correlates with the presence and severity of OSA, such that every 1 point increase in Mallampati Scale score is associated with both a 2.5-fold increased odds for having OSA as well as a 5-unit increase in AHI (29).
**Screening Questionnaires**

Due to considerations of cost, availability and time, the use of overnight PSG to diagnose OSA may not be a realistic routine option in many health care settings. Where access to PSG is limited, screening procedures have been used to assess the likelihood of OSA, so that only those with a high likelihood of having OSA go on to complete a PSG. Questionnaires have been developed to assess the likelihood of a person having OSA, such as the Epworth Sleepiness Scale (ESS) (170), Berlin Questionnaire (BQ) (171) and others (172-174).

Using a scale of 0-24, the ESS assesses self-reported propensity to fall asleep under several conditions commonly encountered in daily living (170) (Appendix 1). A score of 0 represents the least tendency toward sleepiness, whereas a score of 24 represents the greatest tendency; scores > 10 are considered to indicate clinically-significant EDS (170, 175). Among otherwise-healthy OSA patients, there is a modest but significant correlation between ESS score and AHI (170, 175).

The BQ was designed to predict the presence of OSA within the general population (171) (Appendix 2). The BQ is a self-administered questionnaire that assigns a score of 0-3 based on BMI as well as responses to categories of questions regarding history of snoring, daytime sleepiness and hypertension. Patients with scores of 0 or 1 are said to be at low risk of OSA, whereas those with scores of 2 or 3 are said to be at high risk. In evaluating its value against unattended, at-home cardiorespiratory monitoring in a population of 100 primary care patients, the BQ was demonstrated to predict an AHI > 5 with 86% sensitivity and 77% specificity (171).
**Diagnosis**

The standard means of diagnosing OSA is overnight, in-laboratory PSG, attended by a sleep technician (125). PSG consists of electroencephalography (EEG), electrooculography (EOG), submental and anterior tibial electromyography (EMG), air flow, respiratory movement, arterial hemoglobin oxygen saturation (SaO₂) and electrocardiography (ECG). Several variables can be derived from these physiological signals, including wake and sleep time, sleep stages, arousals, the number, frequency and distribution of apneas and hypopneas and the degree of oxygen desaturation.

From PSG data, the physiological severity of OSA can be measured and expressed via the AHI. Sleep laboratories that use AASM criteria classify an AHI of 5 to < 15 as mild OSA, an AHI of 15 to ≤ 30 as moderate OSA and an AHI of > 30 as severe OSA. However, sole consideration of the AHI is insufficient, as clinical evaluation of patients’ symptoms is critical to the diagnosis of OSA.
2.1.6 Treatment

*General Strategies for Treatment of Obstructive Sleep Apnea*

Due to the association between OSA and peripharyngeal adiposity, weight reduction is recommended routinely in overweight and obese OSA patients. Additionally, OSA patients are advised routinely to avoid consuming ethanol or sedative medication before bedtime, as these increase the duration and frequency of obstructive events, likely by dampening genioglossal activity and/or prolonging the time to arousal following obstruction (176-180). In patients with mild OSA, successful implementation of these lifestyle modifications may be sufficient to reduce AHI to < 5 (181). However, substantial weight loss may be difficult to achieve by diet and exercise alone. In addition, patients with moderate-to-severe OSA often require additional treatment(s) to manage this condition.

*Continuous Positive Airway Pressure*

CPAP is the standard form of therapy for most patients with OSA. CPAP delivers pressurized room air to the UA via a nasal or oronasal mask, pneumatically splinting open the UA to prevent its collapse. When titrated to the appropriate pressure, CPAP eliminates apnea, hypopnea, snoring, CIH, exaggeratedly-negative intrathoracic pressure swings and bursts of sympathetic nervous system activity caused by OSA (182-185). Accordingly, CPAP use reduces immediately symptoms of EDS (184, 186-188). The degree of symptom reduction correlates directly with nightly hours of CPAP use (189). Moreover, CPAP use greatly reduces the frequency respiratory arousals, consolidates sleep and increases slow-wave sleep and REM sleep time without decreasing totals sleep time (186, 190-194). Randomized controlled trials reveal that, in addition to improving subjective and objective
measures of daytime sleepiness, regular CPAP use also improves significantly quality of life and cognitive function (195, 196). Additionally, observational studies report that OSA patients on long-term CPAP therapy have lower rates of overall and cardiovascular mortality compared to non-treated patients (21, 197-199).

**Oral Appliances**

An oral appliance that advances the mandible or protrudes the tongue may also be prescribed to treat OSA and/or snoring. These are mouthguard-style devices that allow the mandible to rest passively at a more anterior displacement by anchoring against the upper teeth. Such advancement of the lower jaw is thought to help prevent posterior relapse of the tongue as well as to increase radial traction on the UA wall. Initially, it was presumed that this appliance’s effects were purely anatomic, because advancement of the mandible increases UA size and decreases the static compliance of the pharynx (200). However, mandibular advancement also augments GG, geniohyoid and masseter muscle firing in awake subjects (201) as well as masseter and submental muscle firing in sleeping subjects (202). The pharyngeal section in which cross-sectional area is increased in response to mandibular advancement may vary between patients, affecting either the retroglossal and/or retropalatal areas of the pharynx (203-205). There are a number of different styles of oral appliances, including off-the-shelf models made of thermosensitive materials that patients mold to their own teeth at home, as well as appliances that are custom made by dentists. Custom-made appliances often carry the advantage of being adjustable, so that the anterior displacement of the mandible can be titrated gradually without having to purchase another appliance. The efficacy of oral appliances in treating OSA has not been adequately assessed via a
randomized controlled trial. A large-scale meta-analysis conducted by Hoffstein concluded that oral appliances have a success rate of approximately 50% for reducing the AHI to < 10 (200). The same analysis found a mean reduction in AHI of 42% with oral appliance therapy, compared to a 75% reduction with CPAP. Accordingly, although its efficacy is considerably less than that of CPAP mandibular advancement is a potential alternative for patients with mild-to-moderate OSA who do not tolerate CPAP.

**Postural Therapy**

In some patients, OSA occurs primarily or exclusively while sleeping in the supine position, probably because this position facilitates relapse of the tongue against the posterior pharyngeal wall. In such patients, avoidance of sleeping supine may be an effective therapy for OSA. Strategies to avoid sleeping supine include sewing a tennis ball into the back of a shirt, wearing commercially-available garments with a ball(s) or cylinder(s) sewn in the back, or using an apparatus consisting of a vest fastened to a board placed under a pillow that makes sleeping supine difficult or impossible (206). In a randomized crossover trial in 13 patients with supine-related OSA comparing 2 weeks use of a backpack with a ball in it to CPAP, the backpack eliminated supine sleep and caused a reduction in the AHI similar to that observed with CPAP (207).

**Venous Compression Stockings**

Venous compression stockings (CS), which increase extravascular tissue pressure of the calf and thereby decrease dependent fluid accumulation in the legs, have been suggested recently as a novel treatment for OSA in sedentary patients (105). As will be discussed in Section
2.2.6, prevention of daytime sequestration of fluid in the legs, and thus of subsequent translocation of fluid from the legs to the neck overnight, can reduce the severity of OSA in sedentary subjects.

**Surgery**

Uvulopalatopharyngoplasty and other surgeries that excise portions or all of the uvula and soft palate aim to treat OSA and/or snoring by increasing UA luminal size or by inducing localized fibrosis, stiffening UA soft tissue structures that are vulnerable to collapse. The effectiveness of these surgeries is lower than that of CPAP: although uvulopalatopharyngoplasty has yet to be evaluated in a randomized clinical trial, Hoffstein’s meta-analysis found this surgery to cause a mean reduction in AHI of 30%, compared to a 75% reduction with CPAP (200). In a follow-up questionnaire completed by nearly 200 patients who received uvulopalatopharyngoplasty or a similar surgery, only 37% reported improvement in sleep quality following the surgery, and just 34% claimed to benefit from a reduction in snoring that was sustained for over 2 years (208). Less than half of all subjects claimed they would choose the surgery again if given the chance.
2.2 ROSTRAL FLUID SHIFT

2.2.1 Introduction
Although obesity, peripharyngeal adiposity and large NC are risk factors for OSA, the majority of subjects with OSA are not obese (26). Additionally, measures of body habitus and NC together account for only one-third of the variability in AHI between OSA patients (30-33). Accordingly, factors other than body habitus and NC must play a role in the pathogenesis of OSA. One such factor may be fluid accumulation in the neck and peripharyngeal tissues.
2.2.2 Relationship of Peripharyngeal Edema to Obstructive Sleep Apnea

In 1990, Wasicko and colleagues (209) studied 9 anesthetized, paralyzed and vagotomized cats in which the UA was surgically isolated. Intravenous (IV) administration of vasodilator agents (paparavine or sodium nitroprusside) increased UA collapsibility by 30% (quantified by an increase in Pcrit from baseline). This increase in collapsibility was accompanied by a decrease in UA-XSA, assessed by MRI. In 3 of these cats, the investigators generated static pressure-volume curves by manipulating the volume of a syringe connected to the caudal end of the UA. The curves were similar under both the control and vasodilator conditions, indicating that pharyngeal vasodilation likely increased UA collapsibility as a result of peripharyngeal fluid accumulation, rather than an increase in tissue compliance. The authors speculated that mucosal congestion may be an important mechanism facilitating and exacerbating OSA (209).

Subsequently, these investigators demonstrated in healthy men that topical application of the vasoconstrictor phenylephrine to the UA mucosa decreased $R_{UA}$ (210). However, 3 aspects of this study limited its relevance to the pathogenesis of OSA. First, a pharmacologic intervention was required to induce changes in $R_{UA}$. Second, subjects were studied while awake and seated, rather than while asleep and recumbent. Third, only healthy subjects without OSA were studied. Nevertheless, these findings were important because they suggested that changes in peripharyngeal fluid content, in this case due to vasodilation or vasoconstriction, can alter UA morphology and mechanics.
Anastassov and colleagues analyzed uvulopalatal surgical specimens from 5 OSA patients and found evidence of stromal and subepithelial edema, along with increased vascularity, vascular dilation and vascular congestion (211). However, they were unable to determine whether UA edema was a cause, or a consequence, of OSA. For example, edema could be secondary to inflammation of the UA soft tissues in OSA, caused by local vibratory trauma from snoring and by systemic release of inflammatory mediators from CIH. Moreover, UA mucosal water content as assessed by MRI is significantly decreased in OSA patients following chronic CPAP treatment, suggesting that treating OSA reduces UA edema (212).
2.2.3 Experimentally-Produced Rostral Fluid Shift Studies
Shepherd and coworkers were the first to examine the possible effect of shifting fluid from
the legs to the upper body on UA properties in subjects with OSA (213). With subjects
supine, they evaluated changes in UA-XSA by computed tomography in response to 1) raising the legs to displace fluid into the upper body, and 2) occluding thigh veins with blood
pressure cuffs inflated to 40 mm Hg to reduce venous return to the right heart and reduce
venous volume of the neck. However, they did not measure indices of fluid displacement
from the legs or of fluid movement into the neck, and therefore could not determine whether
they were successful in doing so. Although there were tendencies for leg raising and thigh
vein occlusion to decrease and increase UA-XSA, respectively, these were not statistically
significant. This may have been due either to an inability of either intervention to alter neck
or peripharyngeal fluid volume or to inadequate sample size.

Subsequently, Chiu et al. (1) evaluated the effect on \( R_{UA} \) of rostral fluid shift in non-obese,
healthy subjects by inflating medical anti-shock trousers (MAST) to shift fluid from the legs
to the neck. The MAST extended from ankles to the upper thighs and were inflated to a
pressure of 40 mm Hg. Chiu and colleagues employed a randomized double cross-over study
design to compare this application of lower body positive pressure (LBPP) for 5 minutes to a
5-minute control period, during which the MAST were not inflated. Leg fluid volume (LFV)
of one leg and NC were measured via bioelectrical impedance and mercury strain gauge
plethysmography, respectively. Pressure-sensitive catheters were placed in the nasopharynx
and oropharynx, and transpharyngeal pressure was calculated as the difference between these
two pressures. Airflow was measured by a pneumotachygraph fitted into an airtight facemask,
and \( R_{UA} \) was calculated by dividing transpharyngeal pressure by airflow. Measurements of
LFV, NC and $R_{UA}$ were made at baseline and then after 1 and 5 minutes of the control and LBPP periods. Application of LBPP produced a mean decrease in LFV of one leg of 170 ml. Assuming equal fluid displacement from both legs, a total of approximately 340 ml was displaced. NC increased significantly from baseline by 0.3% and 0.22% after 1 and 5 minutes of LBPP, respectively. This was accompanied by a 40% increase in $R_{UA}$ after 1 minute. This increased further to 100% after 5 minutes of LBPP. Because UA-XSA and $R_{UA}$ are lung-volume dependent, it was possible that LBPP induced an increase in lung water which could have reduced lung volume and this induced an increase in $R_{UA}$. To rule out this possibility, Chiu and colleagues assessed total lung capacity by helium dilution before and after application of LBPP in a subset of the subjects. They found that LBPP did not induce any change in total lung capacity and, therefore, that the LBPP-induced increases in $R_{UA}$ could not be attributed to a decrease in lung volume. Subsequently, Shiota and colleagues, using the same protocol except that UA-XSA measured by ARP was assessed instead of $R_{UA}$, found LBPP caused a significant 9% decrease in UA-XSA (2). Thus, narrowing of the UA was the probable cause of the previously-described increase in $R_{UA}$ (1). Subsequently, Su and colleagues conducted 2 studies investigating the change in UA collapsibility with LBPP assessed by Pcrit during wakefulness (3, 4). In their first study (3), they found that the previously-employed LBPP protocol increased Pcrit compared to control in 13 healthy men. In their following study, they compared the effects of LBPP on Pcrit between 14 healthy men and 13 healthy women. Although LBPP caused similar changes in LFV and NC in both men and women, Pcrit increased significantly in men but was unchanged in women, suggesting a different response between men and women to rostral fluid shift.
Although these LBPP studies demonstrated that experimentally-induced rostral fluid shift could increase NC, $R_{UA}$ and $P_{crit}$, and reduce UA-XSA, they were performed during wakefulness under artificial conditions, and thus it remained unclear whether these results could be extrapolated to sleep under spontaneous conditions.
2.2.4 Relationship Between Spontaneous Overnight Rostral Fluid Shift and Obstructive Sleep Apnea

*Healthy, Non-Obese Men*

Redolfi and colleagues were the first to evaluate whether fluid was displaced spontaneously from the legs overnight, and whether the volume displaced was related to the severity of OSA (46). They studied 23 non-obese, otherwise healthy men who were referred for diagnostic PSG because of a clinical suspicion of OSA. Immediately before and after the PSG, LFV and NC measurements were made, via bioelectrical impedance and tape measure, respectively, with subjects in the supine position. Prior to the PSG, subjects also filled in a questionnaire to document the time spent sitting, standing and lying down each hour from the time they awoke in the morning to the time they visited the sleep laboratory. The experimenters made several important observations. First, LFV decreased spontaneously overnight (mean $\Delta$LFV of one leg = -133 ml). Second, the decrease in LFV was accompanied by a mean increase in NC of 1.0 cm and the $\Delta$NC was inversely proportional to $\Delta$LFV ($r = -0.79$, $P < 0.001$). Third, the AHI correlated strongly with the overnight $\Delta$LFV according to an inverse exponential relationship ($r = -0.802$, $P < 0.001$). Moreover, a similarly strong inverse correlation was observed between $\Delta$LFV and the overnight change in NC, indicating that some of the fluid that left the legs overnight translocated into the neck. Multivariable analysis demonstrated that the overnight changes in NC and LFV were independent correlates of the AHI, and that these 2 variables together explained 68% of the AHI variability between subjects. Fourth, $\Delta$LFV correlated strongly and inversely with the amount of time that subjects reported sitting during the day preceding the PSG ($r = -0.588$, $P < 0.01$). This suggested that, in non-obese men, daytime sequestration of fluid in the lower
extremities, promoted by prolonged sitting with insufficient calf muscle activity to prevent fluid accumulation in the legs, was related to the degree of overnight fluid displacement from the legs and, subsequently, to OSA severity. However, these observations did not establish a causal relationship between overnight rostral fluid shift from the legs OSA.

**Men with Heart Failure**

Subsequently, Yumino et al. extended this concept to the study of 57 men with HF (ejection fraction ≤ 45%) (47). LFV and NC were measured immediately before and after PSG. On the basis of PSG results, subjects were classified as obstructive-dominant (≥ 50% of apneas and hypopneas were obstructive; n = 35) or central-dominant (> 50% of apneas and hypopneas were central; n = 22). Subjects in obstructive- and central-dominant groups were classified as having OSA or CSA, respectively, if their AHI was ≥ 15. Overnight, the obstructive-dominant group experienced a ΔLFV of -173 ml (one leg), a ΔNC of +0.7 cm and an AHI of 27. There were strong inverse relationships between ΔLFV and ΔNC (r = -0.78, P < 0.001) and between ΔLFV and AHI (r = -0.88, P < 0.001), but there was no relationship between AHI and mean transcutaneous PCO$_2$ during sleep in the obstructive-dominant group. The central-dominant group experienced ΔNC and AHI but had twice as great a decrease in LFV as the obstructive-dominant group. The relationships observed between ΔLFV and ΔNC and between ΔLFV and AHI were similar to those observed in obstructive-dominant group. However, in contrast to the obstructive-dominant group, there was an inverse linear relationship between ΔLFV and mean transcutaneous PCO$_2$ during sleep in the central-dominant group. In all subjects, the magnitude of ΔLFV was related not only to the severity of sleep apnea, but also to the type, with increasingly greater decreases in LFV corresponding
to no/mild sleep apnea (AHI < 15, mean ∆LFV = -100 ml), to OSA (AHI ≥ 15, mean ∆LFV = -235 ml), and to central sleep apnea (AHI ≥ 15, mean ∆LFV = -400 ml). Thus, the authors of this study came to the conclusion that overnight rostral fluid shift may be considered a unifying concept for the pathogenesis of both obstructive and central sleep apnea in HF. In patients with OSA, a proportion of the fluid that displaced from the legs reached the neck promoting UA obstruction. In patients with central sleep apnea, a similar volume of fluid accumulated in the neck, as evidenced by the similar increase in NC. However, because the overnight ∆LFV was twice that of patients with OSA, part of the excess fluid must have redistributed elsewhere. The observation that ∆LFV was inversely related to transcutaneous PCO₂ suggests that some of the excess fluid may have accumulated in the lungs, activating pulmonary receptors, augmenting respiratory drive and causing PCO₂ to decrease.

In patients with OSA, it is possible that generation of negative intrathoracic pressure could draw blood from the legs into the thorax, increasing RV filling pressure and thus distending the neck veins. If so, fluid shift from the legs to the neck could be secondary to OSA, rather than a cause of OSA. Therefore, to test the hypothesis that rostral fluid shift from the legs was a primary phenomenon, and not secondary to OSA, in the same study Yumino et al. (47) also evaluated the effect of CPAP on ∆LFV, ∆NC and AHI in 20 of the obstructive-dominant patients with an AHI ≥ 15. CPAP decreased the AHI (from 38 to 12, P < 0.001) and attenuated the overnight increase in NC (from 0.9 cm to 0.2 cm, P< 0.001), but it did not significantly alter ∆LFV. Because ∆LFV was unaffected by CPAP, overnight rostral fluid shift cannot be secondary to the apnea-related generation of negative intrathoracic pressure, since CPAP abolishes such negative intrathoracic pressure (39). Moreover, the observation
that CPAP attenuated the increase in NC suggests that another mechanism of action by which CPAP maintains UA patency and alleviates OSA, besides by acting as a pneumatic splint, is by preventing fluid accumulation in the neck. This mechanism is consistent with findings of a study that showed that in patients with OSA, but without HF, chronic CPAP use was associated with a reduction in peripharyngeal edema and an increase in UA-XSA (212).

Subjects with Hypertension

Friedman et al. (106) evaluated the relationship between spontaneous overnight rostral fluid shift and OSA severity in men and women with either controlled (n = 15) or drug-resistant (n = 25) hypertension. Subjects with drug-resistant hypertension exhibited a larger overnight ∆LFV (-347 ml versus -176 ml; P < 0.001), a greater increase in NC and a higher AHI. When both groups of subjects were considered together, a strong, exponential relationship was found between ∆LFV and the AHI (r = -0.75, P < 0.0001). Moreover, there was no significant association between the AHI and BMI or baseline NC, suggesting that the degree of overnight rostral fluid shift played a more important role than body habitus in the pathogenesis of OSA in these hypertensive subjects.

Thus, in healthy, non-obese men, men with HF and subjects with hypertension, a strong relationship exists between overnight rostral fluid shift and OSA severity. However, since the studies that revealed such relationships were all observational, they did not establish a causative role for overnight rostral fluid shift in the pathogenesis of OSA.
2.2.5 Probable Relationship between Overnight Rostral Fluid Shift and OSA Prevalence in Men and Fluid Overloaded Patients

Men
The prevalence of OSA is 2-3 times higher in men than in women (12, 13, 26, 40). A number of explanations have been proposed. For instance, the visceral/centripetal pattern of adipose distribution found typically in men is thought to contribute more to peripharyngeal adiposity than does the subcutaneous pattern of adipose accumulation in the hips and thighs found typically in pre-menopausal women (58). Also, the collapsible segment of the UA appears to be significantly longer in men than women, even when normalized for body height, suggesting a morphological predisposition of the UA to collapse in men (214). Despite the plausibility of these explanations, none explain conclusively the much higher prevalence of OSA in men (40, 117). Another possible explanation for the higher prevalence in men is a differential response to rostral fluid shift, as first suggested by Su et al.’s findings that LBPP-induced rostral fluid shift increased Pcrit in men but not in women (4).

In another study, conducted in men and women with HF, Kasai and colleagues (117) measured LFV and NC immediately before and after PSG. They showed that, although the magnitude of overnight LFV reduction was similar in men and women (-154 ml versus -161 ml, P=0.547), the overnight increase in NC was much greater in men than in women (1.0 cm versus 0.2 cm, P < 0.001). Furthermore, whereas in men the ΔLFV correlated strongly with increases in both NC (r = -0.771, P<0.001) and with AHI (r = -0.737, P<0.001), no such relationships existed in women. This suggests that, in men, a substantial portion of the overnight fluid movement out of the legs is redistributed into the neck where it can narrow
the UA and contribute to the pathogenesis of OSA. Conversely, in women, a smaller proportion of the overnight movement of fluid out of the legs accumulates in the neck and bears no relationship to OSA severity. These findings also imply that a substantial proportion of the fluid displaced from the legs overnight is sequestered below the neck in women, possibly in the abdominal and/or pelvic capacitance vessels. If so, such substantial fluid sequestration may be one mechanism preventing women from developing OSA as often as men. Compared to men, women have much larger gonadal veins (215) that could act as a fluid reservoir, particularly during pregnancy when women develop fluid retention and leg edema. Fluid sequestration in the pelvic veins, and possibly other capacitance vessels, could prevent fluid movement in the neck and might prevent OSA. This hypothesis merits testing in further studies.

Edematous patients
The prevalence of OSA is substantially higher in patients with HF and ESRD than in the otherwise healthy population (36-41, 117). A common feature of both HF and ESRD is dependent fluid retention (48). Accordingly, a possible explanation for the elevated prevalence of OSA in these patients is that excessive fluid retention predisposes to greater overnight rostral fluid shift into the neck and peripharyngeal tissues, leading to higher rates of UA obstruction during sleep, and thus of OSA. Indeed, such an explanation is supported strongly by the current literature.

As already mentioned, OSA severity has been shown to correlate strongly with overnight ∆LFV in men with HF (47, 117). Moreover, in patients with diastolic HF, intensive fluid
unloading with IV diuretics decreased AHI and increased UA-XSA, respectively, by approximately 25% (216).

Similarly, a few studies have examined the effect of targeted overnight fluid removal via nocturnal dialysis on OSA severity in ESRD patients. Hanly and Pierratos (38) performed PSG in ESRD patients who were on conventional thrice-weekly hemodialysis before, and then at least 6 weeks after patients converted to nightly nocturnal hemodialysis. The mean AHI dropped from 25 on conventional to 8 once on nocturnal hemodialysis. Similarly, Tang et al. (217) demonstrated that nocturnal cycled peritoneal dialysis in ESRD patients with severe OSA significantly reduced AHI in association with a greater reduction in total body water at night compared to 24-hour ambulatory peritoneal dialysis. Thus, the higher prevalence of OSA in ESRD patients appears to be at least partially related to fluid retention, particularly at night. However, since both of the above studies were not randomized, but rather, were before-and-after studies, a causative role of fluid retention in OSA has yet to be demonstrated conclusively in ESRD patients.

Ifitkhar and colleagues published a retrospective analysis (218) of PSGs from 378 patients with severe OSA but without a history of renal, pulmonary or hepatic disease or of HF. Thirty-five percent of the patients had significant edema, defined as the presence of \( \geq 1+ \) edema on a scale of none, trace, 1+, 2+ or 3+ assessed during a physical examination. Furthermore, after correcting for age, BMI and presence of hypertension and/or diabetes mellitus, the AHI was 46% higher in edematous subjects than in non-edematous subjects. However, because they did not measure fluid volumes in various body segments, they were
unable to determine the cause of this relationship. Taken together, the above studies suggest that fluid retention -- regardless of etiology -- and its overnight rostral displacement are related to the presence and severity of OSA.
2.2.6 Effect of Venous Compression Stockings on Overnight Rostral Fluid Shift and Severity of Obstructive Sleep Apnea

To determine whether overnight rostral fluid displacement from the legs contributes to the pathogenesis of OSA, Redolfi and colleagues (105) studied the effects of wearing CS on overnight ∆LFV and AHI in 6 non-obese (BMI < 30 kg/m²) sedentary men (i.e. they reported sitting for at least 10 hours per day). After diagnosis of OSA (AHI ≥ 15) through clinical PSG, subjects underwent repeat overnight PSG, before and after which LFV and NC were measured. After waking up in the sleep laboratory the next morning, subjects donned off-the-shelf, below-the-knee CS designed to apply a pressure of approximately 20 mm Hg. They wore them the entire day, and then returned to the sleep laboratory at night and removed the CS just before a repeat PSG was performed. LFV and NC were again measured before and after PSG. By compressing the capacitance vessels of the legs and increasing hydrostatic tissue pressure, CS prevent accumulation of fluid into the capacitance vessels of the calf and transudation of capillary fluid to the interstitial tissues of the legs. Thus, they counteract gravity-dependent fluid accumulation within the legs (219).

 Compared to the baseline values, daytime CS use decreased the mean pre-sleep values for ankle circumference by 4-6%, and the mean overnight values of ∆LFV by 40% and of ∆NC by 42%. Subsequently, median AHI was decreased by 24% (from 30.9 to 23.4, P = 0.016). Thus, the authors concluded that, in sedentary, non-obese men with OSA, use of CS for one day reduces dependent fluid accumulation in the legs during the day, thereby reducing the spontaneous displacement of fluid from the legs to the neck overnight and consequently reduces the severity of OSA. This provided evidence for a causative relationship between
overnight fluid displacement into the neck and OSA. However, there were limitations to their study, including a small sample size, short duration and non-randomized design that limited the generalizability of their findings.
2.2.7 Rationale for Further Evaluation of Fluid-Mediated Upper Airway Obstruction During Sleep

Although Redolfi et al. provided proof-of-principle that overnight rostral fluid shift can contribute directly to OSA severity (105), there are no experimental data that show that fluid loading can provoke UA narrowing and obstruction to airflow during sleep. Accordingly, further experiments investigating the potential role of fluid loading in UA obstruction during sleep are warranted.

A number of studies have acutely and reversibly induced OSA and/or repetitive UA obstruction in healthy subjects during sleep (220-229). For instance, in one study, repetitive UA occlusions during sleep were induced by application of negative pressure through a nasal mask, with no adverse events reported (228). In a study that examined the association between nasal obstruction and OSA, 7 men slept while their nostrils were completely obstructed with gauze; this induced moderate-to-severe OSA in all subjects but did not evoke any adverse effects (229). In a study of 7 healthy men, 4 different inspiratory resistance loads of increasing resistance (4-25 cm H₂O·l⁻¹·s) were applied periodically every 8 minutes for 4 minutes at a time throughout a full night’s sleep, inducing oxygen desaturations of 1-2%; no clinically-significant symptoms or signs were reported (230). AHI and arousals were not reported.

Additionally, several studies have evaluated the effects of intermittent hypoxia (IH) in healthy subjects. For example 18 healthy subjects were exposed to 8-9 hours of IH per night...
for either 14 (n = 10) or 28 (n = 8) consecutive nights (223). IH was induced by having the subjects sleep in tents with ambient PO$_2$ values low enough to produce a baseline SaO$_2$ of 82-85%, while 15-second boluses of 100% oxygen were delivered via nasal cannulae every 2 minutes. This induced mean oxygen desaturations to 85% SaO$_2$ at a frequency of 30 desaturations per hour. The authors reported that subjects tolerated the exposure well, and the only adverse effect of the study that could be attributed to the IH was mild morning headache.

In summary, the above evidence provides a strong rationale to conduct a study to evaluate the effects of fluid loading on UA size and obstruction during sleep. Since other experimental means of inducing UA obstruction, apneas, hypopneas and IH have been shown to be safe, such a study should be medically and ethically acceptable.
2.3 EXPERIMENTAL FLUID LOADING

One of the challenges of determining whether fluid loading during sleep will narrow the UA and induce UA obstruction is finding a practical method for increasing nuchal or peripharyngeal fluid volume during sleep. Application of LBPP by inflating MAST induces peripharyngeal fluid accumulation in awake subjects (1-4). However, we have found in preliminary experiments that inflation of MAST during sleep invariably wakes the subject up, and so this method does not appear suitable for our purposes. Moreover, although use of MAST for < 1 hour is safe, multiple case reports (231-233) describe patients developing compartment syndromes in the legs in association with just a few hours of MAST use, emphasizing the unsuitability of using MAST in sleeping subjects for our purposes.

An alternative technique for fluid loading of the neck is an IV fluid infusion during sleep. In order to avoid induction of adverse electrolyte imbalances, IV fluid administered in large quantities must be isotonic to plasma. Normal saline, comprised of 0.9% NaCl in water, is isotonic to plasma, and is often used for treatment of non-hemorrhagic hypovolemia (58, 234) and in studies examining the physiological effects of acute systemic fluid expansion in euvolemic subjects.
2.3.1 Safety of Rapid Intravenous Administration of Normal Saline

The main clinical concern associated with fluid overload is the development of pulmonary edema, which can induce dyspnea, impair pulmonary gas exchange and, in extreme cases, lead to death if untreated.

A number of studies have demonstrated that a rapidly-administered saline bolus of 22 ml/kg body weight is safe in healthy volunteers (235-237). Additionally, there is considerable clinical evidence suggesting that infusion of approximately 20 ml/kg is well below the threshold to cause harm. In a retrospective analysis of post-surgical fluid management, iatrogenic fluid overload was defined as an acute gain in body weight >10% as a result of IV fluid administration (238). This corresponds to an acute saline infusion dose of 100 ml/kg; beyond this threshold, mortality was significantly increased. In other retrospective analyses of post-surgical patients, the mean IV fluid volume necessary to produce pulmonary edema was either 7.0L in the first post-operative day (239), or 2.9L/day over a mean duration of 4.7 days post-operatively (240). Thus, a single 22 ml/kg dose of normal saline, corresponding to 1.75L in an 80-kg subject, is far below the threshold to induce pulmonary edema, even if administered over the span of 30-60 minutes.

In addition to fluid management and resuscitation, rapid infusion of a large volume of normal saline has been used to diagnose primary aldosteronism for over 30 years (241-243). The test, known as the saline infusion test, involves rapid IV administration of 2L of normal saline to patients with moderate-to-severe hypertension. To examine patients’ basal hormonal responses to fluid loading, patients are typically withdrawn from antihypertensive medication for 1-2 weeks prior to this test. Thus, the saline infusion test involves carrying out acute fluid
loading within a population of patients at greater risk for developing pulmonary edema than normotensive subjects. A study analyzing effects of the saline infusion test in over 1000 hypertensive patients found the adverse event rate to be very low: < 0.5%. Adverse effects were minor and consisted exclusively of headache and chest pain unassociated with ECG abnormalities (241). Similar findings were reported in other studies involving 500 patients who underwent the saline infusion test (242, 243).

In summary, the rapid IV infusion of either 2L or 22 ml/kg of saline has been shown not to induce dyspnea or pulmonary edema in euvoletic subjects with or without hypertension, and therefore should be safe in healthy, non-hypertensive, euvoletic subjects.
2.3.2 Compression Stockings

Approximately 75% of systemic fluid volume resides in the venous circulation (244). Most of the fluid administered IV would therefore be expected to distribute rapidly to the highly-compliant capacitance veins. Distribution of fluid to the jugular veins causing venous distension could cause UA narrowing, particularly at the level of the mandible, where jugular distension could displace the lateral wall of the UA medially and impinge on the pharynx. Conversely, distribution and sequestration of infused fluid into other capacitance vessels, such as those located in the legs, would reduce the volume of fluid available for peripharyngeal accumulation. Therefore, reducing the sequestration of infused fluid into the capacitance vessels of the calf should increase the likelihood for IV fluid to accumulate around the UA and cause it to narrow.

When subjects assume an upright position as in sitting or standing, an effect of gravity on the column of blood from the heart to the legs is to produce an intravascular hydrostatic pressure gradient that increases caudally from the heart. In accordance with Starling’s forces, this promotes fluid to accumulate in the interstitium surrounding the capillaries of the capacitance veins of the calf, particularly if this increased capillary hydrostatic pressure is not adequately met by interstitial tissue pressure supplied by contraction of the calf muscles. By increasing interstitial tissue pressure, CS reduce cross-sectional area of the superficial and deep veins of the calf by approximately 20%, thereby reducing capacitance of these veins (245, 246). The increased interstitial tissue pressure also counteracts capillary leakage, favoring fluid flow from the interstitium into the vasculature. Accordingly, CS prevent dependent fluid accumulation and hemostasis in the calf that may occur in the upright position due to the
effect of gravity on hemodynamics (105, 219, 245, 247). Due to these same mechanisms of reducing venous capacitance and increasing interstitial hydrostatic pressure CS would likely reduce the volume of fluid sequestered in the calf during recumbent IV fluid loading.

CS use is safe, with no adverse effects reported for acute CS use, even in conjunction with hypervolemia. For example, among a population of hypervolemic patients with decompensated HF, CS did not alter supine BP or SaO$_2$ (248). Moreover, unlike MAST, CS are sufficiently comfortable for use during sleep.

In summary, the use of CS appears to be a safe technique for preventing fluid accumulation in the legs and for increasing the likelihood that IV fluid loading will induce peripharyngeal fluid accumulation during sleep.
3. RATIONALE AND HYPOTHESES

OSA is more common in edematous patients than in the general population, despite lower body weight (36-39, 41, 117). In healthy men (46), men with HF (47) and subjects with hypertension (106), OSA severity correlates with the volume of fluid that translocates spontaneously from the legs to the neck overnight. In sedentary men, attenuation of this translocation reduces OSA severity (105). In healthy men, but not in healthy women, experimentally-produced rostral fluid shift and consequent nuchal fluid accumulation increases UA collapsibility(3, 4). Thus, fluid accumulation in the neck/peripharyngeal tissues may induce UA obstruction in healthy men.

Our hypotheses are that in otherwise-healthy men with mild or no sleep apnea, IV saline administration coupled with the wearing of CS will:

1) Induce nuchal/peripharyngeal fluid accumulation and UA narrowing, as evidenced by an increase in NC and a reduction in UA-XSA, respectively.

2) Induce UA obstruction during sleep, as evidenced by an increase in the AHI.
4. METHODS

Subjects

Inclusion criteria were men 18-60 years of age. Exclusion criteria included gross obesity (BMI ≥35 kg/m²); blood pressure (BP) ≥ 140/90 mm Hg; BQ score >1; ESS score >10; self-reported history of cardiovascular, renal, neurological or respiratory diseases, or of sleep apnea.

Screening Procedure

To determine eligibility, potential subjects completed a screening procedure immediately after consenting to the study. During this procedure, subjects completed the ESS and BQ (Appendices 1-2) and were questioned with regards to a history of cardiovascular, renal, neurological or respiratory diseases, or of sleep apnea. Height and body weight were measured in order to calculate BMI. BP was measured using an automatic sphygmomanometer (BPM-200; BpTRU Medical Devices, Coquitlam, BC). Three consecutive readings were taken while subjects were seated upright; the last 2 readings were averaged to give mean BP. Only those subjects whose systolic BP was <140 mm Hg and whose diastolic BP was <90 mm Hg during the initial screening procedure, as well as at the beginning of each study session, were allowed to participate.

Sleep Deprivation

In order to facilitate daytime sleep, subjects were asked to restrict their sleep to ≤ 4 hours the night before each study session. Additionally, subjects were instructed to refrain from
tobacco, caffeine, alcohol, sedative, electrolyte drink and recreational drug intake for 24 hours prior to each study session.

_Acoustic Reflection Pharyngometry_

To measure UA-XSA, ARP (Eccovision Acoustic Pharyngometer; Sleep Group Solutions, North Miami Beach, FL) was employed immediately preceding and immediately following each sleep period, while subjects were awake and supine. Subjects were connected to the pharyngometer via a modified, scuba-type mouthpiece designed to prevent movement of the tongue. As initial calibration of the pharyngometer required nasal breathing, subjects did not wear nose clips. The pharyngometer emitted soundwave bursts at 5 pulses/second while subjects were instructed to breathe orally at normal tidal levels. The soundwaves reflected off the surfaces of the UA back into pharyngometer, where they were recorded by 2 microphones embedded in the unit. By comparing incident and reflected soundwaves, the acoustic pharyngometer is able to generate traces representing cross-sectional area as a function of distance from the teeth. Two technically-acceptable traces (superimposable and with variability ≤5%) were taken at the end of resting expiration. During calibration, the velum was located by instructing subjects to breathe nasally, whereas the glottis was located via the “silent O” manoeuvre, in which subjects mimicked saying the letter “O” without vocalization. Both these techniques elicit characteristic valleys in the area-distance tracings that can be used by an experienced ARP operator to determine the precise locations of the velum and glottis (249). UA-XSA was calculated as the mean cross-sectional area from the velum to the glottis. The resolution of the pharyngometer was 0.43 cm, indicating that approximately 25 measurements would be made in a typical 10-11 cm long pharynx. ARP
generates reproducible data (250, 251) and has been validated against both radiographic and MRI techniques for determining UA-XSA (252, 253). Sophisticated imaging software (SGS 3-D Airway Software, Sleep Group Solutions, North Miami Beach, FL), was used to generate images of the UA from area-distance plots.

*Compression Stockings*

Off-the-shelf, below-the knee CS (Sigvaris; Ganzoni & Cie. AG, St. Gallen, Switzerland) were placed on subjects’ legs prior to the saline infusion session of the protocol. The stockings are designed to apply 15-20 mm Hg of pressure to the legs.

*Neck Circumference*

NC was determined with a measuring tape at the superior border of the cricothyroid cartilage while subjects were supine. A line was drawn at this level by a marker pen so that measurements after sleep were taken at exactly the same level as before sleep.

*Intravenous Saline Infusion*

Before sleep, subjects were cannulated via a 20-gauge IV catheter inserted into a superficial forearm vein to infuse normal saline. In the control session, subjects were administered the minimal saline volume required to keep the vein open throughout the entire session. In the intervention session, subjects were started on minimal saline, and upon N2 sleep onset were switched to a bolus dose of approximately 22 ml saline/kg body weight. This bolus dose was administered at the maximum rate of gravity infusion. As the 20-gauge catheter was rated at 60 ml/minute maximum flow, the time needed to complete the 1750 ml bolus for an 80-kg
subject was approximately 35 minutes. Once the bolus was delivered, IV infusion rate reverted to minimal saline for the duration of the study session.

**Polysomnography**

The signals collected in the present study were EEG (leads: C3/A2, C4/A1, O2/A1, F3/A1, F4/A2), EOG, submental EMG, ECG, respiration and SaO$_2$. All signals were collected at a sampling frequency of 256 Hz using commercially-available sleep laboratory software (Sandman Elite; Embla Systems, Ottawa, ON). Sleep/wake stages were scored in 30-second epochs using American Academy of Sleep Medicine criteria (6).

Respiratory motion was monitored continuously using respiratory inductance plethysmography (Respirtrace; Ambulatory Monitoring, Inc., White Plains, NY). The plethysmography device consists of 2 elasticized bands, into which are embedded wires in a zigzag configuration that transduce mechanical strain into an electrical AC signal. One band is placed around the subject’s ribcage just below the axillae and the other is placed around the subject’s abdomen between the ribcage and iliac crests to quantify motion of the ribcage and abdomen, respectively (thoracoabdominal motion) and from these signals, $V_T$ was calculated as follows. After the RIP bands were applied, subjects performed three tidal breaths into a spirometer in both supine and upright seated positions. This allowed calibration of the RIP-calculated $V_T$ to within ±10% of spirometric values. Nasal airflow was measured by nasal pressure cannulae (BINAPS; Salter Labs, Arvin, CA) placed in the nares. SaO$_2$ was monitored continuously via a pulse oximeter (Nellcor; Nellcor Puritan Bennett, Inc, Pleasanton, CA) placed on the earlobe or, if subjects found the earlobe oximeter to be cumbersome for sleep, on the index finger. ECG data were collected via a lead-1 ECG.
Scoring of Respiratory Events

Obstructive apneas and hypopneas were defined as a $\geq 90\%$ reduction in $V_T$ and a 50-90\% reduction in $V_T$ from baseline, respectively, lasting at least 10 seconds with out-of-phase thoracoabdominal motion or flow limitation on the nasal pressure tracing. Central apneas and hypopneas were defined similarly, except that during apnea there was no thoracoabdominal motion, and during hypopnea, thoracoabdominal motion was in-phase and there was no evidence of airflow limitation in the nasal pressure tracing. The frequency of apneas and hypopneas per hour of sleep was defined as the AHI.

Daytime PSG has been validated against overnight PSG for the diagnosis of OSA, both with (254) and without (255, 256) sleep deprivation to 4 hours of sleep the night before.

Experimental Protocol

A schematic of the screening and experimental protocols is presented in Figure 3.
Subjects
Inclusion Criteria: Men aged 18-60 years; Body mass index < 35 kg/m²; BP < 140/90; Berlin Questionnaire ≤1; Epworth Sleepiness Scale ≤10
Exclusion Criteria: Self-reported history of cardiovascular, renal, neurological or respiratory diseases or of sleep apnea; AHI > 30 during control

Sleep Deprivation < 4 hrs/night

Randomization

Control

UA −XSA, NC measured
Minimal volume of saline necessary to keep vein open administered throughout session
Compression stockings not worn
AHI quantified

UA −XSA, NC measured

Saline Infusion

Pre-Sleep

UA −XSA, NC measured
22 ml/kg body weight saline administered over 30-50 minutes at onset of N2 sleep
Compression stockings worn throughout session
AHI quantified

Daytime Sleep Study

Post-Sleep

UA −XSA, NC measured

Washout (Approx. 1 week)

Cross-over to Remaining Arm of the Study

Figure 3: Schematic of screening and experimental protocols.
Abbreviations: BP, blood pressure; UA-XSA, upper airway cross-sectional area; NC, neck circumference; AHI, apnea-hypopnea index.
Subjects underwent screening after consenting to the study. A randomized, controlled, double-crossover study design was employed; eligible subjects were scheduled to complete their first study session shortly after screening, with the second session occurring approximately 1 week later.

The day following sleep deprivation, subjects arrived at the Toronto Rehabilitation Institute Sleep Research Laboratory between the hours of 11:30 and 14:30. BP was measured in the upright, seated position, following which subjects were instrumented for PSG. Subjects then laid down supine on a sleep laboratory bed and NC and UA-XSA were measured. A 20-gauge IV catheter was then inserted into a superficial forearm vein and attached to two, 1-litre bags of normal saline warmed to approximately 37° C. Subjects were then randomized by coin toss to complete either the control or saline infusion intervention first. After completion of the first protocol, subjects underwent a washout period of approximately one week, after which they returned to the laboratory to complete the remaining arm of the study.

In both sessions, subjects were allowed to sleep for as long as they wished, to a maximum of 4 hours. All measurements performed prior to sleep were then replicated.

The study protocol was approved by the local Research Ethics Board of the Toronto Rehabilitation Institute and all subjects provided written informed consent prior to their participation in the study. Subjects were recruited via advertisement.

*Statistical Analyses*
Continuous variables were compared using two-tailed, paired t-tests for variables with normally-distributed data and Wilcoxon signed rank test for variables with non-normally distributed data. The time-intervention interaction terms for changes in NC and UA-XSA were assessed by two-way repeated ANOVA with crossover design. Fisher’s exact test was used to compare categorical data between different groups. Data are presented as mean ± SD unless otherwise stated. A two-sided P value of 0.05 was considered significant. Univariate linear regression analyses were performed for age versus AHI, BMI versus AHI, BMI versus NC, BMI versus UA-XSA and NC versus UA-XSA. Statistical analyses were performed using GraphPad Prism for Windows software version 5.0 (GraphPad Software, Inc., San Diego, CA).
5. RESULTS

Subjects

Eighteen men signed the consent form. Of these, 2 met exclusion criteria prior to randomization, 2 were lost to follow-up prior to randomization, 2 were randomized to complete the control session first, after which they were excluded due to unsuspected severe OSA (AHI > 30) and 3 declined to participate after completing the control session.

The remaining 9 healthy men underwent the full protocol. Individual and mean subject characteristics are shown in Table 1.

Table 1: Subject Characteristics

<table>
<thead>
<tr>
<th>Subject #</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>Systolic BP, mm Hg</th>
<th>Diastolic BP, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23</td>
<td>32</td>
<td>107</td>
<td>71</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>23.5</td>
<td>113</td>
<td>74</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>26</td>
<td>113</td>
<td>72</td>
</tr>
<tr>
<td>4</td>
<td>24</td>
<td>22.3</td>
<td>113</td>
<td>70</td>
</tr>
<tr>
<td>5</td>
<td>46</td>
<td>32.1</td>
<td>120</td>
<td>82</td>
</tr>
<tr>
<td>6</td>
<td>24</td>
<td>28.5</td>
<td>105</td>
<td>73</td>
</tr>
<tr>
<td>7</td>
<td>36</td>
<td>27.3</td>
<td>121</td>
<td>75</td>
</tr>
<tr>
<td>8</td>
<td>45</td>
<td>28.6</td>
<td>103</td>
<td>74</td>
</tr>
<tr>
<td>9</td>
<td>36</td>
<td>19.9</td>
<td>108</td>
<td>71</td>
</tr>
<tr>
<td>MEAN ± SD</td>
<td>32.4 ± 8.9</td>
<td>26.7 ± 4.2</td>
<td>111.4 ± 6.3</td>
<td>73.6 ± 3.6</td>
</tr>
</tbody>
</table>
Self-Reported Pre-Sleep Restrictions

All subjects were asked to restrict their sleep to less than approximately 4 hours the night prior to each study session, and the reported pre-session sleep times did not differ between the control and saline infusion sessions (3.5 ± 1.4 hours versus 3.3 ± 1.4 hours; P = 0.228). Prior to each session, all subjects denied consuming any medications, recreational drugs, alcohol or caffeine on the day of either session. Additionally, all subjects denied suffering from any degree of nasal congestion prior to either session.

Saline Administration

Five subjects were randomized to complete the control session first. By design, subjects were infused with a much larger volume of saline in the intervention compared to the control session (1850 ± 280 ml versus 80 ± 40 ml; P < 0.0001). All subjects tolerated the control and saline infusion sessions well. Of the 9 intervention sessions conducted, 3 had to be briefly interrupted by a subject’s need to void following the saline bolus infusion. After these subjects urinated, they returned immediately to bed, went back to sleep and continued the session without incident.
Neck Circumference

Values for NC are displayed in Figure 4. The mean pre-sleep NC did not differ significantly between control and intervention sessions (P > 0.70). During both the control and intervention sessions, NC increased significantly from pre- to post-sleep measurements (P = 0.021 and P < 0.001, respectively). The time-intervention term as assessed by two-way repeated measures ANOVA was statistically significant (P < 0.01).

![Figure 4: NC values for each subject immediately before and after each sleep period in both the control and saline infusion sessions. NC increased significantly during both the control and saline infusion sessions, with a significantly larger increase occurring during the saline infusion session. * P < 0.05 versus pre-sleep; † P < 0.001 versus pre-sleep; ‡ P < 0.01 versus control
Abbreviation: NC, neck circumference](image-url)
Strong correlations were also found between BMI and NC for all four conditions (i.e. pre- and post-sleep during control session, and pre- and post-sleep during the saline infusion session) (Figure 5). There were no significant relationships between BMI and the degree of change in NC across either the control or intervention sessions.

Figure 5: BMI versus NC collected immediately before and after both the control and saline infusion sessions. Under all 4 conditions, BMI correlated strongly with NC.
Abbreviations: BMI, body mass index; NC, neck circumference
Upper Airway Cross-Sectional Area

An area-distance plot of the UA obtained in one subject immediately before and after saline intervention is shown in Figure 6. From this plot, images of the UA were generated (Figure 7). In this subject, there was a marked reduction in UA-XSA in response to saline infusion.

Figure 6: Area-distance curves generated by acoustic reflection pharyngometry in one subject immediately before and after saline intervention. UA-XSA decreased by 32% following saline infusion (from 3.8 cm$^2$ to 2.6 cm$^2$).
Abbreviation: UA-XSA, upper airway cross-sectional area
Figure 7: Representative images of the upper airway, derived from area-distance curves in Figure 6. Abbreviation: UA-XSA, upper airway cross-sectional area.
Values for UA-XSA are shown in **Figure 8**. The mean pre-sleep UA-XSA did not differ significantly between control and saline infusion sessions (P > 0.90). There was no significant difference between pre- and post-sleep UA-XSA during the control period. In contrast, during the intervention, UA-XSA decreased significantly from pre- to post-sleep (P = 0.014). The time-intervention term as assessed by two-way repeated measures ANOVA was statistically significant (P = 0.015).

**Figure 8**: UA-XSA values for each subject immediately before and after each sleep period in both the control and saline infusion sessions. UA-XSA decreased significantly during the saline infusion session, and the reduction in UA-XSA was significantly greater in the saline infusion session (15%) than the control session (3%).

Horizontal bars indicate mean
* P < 0.05 versus pre-sleep; ‡ P < 0.05 versus control

Abbreviation: UA-XSA, upper-airway cross-sectional area
There were no significant relationships between UA-XSA and BMI, NC or AHI measured at any time point.

*Sleep Architecture*

Sleep variables are displayed in Table 2. Total sleep time, sleep onset latency, REM sleep onset latency, number of arousals per hour of sleep (arousal index) and distribution of sleep amongst the various stages were similar between the control and saline infusion sessions. However, sleep efficiency was significantly lower during the intervention than the control session (P = 0.031).

**Table 2: Sleep Architecture Data**

<table>
<thead>
<tr>
<th></th>
<th>Control (N = 9)</th>
<th>Saline Infusion (N = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time in bed after lights out, minutes</td>
<td>157 ± 47</td>
<td>172 ± 36</td>
<td>0.300</td>
</tr>
<tr>
<td>TST, minutes</td>
<td>144 ± 39</td>
<td>131 ± 38</td>
<td>0.390</td>
</tr>
<tr>
<td>Sleep efficiency, %</td>
<td>87.2 ± 6.7</td>
<td>79.8 ± 11.2</td>
<td><strong>0.031</strong></td>
</tr>
<tr>
<td>Sleep onset latency, minutes</td>
<td>2.4 ± 2.4</td>
<td>5.3 ± 8.1</td>
<td>0.199</td>
</tr>
<tr>
<td>REM latency, minutes</td>
<td>89.6 ± 46.5</td>
<td>96.4 ± 78.6</td>
<td>0.822</td>
</tr>
<tr>
<td>Arousal index, # per hour of sleep</td>
<td>17.2 ± 20.2</td>
<td>25.4 ± 27.1</td>
<td>0.326</td>
</tr>
<tr>
<td>N1 sleep, % TST</td>
<td>10.5 ± 4.8</td>
<td>11.5 ± 6.4</td>
<td>0.692</td>
</tr>
<tr>
<td>N2 sleep, % TST</td>
<td>65.3 ± 16.4</td>
<td>65.5 ± 16.2</td>
<td>0.975</td>
</tr>
<tr>
<td>N3 sleep, % TST</td>
<td>14.7 ± 12.7</td>
<td>14.3 ± 13.6</td>
<td>0.941</td>
</tr>
<tr>
<td>REM sleep, % TST</td>
<td>9.5 ± 8.3</td>
<td>8.4 ± 10.5</td>
<td>0.797</td>
</tr>
<tr>
<td>Subjects who had N3 sleep, n</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Subjects who had REM sleep, n</td>
<td>7</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>Supine sleep, %TST</td>
<td>100 ± 0</td>
<td>98 ± 7</td>
<td>0.347</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD unless indicated otherwise. Abbreviations: TST, total sleep time; REM, rapid eye movement sleep; N1, N2 and N3, stages of non-rapid eye movement sleep.
Oxygen Saturation

The mean SaO\textsubscript{2} throughout the total sleep time did not differ between the control and intervention sessions (96.2 ± 1.9\% versus 96.2 ± 1.7\%; P = 0.978). The minimum SaO\textsubscript{2} throughout the total sleep time also did not differ between the control and intervention sessions (88.9 ± 5.3\% versus 90 ± 5.6\%; P = 0.623).

Apnea-Hypopnea Index

The AHIs are shown in Figure 9. There was no significant difference in AHI between the control and intervention sessions. There was a large degree of both between- and within-subject AHI variability. Compared to the control session, 4 subjects had a higher AHI, 3 had a lower AHI and 2 had no appreciable change in the AHI during the saline infusion session. Two subjects had a control AHI > 30 despite having low BQ scores of 0 and low ESS scores of 5 and 1 that should have been associated with a low likelihood for OSA in otherwise-healthy men. These subjects were randomized to the intervention session first.
The total AHI was then classified into various subtypes, based on apnea-hypopnea type and on the stages of sleep in which respiratory events occurred. Table 3 shows that there were no significant differences in the obstructive AHI or in the AHI of the various stages of sleep between the control and saline infusion sessions.
Table 3: Apnea-Hypopnea Indices

<table>
<thead>
<tr>
<th></th>
<th>Control (N = 9)</th>
<th>Saline Infusion (N = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total AHI, events/hr</td>
<td>19.5 ± 22.7</td>
<td>30.3 ± 29.5</td>
<td>0.249</td>
</tr>
<tr>
<td>REM AHI, events/hr</td>
<td>28.0 ± 26.1</td>
<td>36.9 ± 20.4</td>
<td>0.486</td>
</tr>
<tr>
<td>NREM AHI, events/hr</td>
<td>17.4 ± 23.6</td>
<td>28.9 ± 30.7</td>
<td>0.231</td>
</tr>
<tr>
<td>Obstructive AHI, events/hr</td>
<td>15.8 ± 22.7</td>
<td>30.3 ±29.5</td>
<td>0.235</td>
</tr>
<tr>
<td>Obstructive REM AHI, events/hr</td>
<td>27.3 ± 26.6</td>
<td>35.6 ± 18.7</td>
<td>0.500</td>
</tr>
<tr>
<td>Obstructive NREM AHI, events/hr</td>
<td>13.7 ± 23.3</td>
<td>25.0 ± 30.5</td>
<td>0.225</td>
</tr>
<tr>
<td>Central AHI, events/hr</td>
<td>3.7 ± 7.5</td>
<td>3.7 ± 5.9</td>
<td>0.923</td>
</tr>
</tbody>
</table>

Values are presented as mean ± SD, except for central AHI values, which are presented as mean ± SE.

Abbreviations: AHI, apnea-hypopnea index; REM, rapid eye movement sleep; NREM, non-rapid eye movement sleep.

Interestingly, the only 2 subjects who were over the age of 40 had the greatest increases in AHI during the saline infusion session (from 14 during control to 64 during saline infusion, and from 19 during control to 78 during saline infusion). Accordingly, linear regression analysis between subject age and total AHI during the saline infusion was performed. Figure 10 shows a strong, direct relationship between these two variables (r = 0.80, P < 0.01); a similar relationship was not found between age and AHI during the control arm (r = 0.22, P = 0.57). There were no significant relationships between BMI and AHI in either session.
Figure 10: This plot shows a strong, direct relationship between age and the total AHI during the saline infusion session. There was no such relationship during the control session. Abbreviation: AHI, apnea-hypopnea index
6. DISCUSSION

The major findings of our study are that, compared to the control session, saline infusion and CS induced a greater increase in NC, a greater decrease in UA-XSA and lower sleep efficiency. However, saline infusion had no significant effect on the AHI. This indicated that saline infusion and CS increased fluid accumulation in the neck sufficient to narrow the UA but, in these subjects, insufficient to induce or worsen sleep apnea.

*Neck Circumference*

A strong correlation between BMI and NC measured by tape measure has been well established in previous studies (30, 257). Therefore, the strong linear correlations between BMI and NC measured pre- and post-sleep in the control and saline infusion sessions supports our use of a tape measure to measure NC as valid.

Although NC increased by only 0.1 cm between pre- and post-sleep during the control session, this increase was statistically significant. This increase is consistent with the findings of Redolfi *et al.*, who observed in non-obese healthy men that there was a similarly small increase in NC over several hours before and after an overnight PSG in those who did not have OSA (46). This is probably attributable to a gravitational effect causing fluid accumulated in the legs while upright to be displaced to the upper body and neck while recumbent (258).
During the saline infusion session, the increase in NC was much greater than that observed
during the control. This finding alone confirms that some of the infused saline accumulated
in the neck. This increase in NC is likely attributable primarily to the IV saline infusion,
rather than to the use of CS. The application of 40 mm Hg LBPP to the legs from the ankles
to the upper thighs in healthy, awake subjects induces an acute increase in NC of 0.04 – 0.12
cm (0.1-0.3%.) that plateaus within 1-5 minutes (1-4). Thus, the CS used during saline
infusion, which only extended to the knee and were designed to apply only 15-20 mm Hg of
pressure, probably made a minimal contribution to the 0.6 cm (1.4%) increase in NC that
occurred from the beginning to the end of the saline infusion sessions. Rather, the main
action of the CS was likely to prevent the infused fluid from accumulating in the legs by
increasing interstitial and IV pressure, thereby promoting accumulation of this fluid rostral to
the legs.

*Upper Airway Cross-Sectional Area*

Between pre- and post-sleep, UA-XSA decreased significantly more during the saline
infusion session than during the control session. In conjunction with the increase in NC
during saline infusion, this reduction in UA-XSA demonstrates that IV saline loading
coupled with the use of CS induced nuchal and/or peripharyngeal fluid accumulation that
narrowed the UA, presumably by increasing peripharyngeal tissue pressure (102, 103). The
fluid that reached the neck may not have translocated from the circulation, in which case the
reduction in UA-XSA would likely have been caused by distension of the nearby jugular
veins. Conversely, the fluid may have translocated from the circulation in volumes adequate
to cause interstitial edema in the peripharyngeal tissues, thereby reducing UA-XSA. From
our data, we are unable to discern whether one mechanism was more likely. Regardless, this confirms our first hypothesis that the combination of IV saline loading and CS would reduce UA-XSA in association with an increase in NC.

The reduction in UA calibre caused by our intervention was greater than that caused by LBPP in a previous study. Shiota et al. (2) assessed UA-XSA via ARP during application of 40 mm Hg LBPP to healthy awake subjects in the supine position. They found that 5 minutes of LBPP decreased UA-XSA by 0.18 cm$^2$ (9%). In the present study, our intervention decreased UA-XSA by 0.43 cm$^2$ (13%). Thus, the reduction in UA calibre caused by IV fluid loading and CS use was approximately 1.4-fold greater than that caused by LBPP. As UA-XSA is lung-volume dependent, it is possible that the infused fluid did not directly decrease UA-XSA at the level of the pharynx, but instead infiltrated the lungs, thereby reducing lung volume and UA-XSA. Since we did not assess lung volume, we are unable to rule out this possibility objectively. However, infiltration of fluid into the lungs may increase the frequency of central apneas and hypopneas or decrease mean or minimum SaO$_2$, both of which did not happen during our intervention. Moreover, Chiu et al. assessed total lung capacity by helium dilution before and after application of LBPP and found no effect, suggesting that rostral fluid accumulation can reduce UA-XSA without reducing lung volume.
Apnea-Hypopnea Index

Although saline infusion and CS induced a greater increase in NC and a greater reduction in UA-XSA than during the control session, it had no significant effect on the AHI. Thus, our second hypothesis is not confirmed.

There are several possible explanations for why our intervention did not increase the AHI. One possibility is that the volume of fluid infused was not great enough to induce sufficient degrees of nuchal fluid accumulation and peripharyngeal tissue pressure elevation to cause a critical increase in UA collapsibility. A second possibility is that although factors that increase UA extraluminal tissue pressure, such as peripharyngeal adiposity or peripharyngeal fluid accumulation, may promote OSA, they may not in themselves induce OSA if the UA is not already anatomically narrow and/or intrinsically compliant. Indeed, Dempsey et al. (80) propose that anatomical predisposition for airway closure is an essential component for the development of OSA. For example, several studies have demonstrated that the UA of subjects with OSA is narrower, more compliant and more collapsible than in subjects without OSA (58, 64, 65, 71, 98-100). The mean baseline UA-XSA values in our subjects were 2.92-2.93 cm$^2$. In other studies in which UA-XSA was measured in awake subjects using ARP, apneic patients had mean supine UA-XSA values of 1.43 - 1.94 cm$^2$, whereas non-apneic subjects had values of 1.89 – 2.68 cm$^2$ (2, 259, 260). Thus, the baseline UA-XSA of our subjects was far closer to those of non-apneics than those of apneics, as would be expected. Accordingly, another plausible explanation for why our intervention did not induce significant UA obstruction is that our subjects were not anatomically predisposed toward the development of UA obstruction during sleep. In addition to having non-narrow UAs, it is
possible that our subjects had UAs that were relatively non-compliant (71, 72, 77). However, we did not measure UA compliance, and so have no data relevant to this possibility.

A third possible explanation for the lack of effect of our intervention on the AHI was the relative youth of our subjects. OSA prevalence and severity increase with increasing age (12, 15, 26, 112). The mean age of our subjects was 32 years, with approximately half of our subjects in their 20s. Thus, they were younger than most OSA patients, whose mean age at diagnosis is usually 45-55 years (12, 15, 26, 112). Compared to healthy, non-apneic men aged 18-25 years, healthy, non-apneic men aged 42-67 years exhibit increased UA collapsibility during sleep independent of neuromuscular factors (113, 114). This relationship between increased age and increased UA collapsibility is also independent of BMI, and appears to plateau past age 45 years (261). Our findings appear compatible with these findings, since subjects in our study ≥ 45 years of age exhibited a > 4-fold increase in AHI during the intervention, compared to a 1.5-fold increase in the other 7 subjects < 45 years of age. Although this increase in AHI in this older subset of our subjects was not statistically significant, it does suggest that older individuals may be more susceptible to the adverse effects of fluid overload on UA anatomy and physiology and therefore may be more likely to develop OSA in response to fluid overload than younger individuals. Moreover, age correlated strongly with interventional but not control AHI ($r = 0.80, P < 0.01$ versus $R = 0.22, P > 0.5$); this suggests that older subjects in our study had a tendency toward UA obstruction during sleep that was elicited by our intervention. The hypothesis that older individuals are more prone to develop UA collapse during sleep in response to fluid loading than younger individuals merits further investigation in larger samples.
There are several factors through which normal ageing might increase UA collapsibility. Although our data do not support a relationship between increased age and UA narrowing, a study in which ARP was conducted in 60 males aged 16-74 years demonstrated a modest inverse relationship between age and supine UA-XSA ($r = -0.30 \ P < 0.01$) (262), suggesting that the UA becomes narrower with age. An age-related increase in length of the collapsible segment of the UA would increase UA collapsibility, however this phenomenon has been demonstrated only in women (263) and therefore may not be applicable to the men in our study. Studies in rats have found that normal ageing is associated with an alteration of UA dilator muscle fibre type such that populations of fast glycolytic fibres (type IIb; easily fatigable) increase while those of fast oxidative fibres (type IIa; fatigue-resistant) decrease, resulting in increased fatigability of these muscles with age (264, 265). Indeed, this muscle fibre alteration has been demonstrated to occur to a greater degree in OSA patients compared to non-apneic controls (266-269).

As mentioned previously, the increase in UA collapsibility with age does not appear to involve age-related changes in the reflex responsiveness of UA dilator muscles to negative intraluminal pressure. Although the decrease in UA dilator muscle activity at sleep onset is of a greater magnitude in older than younger men, this discrepancy is eliminated when CPAP is administered throughout sleep onset, suggesting that age differences in UA dilator muscle activity are secondary to differences in UA narrowing and $R_{UA}$ (113, 114). Thus, it is unlikely that the degree of reflexive activation of UA dilator muscles by saline-induced increases in extraluminal tissue pressure would be greater in younger than older subjects.
Sleep Efficiency

Sleep efficiency was lower in the intervention than in the control session, such that during the intervention subjects required more time in bed to achieve the same amount of sleep. A possible explanation for this is that the decrease in SE was secondary to an increase in SNS activity caused by fluid loading and bladder distension. Bladder distension increases sympathetic outflow and blood pressure, which return toward normal immediately following micturition (270). Increased sympathetic activity and the proprioceptive discomfort of bladder distension could increase the level of alertness and make it more difficult to sleep. Our data tend to support this explanation, since sleep was interrupted by the need to void in 3 out of 9 subjects following the completion of the bolus infusion during the intervention, whereas a need to void did not interrupt sleep for any subject during the control session. However, an elevation in SNS activity prior to micturition could be reflected as an increase in arousal index and/or a decrease in total sleep time, both of which were not significantly different between control and intervention. Moreover, the 3 subjects who voided during the intervention had a mean sleep efficiency of 85%, compared to 79% in the 6 remaining subjects. Thus, the lower sleep efficiency during the intervention does not appear to be related to subjects interrupting the session to void. In this regard, it is interesting to note that in fluid retaining states, such as HF and refractory hypertension, sleep efficiency is also reduced (44, 271) Thus, although we did not identify the reason for reduced sleep efficiency during the intervention session, these results are consistent with previous data indicating that some fluid retaining states are associated with reduced sleep efficiency even in the absence of sleep apnea.
Subjective Response to Saline Loading

Subjects tolerated the fluid infusion well. The most common source of discomfort was the 20-gauge IV cannulation at the onset of each session; there were no complaints of dyspnea or shortness of breath at any time during the study. As the saline was warmed to approximately 37° C prior to infusion, subjects indicated that they did not feel the saline being infused. All subjects reported voiding within approximately 10 minutes after the intervention session had ended. All of the 3 subjects who declined to complete the second session of the study were randomized to the control session first, and thus did not experience the saline bolus infusion before withdrawing. Thus, our intervention was safe, and appeared to cause a reasonably low level of discomfort in our subjects.
7. LIMITATIONS

Possible Selection Bias

Under the advisory of our Research Ethics Board, we did not conduct the intervention session in subjects who, upon being randomized to first complete the control, were found to have an AHI ≥ 30, as they were concerned that the intervention could have caused an unsafe degree of UA obstruction in these subjects. However, subjects who were randomized to complete the intervention first were allowed to complete the control regardless of their interventional AHI. Accordingly, this may have introduced some degree of selection bias to our subject population.

Utility of Screening Measures

An unanticipated finding of the study was the low predictive value of the combined use of the ESS and BQ for the likelihood of OSA. Of 9 subjects, all of whom met inclusion and exclusion criteria, control AHI was > 15 in 4 subjects, indicative of at least a moderate degree of OSA. In turn, 2 of these 4 subjects had a control AHI of > 30, indicative of severe OSA. Paradoxically, although these 2 subjects had the highest control AHI values, they had some of the lowest ESS and BQ scores among our subject population, indicative of low likelihoods of OSA.

There are 2 reasons for why we chose to predict the likelihood of OSA for each subject using the ESS, BQ and self-reported history of OSA, instead of using PSG to clinically assess the presence or absence of OSA prior to the study. First, we wished to limit the inconvenience to our subjects. Our protocol required subjects to visit our laboratory 3 times: once to provide
consent and to be screened for inclusion/exclusion criteria and 2 additional times for the study sessions – in total, this required a time commitment of approximately 10-12 hours for each subject. We felt that asking the subjects to visit the laboratory an additional instance to undergo an overnight PSG would make the study too inconvenient for most subjects and would thereby unduly restrict subject recruitment. Second, during the evening our sleep laboratory typically is fully occupied by patients undergoing diagnostic PSGs, many of whom must wait several weeks to undergo such a procedure, and displacement of these patients was not a realistic option. We therefore chose to screen based on predictions of subjects’ OSA statuses rather than based on PSG evidence of their OSA status.

We chose to use the ESS and BQ to predict patients’ OSA status because both are tools designed specifically for detecting the signs and symptoms of OSA for screening purposes. An ESS score > 10 is moderately associated with the presence of OSA in otherwise-healthy subjects (170, 175), and such findings support the popular use of the ESS in sleep clinics today. Likewise, in a study conducted by Netzer et al. among 100 patients who underwent PSG, the BQ predicted an AHI > 5 with 86% sensitivity and 77% specificity (171). Thus, we felt that the combined use of the ESS and BQ as a surrogate for PSG screening was justified.

The perceived underperformance of the ESS and BQ may be partially attributable to our subjects’ posture during sleep. The intravenous forearm vein catheter, in addition to the usual electrodes for PSG, may have made our subjects avoid changing positions during sleep, as all subjects slept almost exclusively in the supine position during both the control and intervention sessions. Supine sleep favors posterior displacement of the tongue due to gravity,
and the supine position is associated with a significantly greater AHI than lateral/prone sleep in over 50% of all OSA patients and over 60% of patients with mild-to-moderate OSA (272, 273). Thus, although the mean control AHI for our subjects was indicative of mild-to-moderate OSA, this may not have been representative of a sleep period in which posture was not restricted, possibly explaining why subjects’ BQ and ESS scores were not predictive of the presence or absence of OSA.
8. CONCLUSIONS

In healthy men between the ages of 23-46 years with no history of previously diagnosed OSA and with a low likelihood of having OSA as assessed by the ESS and BQ, IV saline loading coupled with the use of CS during sleep induced nuchal and peripharyngeal fluid accumulation, as evidenced by an increase in NC and a decrease in UA-XSA compared to control. However, such fluid accumulation and UA narrowing did not induce an increase in AHI during the intervention session compared to the control session. An interesting observation was that the intervention was accompanied by a marked increase in AHI in the men aged ≥ 45 years, perhaps due to age-related predisposition to UA collapse. This observation suggests the hypothesis that older men may be more susceptible to UA obstruction due to nuchal and peripharyngeal fluid accumulation than younger men, a hypothesis that could be evaluated in future studies.
9. FUTURE DIRECTIONS

The findings and limitations of the current study provide a strong rationale to continue along this line of investigation. One possibly fruitful strategy suggested by our observations would be to determine if there are age-related effects of UA fluid loading. Our data suggest that older subjects may be more susceptible to UA obstruction and an increase in AHI than younger subjects in response to UA fluid loading. One could undertake a similar protocol and divide subjects according to age, for example a group \( \geq 45 \) years versus a group 18-45 years, to test the hypothesis that IV saline plus CS will increase AHI to a greater extent in older subjects than in younger subjects. Ideally, such a protocol would also incorporate an overnight PSG into the pre-study screening protocol, so that each participant would be confirmed not to have OSA. Full overnight PSGs for the control and intervention arms would also be better, as this would probably increase total sleep time, and especially REM sleep when induction of UA obstruction would be more likely.

Finally, since it has been previously demonstrated that rostral fluid shift by LBPP increased UA collapsibility (i.e. Pcrit) in men but not in women (4), it would also be interesting to compare the effects of saline infusion plus CS in men and women to test the hypothesis that it would reduce UA-XSA and increase AHI to a greater extent in older men than in older women.
10. GLOSSARY

**Hazard ratio, adjusted** – The ratio of a probability of an event, such as morbidity or mortality, occurring in an exposed group, such as a population of subjects with a certain disease compared to that of a non-exposed group, such as a control population. The hazard ratio can be adjusted via a multivariate analysis to correct for known confounding variables.

**Incidence** – The number of people presenting with new cases of a certain disease or condition within a population over a specified length of time.

**Nuchal** – Pertaining to the anatomical area of the neck.

**Odds ratio, adjusted** – Like the hazard ratio, the odds ratio is used to express the probability of an event occurring in one group compared to another group. However, unlike the hazard ratio, the odds ratio is suitable to analyze both high and low probability events, as opposed to only low probability events. The odds ratio can also be adjusted via a multivariate analysis to correct for known confounding variables.

**Peripharyngeal** – Pertaining to the anatomical area surrounding the pharynx.
**Poiseulle's Law** – Under conditions of constant flow, the resistance of flow through a tube is inversely proportional to the radius of the tube raised to the fourth power (resistance = k/radius⁴). Poiseulle’s Law can be combined with a hydraulic analogy of Ohm’s Law (flow = Δ pressure/ resistance) to state that, under conditions of constant pressure, flow in a tube is proportional to the radius of the tube raised to the fourth power (flow = k*radius⁴).

**Prevalence** – The total number of people who have a certain disease or condition within a population at one specific point in time.

**Sensitivity** – the measure of a test’s ability to correctly identify a positive result. In the case of the Berlin Questionnaire, sensitivity refers to the ability of the questionnaire to correctly predict an apnea-hypopnea index of ≥ 5 in a subject. The formula for sensitivity is as follows:

\[
\text{Sensitivity} = \frac{\# \text{ of true positives}}{\# \text{ of true positives} + \# \text{ of false negatives}}
\]

**Specificity** – the measure of a test’s ability to correctly identify a negative result. In the case of the Berlin Questionnaire, specificity refers to the ability of the questionnaire to correctly predict an apnea-hypopnea index of < 5 in a subject. The formula for specificity is as follows:

\[
\text{Specificity} = \frac{\# \text{ of true negatives}}{\# \text{ of true negatives} + \# \text{ of false positives}}
\]
Starling's Forces – These include the hydrostatic and oncotic pressures of the capillary and interstitium. The pressure gradient among Starling’s forces determines fluid flow across the capillary membrane.
11. APPENDICES

11.1 Appendix 1: Epworth Sleepiness Scale

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired? This refers to your usual way of life in recent times. Even if you have not done some of these things recently try to work out how they would have affected you. Use the following scale to choose the most appropriate number for each situation:

0 = would never doze
1 = slight chance of dozing
2 = moderate chance of dozing
3 = high chance of dozing

(Answer each question 0, 1, 2, or 3)

<table>
<thead>
<tr>
<th>Situation</th>
<th>Score 0-3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting and reading</td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
</tr>
<tr>
<td>Sitting inactive in a public place (i.e. a theatre or in a meeting)</td>
<td></td>
</tr>
<tr>
<td>As a passenger in a car for an hour without a break</td>
<td></td>
</tr>
<tr>
<td>Lying down to rest in the afternoon when circumstances permit</td>
<td></td>
</tr>
<tr>
<td>Sitting and talking to someone</td>
<td></td>
</tr>
<tr>
<td>Sitting quietly after a lunch (without having drunk any alcohol)</td>
<td></td>
</tr>
<tr>
<td>In a car, while stopped for a few minutes in traffic</td>
<td></td>
</tr>
<tr>
<td><strong>Total Score</strong></td>
<td></td>
</tr>
</tbody>
</table>
11.2 Appendix 2: Berlin Questionnaire

**CATEGORY 1**

1. **Do you snore?**
   _ a. Yes
   _ b. No
   _ c. Don’t know

   *If you snore:*

2. **Your snoring is:**
   _ a. Slightly louder than breathing
   _ b. As loud as talking
   _ c. Louder than talking
   _ d. Very loud – can be heard in adjacent rooms

3. **How often do you snore**
   _ a. Nearly every day
   _ b. 3-4 times a week
   _ c. 1-2 times a week
   _ d. 1-2 times a month
   _ e. Never or nearly never

4. **Has your snoring ever bothered other people?**
   _ a. Yes
   _ b. No
   _ c. Don’t Know

5. **Has anyone noticed that you quit breathing during your sleep?**
   _ a. Nearly every day
   _ b. 3-4 times a week
   _ c. 1-2 times a week
   _ d. 1-2 times a month
   _ e. Never or nearly never

**CATEGORY 2**

6. **How often do you feel tired or fatigued after your sleep?**
   _ a. Nearly every day
   _ b. 3-4 times a week
   _ c. 1-2 times a week
   _ d. 1-2 times a month
   _ e. Never or nearly never

7. **During your waking time, do you feel tired, fatigued or not up to par?**
   _ a. Nearly every day
   _ b. 3-4 times a week
   _ c. 1-2 times a week
   _ d. 1-2 times a month
   _ e. Never or nearly never

8. **Have you ever nodded off or fallen asleep while driving a vehicle?**
   _ a. Yes
   _ b. No
If yes:
9. **How often does this occur?**
   - a. Nearly every day
   - b. 3-4 times a week
   - c. 1-2 times a week
   - d. 1-2 times a month
   - e. Never or nearly never

**CATEGORY 3**
10. **Do you have high blood pressure?**
   - Yes
   - No
   - Don’t know

**Scoring the Berlin Questionnaire**

The questionnaire consists of 3 categories related to the risk of having sleep apnea. Patients can be classified into High Risk or Low Risk based on their responses to the individual items and their overall scores in the symptom categories.

**Categories and scoring:**

**Category 1:** items 1, 2, 3, 4, 5.
  - Item 1: if ‘Yes’, assign 1 **point**
  - Item 2: if ‘c’ or ‘d’ is the response, assign 1 **point**
  - Item 3: if ‘a’ or ‘b’ is the response, assign 1 **point**
  - Item 4: if ‘a’ is the response, assign 1 **point**
  - Item 5: if ‘a’ or ‘b’ is the response, assign 2 **points**

**Add points. Category 1 is positive if the total score is 2 or more points**

**Category 2:** items 6, 7, 8 (item 9 should be noted separately).
  - Item 6: if ‘a’ or ‘b’ is the response, assign 1 **point**
  - Item 7: if ‘a’ or ‘b’ is the response, assign 1 **point**
  - Item 8: if ‘a’ is the response, assign 1 **point**

**Add points. Category 2 is positive if the total score is 2 or more points**

**Category 3** is positive if the answer to item 10 is ‘Yes’ OR if the BMI of the patient is greater than 30.

**High Risk:** if there are 2 or more categories where the score is positive.
**Low Risk:** if there is only 1 or no categories where the score is positive.

*Adapted from: Table 2 from Netzer, et al., 1999. (Netzer NC, Stooils RA, Netzer CM, Clark K, Strohl KP. Using the Berlin Questionnaire to identify patients at risk for the sleep apnea syndrome. Ann Intern Med. 1999 Oct 5;131(7):485-91)*
REFERENCES


