ROLE OF EXTRACELLULAR FLUID VOLUME IN INDUCING OR AGGRAVATING OBSTRUCTIVE SLEEP APNEA-HYPOPNEA IN PATIENTS WITH RESISTANT HYPERTENSION

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ABSTRACT

Accumulating evidence suggests that volume overload in drug-resistant hypertension (RH) may contribute to the high prevalence of obstructive sleep apnea-hypopnea (OSAH). Upon recumbency, leg fluid volume moves rostrally causing an increase in nuchal and periphrayan geal fluid content, subsequently obstructing airflow. Rostral fluid displacement following lower body positive pressure (LBPP) application and occurring spontaneously overnight were evaluated in subjects with RH (n = 25) and controlled hypertension (n = 15). In both groups, the reduction in mean upper airway cross-sectional area with LBPP strongly related to the amount of fluid displaced from the legs (R^2 = 0.41; p<0.0001), although its magnitude was greater in the RH group (p=0.001; adjusted for propensity score). In both groups, the apnea-hypopnea index strongly related to the amount of fluid spontaneously displaced from the legs during sleep (R^2 = 0.56; p<0.0001), although its magnitude was greater in the RH group (p=0.01; adjusted for propensity score).
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LIST OF ABBREVIATIONS

ABPM – Ambulatory blood pressure monitoring

AHI – Apnea-hypopnea index

BMI – Body mass index

BP – Blood pressure

CH – Controlled hypertension

CI – Confidence interval

CPAP – Continuous positive airway pressure

LBPP – Lower body positive pressure

NT – Normotension

OSAH – Obstructive sleep apnea-hypopnea

R² – Squared correlation coefficient

RH – Resistant hypertension

TBW – Total body water
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CHAPTER 1. INTRODUCTION AND GENERAL AIMS

Hypertension represents a key risk factor for cardiovascular and renal disease and affects one in four adults worldwide.\(^1\) Resistant hypertension (RH) carries a markedly heightened risk profile with 11% of patients referred for uncontrolled hypertension and having at least one follow-up visit meeting its criteria.\(^2\) We surmise that a principal basis for apparent depressor resistance among these patients rests on extracellular fluid volume expansion; unfortunately, however, clinical determination of volume status is limited.\(^3\)

Older individuals are more likely to be salt-sensitive than younger persons, especially among hypertensives.\(^4\) Of note, non-dipping of BP during sleep may be a manifestation of excess intravascular volume as both dietary sodium restriction and diuretic therapy have been shown to restore dipping status in salt-sensitive hypertensives.\(^5,\) \(^6\) Moreover, two additional observations within this patient population have recently emerged. First, studies reveal that the renin-angiotensin-aldosterone axis is abnormally regulated in many salt-sensitive hypertensives as well as subjects with RH. In fact, approximately 20% of RH patients fulfill the criteria for primary hyperaldosteronism.\(^7\) Second, patients with RH have a high prevalence of obstructive sleep apnea-hypopnea (OSAH).\(^8\) In light of these findings, we conjecture that the expanded extracellular fluid volume in RH may be causally related to OSAH. Upon recumbency, leg fluid volume moves rostrally causing an increase in nuchal and peripharyngeal fluid content, subsequently obstructing airflow. Acutely, this has been demonstrated in our laboratory in which lower body positive pressure (LBPP) was
applied to healthy non-obese subjects with resultant increases in neck circumference, upper airway resistance and upper airway collapsibility, and decreases in upper airway cross-sectional area.\(^{(9-11)}\) As such, we conducted two related studies, which we termed the “Nocturnal blood pressure study” and the “Fluid shift study” to highlight that volume overload may be a pathogenic factor in RH, manifesting with non-dipping of BP during sleep and an exaggerated rostral fluid shift with resultant OSAH.
CHAPTER 2. LITERATURE REVIEW

2.1 RESISTANT HYPERTENSION

2.1.1 Prevalence

Hypertension affects one in four adults worldwide and is a major risk factor for cardiovascular disease.\(^1\) Within the hypertensive population, refractory or drug-resistant hypertension (RH) is emerging as a significant and growing problem in managing hypertension. Older studies suggested that the prevalence of RH ranges from 3% to 5%.\(^{12, 13}\) However, in a recent chart review conducted at a hypertension centre, 11% of patients referred for uncontrolled hypertension with at least one follow-up visit were found to meet criteria for RH.\(^2\) A number of patient characteristics have been associated with RH including older age, obesity, black race, diabetes mellitus and chronic kidney disease.\(^{14}\)
2.1.2 Diagnosis and prognosis

RH has been conventionally defined as persistently elevated blood pressure (BP) (≥ 140/90 mmHg in most patients, ≥ 130/80 mmHg in patients with diabetes mellitus or chronic kidney disease) despite good adherence with an antihypertensive drug regimen of ≥ 3 agents at maximal or near-maximal doses including a diuretic.\(^{(15)}\) BP remains uncontrolled most often because of persistent elevations in systolic BP with the disparity in systolic versus diastolic BP control widening with increasing age.\(^{(16, 17)}\) More recently, a consensus paper suggested that patients whose BP values are controlled below these levels but who require ≥ 4 agents (coined ‘controlled RH’) also be considered as having RH.\(^{(14)}\) RH must be distinguished from pseudoresistance as a result of poor BP measurement technique, white-coat hypertension, co-administration of interfering substances (e.g. non-steroidal anti-inflammatory drugs), patient nonadherence with prescribed treatment and clinical inertia where physicians fail to intensify treatment when therapeutic goals are not met.\(^{(14)}\) In some instances, the cause is readily apparent such as excessive alcohol intake or a secondary form of hypertension. In many instances, however, the etiology is not obvious. Prognostically, this is a high risk group; RH patients exhibit an increased incidence of cardiac and extracardiac hypertensive target organ damage.\(^{(18)}\)
2.1.3 Pathogenesis

2.1.3.1 Occult intravascular volume expansion

A defining feature of RH is the inclusion of a diuretic in its treatment. This prerequisite originally stemmed from older studies demonstrating an increase in plasma volume, measured directly using radioactive iodine-labelled albumin and a fall in BP with diuretic therapy.\(^{(19-22)}\) Age and the dipping pattern of BP (≥ 10% fall in nocturnal BP) are also important considerations in evaluating patients for RH. Older individuals are more likely to be salt-sensitive, defined as having a hypertensinogenic effect on a high sodium diet, than younger persons.\(^{(4)}\) This increase in salt sensitivity of BP with age also occurs more frequently in hypertensive than normotensive individuals and may account, at least in part, for the higher prevalence of uncontrolled hypertension in persons older than 65 years observed in analysis of the NHANES III (3\(^{rd}\) National Health and Nutrition Examination Survey) data.\(^{(23)}\) Loss of the normal dipping pattern of BP during sleep may be a manifestation of intravascular volume excess. This was demonstrated in a series of studies that examined the effects of aggressive diuretic therapy or severe dietary sodium restriction on sleep-wake BP rhythm. These interventions were found to shift the non-dipping pattern to normal in salt-sensitive hypertensive patients.\(^{(5, 6)}\) Of note, 66% to 69% of RH patients were found to be non-dippers.\(^{(24, 25)}\)

While it is clear that optimizing volume status is an important aspect of treating RH, this, of course, relies on an accurate assessment of intravascular volume. Unfortunately though, the clinical determination of intravascular volume is
notoriously unreliable\(^{3}\) with the achievement of euvolemia among hypertensive patients with diuretic treatment often eluding even astute clinicians. This was best demonstrated in a 3-month interventional study of 117 drug-resistant hypertensive patients randomized to receive antihypertensive drug therapy based on the judgment of a certified specialist in clinical hypertension (specialist care) or dictated by non-invasive hemodynamic measurements (including cardiac output, systemic vascular resistance and cardiopulmonary blood volume) obtained by thoracic electrical bioimpedance and a predefined algorithm (hemodynamic care). At entry, 91% of patients were taking a diuretic. After 3 months of treatment, BP was reduced by intensified treatment in both treatment groups. However, those receiving ‘hemodynamic care’ had significantly lower BP and better BP control (56% vs. 33%) than those receiving ‘specialist care’. Covert volume expansion seemed to account for the better BP outcome in the ‘hemodynamic care’ group as the final diuretic dosing was significantly higher in that cohort and there were no between-group differences in non-diuretic antihypertensive medication usage.\(^{26}\)

Several methods have been used to assess intravascular volume including various plasma biochemical markers (e.g. renin and atrial / brain natriuretic peptides), bioimpedance analysis and inferior vena cava diameter. Unfortunately, no single method has emerged as a gold standard given theoretical and practical limitations inherent in each; for example, inferior vena cava diameter, which is echocardiographically determined, is costly, not routinely employable and is subject to significant interpatient variability and interoperator error.\(^{27}\) Plasma renin has been
used as a clinical marker of volume expansion and to guide selection of antihypertensive drugs. However, numerous drugs and other conditions can affect its interpretation, thus markedly reducing its utility as a blood volume marker. Plasma atrial / brain natriuretic peptide levels vary with cardiac atrial / ventricular filling pressures and have been used to assess volume status in heart failure patients. Their use in other patient populations such as persons with obstructive sleep apnea-hypopnea (OSAH) have provided mixed results possibly because of the confounding effects of obesity.\textsuperscript{(28-33)} Moreover, the levels are not able to reliably distinguish patients with mild intravascular volume expansion from those who are euvolemic or even hypovolemic. In addition, elevated plasma brain natriuretic peptide levels may indicate the presence of diastolic dysfunction, a common left ventricular response to chronic uncontrolled hypertension, rather than reflecting intravascular volume status in the RH population.\textsuperscript{(34, 35)} Bioimpedance analysis has been evaluated in many studies for the estimation of volume status because of its ease of use, low cost, reproducibility and non-invasive nature. Many studies have shown a strong direct correlation (Pearson’s correlation coefficients $> 0.9$) between this method and total body water (TBW) as determined by conventional isotopic dilution techniques, which are considered the gold standard.\textsuperscript{(27)} Using better measures of agreement, a cross-sectional study of renal transplant recipients demonstrated an intraclass correlation coefficient of 0.943 and a mean difference of 0.7 L (95% confidence interval [CI], 0.2-2 L) via a Bland-Altman plot between isotope dilution and bioimpedance analysis.\textsuperscript{(36)} As
mentioned above, this test has been successfully used to direct antihypertensive care in patients with RH including diuretic treatment.\(^{(26)}\)

The mechanisms whereby volume expansion occurs in RH are largely unknown and likely heterogeneous. Effective control of circulating volume is a complex physiologic bodily process. Changes in plasma volume are sensed by strategically located vascular stretch receptors (carotid sinus baroreceptors, cardiopulmonary baroreceptors, juxtaglomerular apparatus) that are linked to a series of effectors. The kidney is the primary long-term effector that regulates sodium and water excretion. Through hemodynamic and neurohormonal influences, it responds appropriately to changes in effective circulating volume. For example, healthy individuals respond to volume expansion by a variety of means including a decrease in renin release, angiotensin II formation and aldosterone synthesis, inhibition of sympathetic neural activity and stimulation of atrial / brain natriuretic peptides all resulting in a compensatory reduction in renal tubular sodium reabsorption. In RH, one or more of these homeostatic mechanisms fail to operate normally resulting in sodium retention, volume expansion and an increase in BP.
2.1.3.2 Primary hyperaldosteronism

It is well known that the renin-angiotensin-aldosterone axis does not operate normally in a sizable fraction of the hypertensive population. Early studies classified patients with essential hypertension into three renin-based categories (low, 25%; normal, 60%; high, 15%) with patients in each category having relatively distinct clinical features.\(^{(37)}\) Low-renin hypertensive patients tended to be older and were more responsive to the BP lowering effect of a diuretic. Subsequent studies found that aging was associated with a reduction in plasma renin activity, although the decrease was greater in hypertensives than in normotensive individuals. The effects of aging on plasma aldosterone were more complex with values remaining unchanged with aging under basal conditions, unlike renin levels. However, with volume expansion, they were higher in hypertensive than normotensive subjects, regardless of age. Moreover, salt-sensitive patients had higher plasma aldosterone values for a given renin level.\(^{(4)}\)

Recent studies suggest that the renin-angiotensin-aldosterone axis is abnormally regulated in many RH patients.\(^{(38, 39)}\) Specifically, in RH patients, plasma renin is commonly suppressed (more than 75% in one study\(^{(40)}\)) as would be anticipated but unexpectedly, the plasma aldosterone level and aldosterone / renin ratio are elevated.\(^{(38, 41)}\) Moreover, the administration of an aldosterone antagonist such as spironolactone or amiloride (which indirectly antagonizes aldosterone through blockade of epithelial sodium channels) markedly reduced BP\(^{(42-44)}\), independent of primary hyperaldosteronism status.\(^{(42)}\) In fact, the best predictor of BP lowering following the institution of spironolactone was the depressed plasma renin level.\(^{(45)}\) In
studies undertaking detailed testing for primary hyperaldosteronism, approximately 20% of RH patients fulfilled the criteria for this diagnosis. Parenthetically, in most instances, the cause was bilateral adrenal hyperplasia rather than an aldosterone-producing adrenal adenoma. These observations suggest that aldosterone excess plays a significant role in the pathogenesis of RH by increasing distal renal tubular sodium reabsorption.
2.1.3.3 Obstructive sleep apnea-hypopnea

OSAH and hypertension are strongly associated and numerous adjusted analyses and case-control studies have confirmed that this association is independent of obesity, a major risk factor for OSAH.\(^{(47-49)}\) It is defined by the frequency of apneas and hypopneas per hour of sleep (i.e. apnea-hypopnea index or AHI) and originally by the presence of hypersomnolence (i.e. OSAH syndrome). However, the classical OSAH symptoms of restless sleep, nocturnal dyspnea, morning headaches and excessive daytime sleepiness are uncommon in hypertensive and heart failure patients with an elevated AHI.\(^{(8, 50)}\) The prevalence of OSAH in a working population, aged 30 to 60 years, was 5% in women and 15% in men, using a threshold value for AHI of \(\geq 10\) events/hour.\(^{(51)}\) In hypertensive patients, prevalence estimates have generally been higher with most studies reporting a range of 30% to 40% using the same AHI threshold value.\(^{(52)}\) Moreover, the prevalence of OSAH is particularly high in RH; it was first reported at 83% overall, 92% in men and 65% in women.\(^{(8)}\) Importantly, in that report, the subjects were drawn from a specialty hypertension clinic and selected exclusively for RH, irrespective of sleep-related symptoms.\(^{(8)}\) These initial findings have been confirmed by other groups.\(^{(53, 41)}\) For example, in a matched case-control study, OSAH, defined as an AHI \(\geq 10\) events/hour, was found to be strongly associated with RH compared to controlled hypertension (CH) with an adjusted odds ratio of 4.8.\(^{(53)}\)

The mechanisms by which OSAH induces hypertension have not been fully elucidated. However, laboratory studies addressing mechanistic pathways for such an
association are disproportionately represented by those that involve the sympathetic nervous system (whether gauged by plasma or urine catecholamines or microneurography), implying that OSAH-induced hypertension may have a predominantly neurogenic basis.\textsuperscript{(54)} For example, in humans, muscle sympathetic nerve activity and/or catecholamine levels have been shown to be elevated in patients with OSAH, even during daytime wakefulness when normoxemic and normocapnic, compared to non-OSAH controls,\textsuperscript{(55)} and these high levels are decreased by the most widely used treatment for OSAH, continuous positive airway pressure (CPAP).\textsuperscript{(56-59)} successful treatment of OSAH with CPAP in patients with RH was associated with a significant fall in both nighttime and daytime BP, implying a possible causal role of OSAH in the pathogenesis of RH.\textsuperscript{(60)} Experimental data have also implicated other potential pathways in mediating hypertension including endothelial dysfunction, systemic and vascular inflammation, elevated endothelin-1 levels, increased arterial stiffness (particularly relevant in older patients), insulin resistance and stimulation of the renin-angiotensin-aldosterone axis with many of these purported mechanisms demonstrating normalization following treatment with CPAP.\textsuperscript{(61)} For example, plasma angiotensin II and aldosterone levels have been reported to be higher in patients with OSAH compared to healthy controls without OSAH or hypertension; moreover, the reduction in plasma angiotensin II levels with CPAP treatment correlated with the daytime and nighttime reductions in BP.\textsuperscript{(29)} In a study of consecutive patients evaluated for RH, subjects at high risk for OSAH had significantly greater 24-hour urinary aldosterone excretion and lower plasma renin activity than those at low risk
for OSAH. These findings suggest that OSAH may contribute to the development of RH by stimulating aldosterone secretion; alternatively, another mechanism whereby aldosterone excess begets RH may be by inducing OSAH through upper airway fluid retention.

Obesity is an important contributing factor to OSAH due to peripharyngeal fat accumulation, which decreases upper airway cross-sectional area and increases its collapsibility. However, increased body weight and neck girth only account for approximately one third of the variability in the AHI. Observations drawn from other patient populations suggest that covert fluid accumulation in the extracellular fluid compartment may be causing or aggravating OSAH and in turn, hypertension. In dialysis-dependent patients, the conversion from conventional to nocturnal hemodialysis was associated with a significant reduction in the AHI overall and virtually eliminated sleep-related breathing disturbances in patients with this condition (half the study population). Withholding a nocturnal hemodialysis session for only one day resulted in a significant worsening of the AHI. In another study of dialysis-dependent patients, nocturnal automated peritoneal dialysis improved AHI compared to continuous ambulatory peritoneal dialysis despite equivalent total (peritoneal + renal) small solute clearance. In discussing the findings of both studies, the investigators raised the possibility that enhanced net ultrafiltration, particularly during sleep, contributed to the improvement in OSAH. A subsequent study by the latter group revealed corresponding increases in pharyngeal volume and cross-sectional area as assessed by volumetric magnetic resonance imaging and
significantly greater net ultrafiltration per night by 1.3 L with nocturnal peritoneal dialysis compared to continuous ambulatory peritoneal dialysis.\(^{65}\) Of note, other potential explanations for these findings include a reversal of uremic neuropathy and/or myopathy (with resultant neuromuscular dysfunction in the upper airway), the correction of uremic acidosis (with its attendant compensatory hypocapnia; however, since acidosis is a respiratory stimulant that affects both respiratory pump muscles and pharyngeal dilators, correction of acidosis is very unlikely to be a cause of the sleep apnea alleviation) and removal of uremic toxins. In fact, in the latter study, the improvement in central sleep apnea was hypothesized as secondary to better nighttime small solute clearance.\(^{65}\) The improvement in BP control with nocturnal hemodialysis has been attributed, at least in significant part, to a decreased extracellular fluid volume and it has been speculated that such a decrease, particularly in the upper airway, may have a salutary effect on the pathophysiology of OSAH.\(^{66}\) A recent study in non-dialysis obese subjects with OSAH and diastolic heart failure demonstrated a reduction in body weight, BP, forced mid-expiratory to forced mid-inspiratory airflow ratio and AHI with an increase in upper airway cross-sectional area following a three day course of furosemide and spironolactone.\(^{67}\) In aggregate then, these findings suggest that the expansion of extracellular fluid volume in RH may be the basis for the high prevalence of OSAH in these patients, which in turn aggravates hypertension.

Support for this concept comes from several other sources. Shepard et al. tested the effects of shifting fluid into the neck by raising the legs and of reducing venous
return by applying venous occlusive tourniquets around the thighs in OSAH patients. Using computed tomography, they found that pharyngeal cross-sectional area tended to decrease in response to leg raising and to increase in response to tourniquet application. However, these changes in upper airway cross-sectional were not significant, perhaps because the interventions did not cause sufficient fluid displacement to alter upper airway geometry. Other investigators showed that the application of lower body positive pressure (LBPP) by antishock trousers, which displaces a similar leg fluid volume in healthy subjects as when transitioning from upright to recumbent, increases central venous pressure in normal subjects. Further, in awake, healthy, non-obese adults, rostral fluid displacement by LBPP application increased neck circumference and upper airway resistance and collapsibility and decreased upper airway cross-sectional area. Moreover, in another study involving 23 otherwise healthy non-obese men with suspected OSAH, the overnight rostral fluid shift correlated best with the AHI, accounting for approximately two thirds of its variability. These latter findings provide the strongest evidence to date that spontaneous overnight rostral fluid redistribution plays a role in the pathogenesis of OSAH.
2.1.3.4 Obesity

It is well known that excess weight is associated with an increased prevalence of hypertension and clinical trials of weight reduction in overweight hypertensive patients have shown marked falls in BP.\(^{(73)}\) Obesity also complicates the treatment of hypertension and is a major risk factor for having uncontrolled hypertension.\(^{(74, 17)}\) In our study of RH patients, 61% were obese (defined as a body mass index \(\geq 30\) kg/m\(^2\); see “Nocturnal blood pressure study”\(^{(75)}\)), whereas in a study of a cohort of more than half a million American adults, aged 50 to 71 years at baseline, the prevalence of obesity was only 22%.\(^{(76)}\) The mechanisms by which obesity reduces the effectiveness of antihypertensive treatment and contributes to RH are unclear. Factors that have been implicated include endothelial dysfunction\(^{(77)}\) and increased sympathetic nerve activity\(^{(78)}\) with the latter often ascribed to the central sympathetic effects of such putative factors as hyperinsulinemia and hyperleptinemia\(^{(79)}\) and possibly aggravated by the co-existence of OSAH and aldosterone excess\(^{(39)}\), which would increase renal tubular sodium reabsorption. Additional antinatriuretic mechanisms in obesity, which operate via enhanced renal tubular sodium reabsorption\(^{(80)}\), consist of systemic and adipose tissue renin-angiotensin-aldosterone system activation\(^{(81, 82, 79, 83)}\) and reduced atrial / brain natriuretic peptide levels\(^{(32, 83)}\). Further, insulin resistance to glucose metabolism, promoted by obesity, results in hyperinsulinemia with increased proximal renal tubular sodium reabsorption\(^{(84)}\) due to relatively preserved renal insulin sensitivity\(^{(85)}\) and a hyperglycemia-induced increase in the filtered glucose load with a
parallel reabsorption of excess filtered glucose and sodium via a sodium-glucose cotransporter\textsuperscript{(86)}. 
2.2 NON-DIPPING AND NOCTURNAL HYPERTENSION

2.2.1 Summary

Awareness of the normal sleep-wake cycle of BP has resulted in an increasing appreciation for the significance of non-dipping, a concept often used to construe nocturnal hypertension. Notwithstanding various limitations, principally related to its reproducibility, the identification of nocturnal hypertension has been shown to predict deaths and/or adverse cardiovascular events both independent of and often superior to daytime BP. Several patient profiles have been associated with its occurrence (e.g. older age, black race, diabetes mellitus, obesity, chronic kidney disease, sleep-related disturbances); moreover, many of these states are associated with sympathoexcitation and/or augmented salt sensitivity suggesting a common pathophysiologic basis. Further, a number of therapeutic strategies (e.g. dietary salt restriction, diuretic therapy, dietary potassium liberalization, weight loss, chronotherapy, nocturnal CPAP) have demonstrated restorative capacity for the normal circadian BP rhythm. Nevertheless, whether greater effort expended on achievement of nighttime BP control above and beyond daytime BP results in a greater improvement in patient outcomes remains to be seen. This subchapter has been accepted for publication in Integrated Blood Pressure Control and reproduced with permission.\(^{(87)}\)
2.2.2 Introduction

An appreciation of the normal sleep-wake cycle of BP and urinary sodium excretion in humans, characterized by a nocturnal fall and diurnal rise, has been well established for several decades.\(^{(88)}\) Consequently, this has resulted in an increased awareness of nocturnal hypertension as well as various sleep BP pattern disturbances, gleaned non-invasively from the recordings provided by 24-hour ambulatory BP monitoring (ABPM). Specifically, the presence of non-dipping of BP during sleep and nocturnal hypertension are being increasingly recognized and regarded as important harbingers of poor cardiovascular prognosis. This subchapter addresses several aspects related to the non-dipping BP pattern and nocturnal hypertension including diagnostic limitations, pathogenesis and associated patient profiles, prognostic significance and therapeutic strategies. Taken together, persistent non-dipping and nocturnal hypertensive status, perhaps secondary to altered diurnal rhythm of urinary sodium excretion and/or nocturnal sympathovagal balance, are strongly associated with deaths, cardiovascular events and progressive loss of renal function, independent of daytime and 24-hour BP. Several pharmacological and non-pharmacological approaches may restore the normal circadian rhythm of BP and urinary sodium excretion; however, whether this translates to a clinically meaningful reduction in unfavourable cardiovascular and renal consequences remains to be seen.
2.2.2.1 Definitions

Nocturnal hypertension has typically been cited synonymously with the presence of non-dipping of BP during sleep. Specifically, non-dipping has been defined as a $< 10\%$ fall in nocturnal BP relative to diurnal BP (i.e. $\frac{\text{daytime BP} - \text{nighttime BP}}{\text{daytime BP}} \times 100\%$)\(^{(89)}\), arithmetically equivalent to a night-to-day BP ratio $> 0.9$. Non-dippers have been further subdivided into either attenuated dippers ($\geq 0\%$ but $< 10\%$ fall) or risers ($< 0\%$ fall). Interestingly, there is also a patient subset, situated at the opposite end of the dipping spectrum, referred to as extreme dippers ($\geq 20\%$ fall) that has been linked with silent and clinical end organ ischemia, perhaps related to nocturnal hypoperfusion and/or an exaggerated morning BP surge\(^{(90-93)}\); however, this entity is not further discussed herein. In other circles, the presence or absence of nocturnal hypertension has been defined using various absolute nocturnal BP cut-offs. According to the American Heart Association Council on High Blood Pressure Research, nocturnal BP $< 115/65$ is deemed optimal, $< 120/70$ is deemed normal and $> 125/75$ is deemed abnormal.\(^{(89)}\) Of note, although high nocturnal BP may be accompanied by a non-dipping pattern, both are not always concomitantly present and the pathophysiologic and clinical significance of each may differ.\(^{(94)}\)
2.2.2.2 Diagnostic limitations

Over the years, a number of diagnostic limitations categorizing dipping status and nocturnal BP have been identified. First, there is no universal agreement on the definition of non-dipping. For example, in some reports, it is defined as higher sleep BP level than awake BP level, rather than the more commonly used definition of a < 10% fall in nighttime BP. Second, analyses involving repeated measurements have questioned the reproducibility of dipping status and diagnosing nocturnal hypertension using cut-off values, given the well-described phenomenon of regression to the mean. On repeated ABPM, expressing the decline in BP during sleep as a continuous variable (e.g. percentage fall in nocturnal BP) was shown to be far more reproducible than its expression as a categorical variable (i.e. using the traditionally adopted 10% cut-off), given a loss of information. This is particularly an issue for defining abnormalities of dipping status as definitions rest on a difference score of individual daytime and nighttime BP levels. In a study of repeated ABPM, agreement in diagnosing nocturnal hypertension on the basis of two measurements was greater using an absolute nocturnal BP threshold value (Cohen’s kappa = 0.485 [systolic BP]) rather than the percentage nocturnal BP fall (Cohen’s kappa = 0.378 [systolic BP]). Because of better metrics, nocturnal hypertension using threshold values was also a better predictor of left ventricular hypertrophy than non-dipping BP pattern. Third, the dipping pattern of systolic BP has been shown to be more reproducible than that of diastolic BP in several studies, although not all, fourth, the methods used to define the nighttime period have varied from
arbitrarily fixed clock times to self-reported sleep times using diary entries or even, polysomnographically verified sleep times. With respect to the arbitrarily fixed clock time method, one improvement has involved the use of narrow clock intervals to exclude the transition period from 0600 to 1000 h\textsuperscript{(105)} or 0700 to 1000 h\textsuperscript{(106)} in the morning and from 2000 to 0000 h\textsuperscript{(105)} or 2300-0100 h in the evening.\textsuperscript{(106)} Recently, it was shown that different definitions significantly affect the classification of nocturnal BP dipping and its relation to left ventricular mass index and urinary albumin excretion as indices of hypertensive target organ damage.\textsuperscript{(98, 107)} Fifth, failing to account for nighttime awakenings\textsuperscript{(108)} and daytime naps\textsuperscript{(109)} has been associated with misclassification of nocturnal hypertension. Additionally, variations in nocturnal body position and diurnal physical activity affect nighttime and daytime BP.\textsuperscript{(110, 111)} Finally, nocturnal BP may be unreliable as a prognostic marker for cardiovascular events and deaths among subjects with perceived sleep deprivation $\geq$ 2 hours during ABPM related to repeated cuff inflations.\textsuperscript{(112)}
2.2.3 Pathophysiology

Although there have been several theories as to the pathogenesis of non-dipping\textsuperscript{(113)}, perhaps the two most cited explanations rest on disturbances in the diurnal rhythm of renal sodium excretion and abnormalities of nocturnal autonomic or sympathovagal balance. Further, the association of non-dipping pattern with patient profiles are relevant to a discussion on mechanisms. Specifically, several patient characteristics have been associated with nocturnal hypertension and non-dipping BP pattern; not surprisingly, many of these states are associated with impaired renal capacity to excrete sodium and/or relative sympathoactivation.
2.2.3.1 Diurnal rhythm of renal sodium excretion

Many studies have shown an association between disturbed diurnal renal sodium excretion and nocturnal hypertension and non-dipping pattern of BP during sleep. In a recent study of a large group of African subjects, those with the lowest day-to-night ratios of urinary sodium excretion had significantly higher nighttime systolic BP and lower systolic BP dipping in both unadjusted and adjusted analyses.\(^{(102)}\) The ‘low daytime sodium excretors’ excreted three times less sodium during the day than during the night, whereas the ‘high daytime sodium excretors’ had a sodium excretion rate that was 46% higher during the day than during the night, although total daily sodium excretion was equivalent in both groups. These findings are in accord with earlier, smaller studies conducted during hospitalization.\(^{(114, 5, 115)}\) In a study of Japanese patients with essential hypertension, non-dippers on a high sodium diet had a significantly higher nighttime systolic BP and mean arterial pressure and showed no nocturnal decrease in BP compared to the dippers.\(^{(5)}\) Furthermore, nighttime urinary sodium excretion was significantly greater in non-dippers than dippers and there was a strong positive relationship between night-to-day ratios of urinary sodium excretion and mean arterial pressure. When the non-dippers were placed on a sodium-restricted diet, there was a significant fall in systolic BP and mean arterial pressure in the non-dippers and there was no longer a significant difference in the night-to-day ratios of urinary sodium excretion between non-dippers and dippers. Given a greater antinatriuresis during the day in the non-dippers, higher nocturnal BP, as a result of pressure-natriuresis with near infinite gain over a wide
range of urinary sodium excretion, may account for the greater proportion of urine sodium excreted at night to maintain total body sodium balance.\(^{(116)}\)

Enhanced salt sensitivity of BP is often found in non-dippers\(^{(116)}\) and has been suggested as mediating nocturnal hypertension.\(^{(114,\, 115)}\) Among black normotensive adolescents, a significantly greater proportion of non-dipping for mean arterial pressure at 50% was identified in those deemed salt sensitive (defined as a $\geq 5$ mmHg BP difference between the high and low dietary salt states) as compared to 18.9% in those deemed salt resistant.\(^{(117)}\) Further, a Japanese study revealed that the nocturnal fall of BP from daytime to nighttime was only significant among salt resistant hypertensives (defined as a $< 10\%$ BP difference between the high and low dietary salt states) unlike their salt sensitive hypertensive counterparts.\(^{(5)}\) This may be related to a disturbed antinatriuretic response to postural change. Specifically, among hypertensives, although the sodium excretion rate was found to be significantly less in the upright than the supine position in both non-dippers and dippers, the difference was magnified in the non-dippers (despite no difference in postural BPs) because of an exaggerated reduction in upright sodium excretion.\(^{(118)}\)

The renal sodium regulatory mechanisms that give rise to nocturnal hypertension and non-dipping pattern remain unclear. In the case of chronic kidney disease, reduced ultrafiltration capacity likely accounts for the blunting of the nocturnal fall in BP along with enhanced natriuresis at night.\(^{(119)}\) However, many individuals with disturbed diurnal renal sodium excretion have normal glomerular function\(^{(102)}\); in these instances, augmented tubular reabsorption of sodium during the
day likely contributes to the genesis of the nocturnal BP and natriuretic abnormalities.\textsuperscript{(116)} In salt sensitive forms of hypertension such as primary aldosteronism, or patients with diabetes mellitus or metabolic syndrome, there is evidence of enhanced tubular sodium reabsorption that may account for non-dipping.\textsuperscript{(115, 119)} Further evidence supporting a role for renal tubular sodium handling comes from a study of patients with essential hypertension showing a differential effect of hydrochlorothiazide, which inhibits sodium transport in the distal nephron, on nocturnal fall in BP in non-dippers compared to dippers; diuretic therapy restored the dipping BP pattern in non-dippers but had no effect in dippers.\textsuperscript{(6)}
2.2.3.2 Nocturnal sympathovagal balance

Nocturnal sympathoexcitation has also been suggested as mediating nocturnal hypertension.\(^\text{(110, 95)}\) This is in contrast to the normal sleep-wake cycle of sympathetic neural activity characterized by sympathoinhibition during sleep (particularly non-rapid-eye-movement sleep).\(^\text{(120, 121, 31)}\) In a cohort of normotensive and hypertensive subjects, non-dippers were observed to have a reduced nighttime fall in urine catecholamine levels and heightened alpha-1-adrenergic receptor responsiveness to phenylephrine (but similar beta-adrenergic receptor responsiveness to isoproterenol), unlike dippers.\(^\text{(122)}\) A recent study found a significant inverse relationship between the degree of sympathetic activation, measured by muscle sympathetic nerve activity, and the magnitude of nighttime fall in BP.\(^\text{(123)}\) In that report, the increase in sympathetic activity was greatest in subjects demonstrating an increase in nocturnal BP (risers). The findings are in accord with results of earlier studies demonstrating a higher mean daytime plasma norepinephrine level in risers\(^\text{(95)}\) and a greater reduction of nighttime BP following administration of the alpha-adrenergic blocker, doxazosin\(^\text{(124)}\). Several reports using power spectral analysis of heart rate variability suggest that a reduction in parasympathetic nervous system activity may also contribute to the non-dipping BP pattern in essential hypertension\(^\text{(125, 126)}\)
2.2.3.3 Associations

A variety of factors including patient characteristics, clinical conditions, and dietary patterns have been reported to be associated with non-dipping. These links often provide some pathophysiologic insights.
2.2.3.3.1 Patient characteristics

Multivariable assessment of a large cohort of untreated hypertensive men and women revealed a highly significant inverse linear correlation between the day-night systolic BP change and age in both sexes.\(^{(127)}\) The prevalence of the non-dipping pattern increased progressively with age, reaching > 40% in subjects \(\geq 70\) years. Several studies have also noted increasing salt sensitivity of BP with increasing age, particularly among hypertensive patients, using provocative maneuvers to either contract or expand extracellular fluid volume.\(^{(4)}\) In fact, the presence of hypertension has been suggested as conferring a lower nocturnal fall in BP\(^{(128)}\), although this has not been unanimously confirmed.\(^{(129)}\) Blacks have been consistently shown to have a greater frequency of both reduced nocturnal fall in BP\(^{(130-132, 23, 133)}\) and salt sensitivity of BP\(^{(4, 134)}\) than whites. Blacks also have higher ambulatory heart rates and lower heart rate variability than non-blacks, possibly reflecting greater impairment in sympathovagal balance in this racial group.\(^{(135)}\) There does not appear to be a sex difference in the prevalence of non-dipping\(^{(127)}\); nonetheless, several studies have shown more adverse health effects in hypertensive women with blunted nocturnal fall in BP. In contrast to men, they have a greater left ventricular mass index\(^{(136, 137)}\) and are more likely to develop a cardiovascular complication\(^{(138)}\).
2.2.3.3.2 Clinical Conditions

Several studies have shown that non-dipping is more commonly found in clinical conditions, including hypertension, diabetes mellitus, impaired glucose tolerance, renal disease, disrupted sleep, and sleep apnea. Overall, it is estimated that approximately 22% of patients with essential hypertension have a non-dipping pattern. \(^{110}\) Among treated hypertensives, non-dipping may also be partly related to the absence of uniform 24-hour therapeutic antihypertensive coverage, perhaps especially in those treated with single morning doses and/or short-acting antihypertensives. \(^{139}\)

The higher prevalence of non-dipping BP pattern in diabetes mellitus \(^ {110}\) does not appear to be a consequence of increased nocturnal awakenings from nocturia due to osmotic diuresis and/or neurogenic bladder. Among subjects reporting having taken an uninterrupted afternoon siesta, diabetics displayed a similar naptime BP decline to their nighttime BP decline, which was blunted as compared to the non-diabetic controls. \(^ {140}\) Insulin resistance, even among non-diabetic subjects, is associated with both a reduced nocturnal fall in BP and enhanced salt sensitivity of BP. \(^ {141}\)

Abnormal diurnal BP variability is present in patients with chronic kidney disease such that they are more likely non-dippers. \(^ {142, 143}\) For example, the mean nocturnal systolic BP change among patients with essential hypertension was found to be a decrease of 12.7 mmHg compared to an increase of 2.7 mmHg among hypertensive patients with renal parenchymal disease, despite matching for age, sex and 24-hour BP. \(^ {144}\) Additionally, the decrease in unadjusted creatinine clearance
following donor nephrectomy was found to correlate with the corresponding increase in the night-to-day BP ratio but not the 24-hour, daytime or nighttime BP.\(^{(145)}\) Non-dipping has also been linked to accelerated loss of renal function. In a retrospective cohort study of outpatients attending a hospital clinic, non-dipping was associated with subsequent deterioration in renal function that was independent of baseline renal function, systolic BP load and other risk factors for renal impairment.\(^{(146)}\) Additionally, in a study of renal transplant recipients, non-dippers and risers had lower glomerular filtration rates, measured by iothalamate clearance, than dippers one year after transplantation; there was no difference in iothalamate glomerular filtration rates at the three week time point post-transplantation.\(^{(147)}\)

Short habitual sleep duration may be associated with non-dipping of nocturnal BP. In two separate studies, untreated hypertensive patients who were non-dippers were found to have significantly shorter durations of sleep by 0.3 hours in men and 0.5 hours in women (according to self-report)\(^{(127)}\) or by 81 minutes (according to wrist actigraphy)\(^{(148)}\), compared to their dipper counterparts; further multivariable analysis in one of these studies showed a positive linear correlation between the day-night systolic BP change and sleep duration.\(^{(127)}\) Moreover, poor sleep quality has also been suggested as being related to nocturnal hypertension. In a study of normotensive and hypertensive subjects with an AHI < 10, the degree of nocturnal BP dipping was positively linked with the percentage of total sleep time occupied by stage 4 non-rapid eye movement sleep (reflecting depth of sleep) and negatively linked with the percentage of wake time following sleep onset (reflecting sleep fragmentation).\(^{(59)}\)
Similar findings of less time spent in stage 4 non-rapid eye movement sleep and sleep fragmentation with micro/arousals along with other qualitative disturbances have also been reported by others.\(^{(149, 53, 150)}\) Notwithstanding the foregoing relations, sleep-disordered breathing, particularly OSAH, has been strongly associated with non-dipping.\(^{(151, 152, 47, 153)}\) Notably, the vast majority of mechanistic studies linking OSAH and hypertension are disproportionately represented by those implicating sympathoactivation (whether gauged by plasma or urine catecholamines or microneurography), independent of obesity. For example, a recent study involved normotensive subjects who were classified as lean or obese with or without OSAH (according to BMI, waist-to-hip ratio and AHI) and matched for age and sex. Relative to the non-OSAH lean group, muscle sympathetic nerve activity was significantly and similarly greater in both the OSAH lean group and in the non-OSAH obese group; the increase was even more pronounced in the OSAH obese group.\(^{(154)}\)
Several studies have recognized an increased prevalence of non-dipping status in obese patients. Among normotensive women, a blunted nocturnal drop in BP was observed in obese compared to non-obese, age-matched subjects. Salt sensitivity also appears to be more common in obese individuals. For example, in a study of obese and non-obese adolescents, when the obese group was changed from a high salt to a low salt diet there was a greater fall in their BP compared to non-obese group. This increased sensitivity to dietary salt intake may be a manifestation of the hyperadrenergic state that is a feature of abdominal obesity and often ascribed to the central sympathetic effects of hyperinsulinemia and hyperleptinemia. The prevalence of OSAH also progressively increases as BMI and associated markers of obesity (e.g. neck circumference, waist-to-hip ratio) increase. Dietary monovalent cation intake patterns, characterized by a high sodium or low potassium content, are also recognized as favouring toward a non-dipping pattern, particularly in salt sensitive individuals. The hypertensinogenic effects of a low potassium diet have been attributed, at least partly, to renal sodium retention. In the Dietary Approaches to Stop Hypertension (DASH) Trial, the BP of individuals randomly assigned to the ‘combination’ (DASH) diet that emphasized low fat dairy products, fruits and vegetables, thus containing high amounts of calcium and potassium, was significantly lower during both day and night compared to the BP of those consuming the ‘typical American’ diet; however, the high quality (DASH) diet did not significantly affect dipping status. This is a surprising finding in view of the
evidence from the same group of investigators that the DASH diet increased, thus improving, the pressure-natriuresis slope (i.e. reduced salt-sensitivity).\textsuperscript{(160)} Possibly misclassification error from the use of a fixed clock method to determine dipping status might explain the negative result.
2.2.4 Epidemiological studies

Numerous observational studies have emerged addressing the prognostic significance of nocturnal hypertension on mortality and cardiovascular disease.
2.2.4.1 Considerations

The presence of nocturnal hypertension in one individual but not the other would be expected to connote a greater 24-hour BP load even if daytime BP levels were otherwise equivalent in the two individuals. Certainly then, one would not be surprised to find a larger burden of hypertensive target organ damage in the former individual. What remains less clear is whether nighttime BP is able to predict such vascular insult independent of daytime or 24-hour BP and perhaps further, whether its predictive ability is superior to that provided by daytime BP. If such an association is truly not spurious, several explanations could be held accountable. First, a proper interpretation of any observational study must be tempered by the potential for residual confounding. For example, if nocturnal hypertension were simply a marker for a disease condition (e.g. OSAH) linked to deaths and cardiovascular events, then any association identified with nocturnal hypertension may be inaccurately estimated if the given state and/or condition were either undefined or poorly defined. In addition, it may not be nocturnal hypertension per se but rather its underlying pathophysiologic state (e.g. heightened sympathetic neural tone) that bestows vascular injury. Second, a cause-effect relation may indeed apply. The sleep state is typically associated with the minimum BP necessary to maintain organ perfusion; perhaps then, if also given a permissive hormonal milieu, susceptibility to vascular target organ damage may demonstrate circadian variability for a given BP load marked by an increase during sleep.$^{161, 162}$ Moreover, for the cerebrum, its vasculature may be less protected from hydrostatic (gravitational) forces when recumbent during sleep than
when erect or sitting.\textsuperscript{(163)} Third, an effect-cause relation can be argued instead, particularly with cross-sectional and case-control studies. Specifically, nocturnal hypertension may simply reflect more extensive target organ damage placing the patient in a high-risk group, as opposed to having a direct effect. Fourth, in view of a narrower dispersion of nocturnal BP values compared to diurnal BP values, partly related to ambulatory physical activity\textsuperscript{(164)}, nighttime BP would be expected to perform mathematically, but not necessarily biologically, better as a predictor than daytime BP.\textsuperscript{(94)}
2.2.4.2 Summary of findings

On the basis of ABPM performed 1 to 5 years earlier, a case-control study of hypertensive subjects revealed a significantly lower nocturnal fall in BP among cases as compared to controls (based on the occurrence of a fatal or nonfatal cardiovascular event) but only in women, despite matching for several covariates including daytime BP.\(^{(138)}\) However, the vast majority of more recent studies focusing on the prognostic implications of nocturnal hypertension have been prospective cohort in design. Of course, there is also a robust body of literature that speaks to the association between nocturnal hypertension and a multitude of unfavourable structural (e.g. cardiac chamber dilatation, ventricular hypertrophy, carotid intima-media thickening, silent lacunae, leukoaraiosis, brain microbleeds) and functional (e.g. diastolic dysfunction) cardiovascular consequences.\(^{(165, 90, 166, 18, 167-170)}\) At least 16 larger studies (sample size > 500) involving 14 varied patient populations have examined the clinical endpoints of all-cause mortality, cardiovascular mortality and/or cardiovascular morbidity as related to nocturnal BP.\(^{(171-186)}\) However, additional adjustments for either daytime or 24-hour BP were not uniformly made in such studies to allow for a determination of the independent prognostic significance of nighttime BP. Regardless, the bulk of the existing data suggests that nocturnal hypertension, whether expressed as a categorical or continuous predictor, is strongly associated with deaths and/or cardiovascular events. For example, in the Japanese general population, a 5% attenuation in the nocturnal BP fall was associated with a 20% increase in cardiovascular mortality, even among individuals with a normal 24-hour BP (defined as < 135/80).\(^{(174)}\) In fact,
several studies have also found that nighttime BP, independent of daytime BP, may predict death and/or adverse cardiovascular outcomes better than daytime BP.\(^{(173, 177, 179, 183, 184, 186)}\) For instance, among untreated hypertensive patients, an increase of 10 mmHg in daytime or nighttime BP was associated with hazard ratios for cardiovascular mortality of 1.12 (daytime systolic) compared to 1.21 (nighttime systolic) and 1.04 (daytime diastolic; not significant) compared to 1.19 (nighttime diastolic), after adjustment for the other BP.\(^{(177)}\) In a substudy of the Systolic Hypertension in Europe (Syst-Eur) trial, a 10-mmHg increase in nighttime systolic BP was more predictive for all-cause deaths (adjusted hazard ratio, 1.21), cardiovascular deaths (adjusted hazard ratio: 1.21) and fatal and non-fatal cardiovascular events (adjusted hazard ratio: 1.26) than a comparable increase in daytime systolic BP (adjusted hazard ratios: 0.93, 1.03 and 0.94, respectively; not significant).\(^{(173)}\)
2.2.5 Therapeutic strategies

Whether greater effort should be expended on achieving nighttime BP control and reversal of the non-dipping pattern over and above the attainment of daytime and 24-hour BP control so as to reap a greater reduction in deaths and cardiovascular events remains uncertain. Nonetheless, several strategies have been suggested as preferentially reducing nighttime BP with restoration of the normal circadian BP rhythm.

Both therapeutic lifestyle changes and pharmacological approaches are associated with conversion from non-dipper to dipper status. Two Japanese studies revealed that therapeutic interventions, namely dietary salt restriction and diuretic therapy, which reduce total body sodium content were able to restore the normal dipping pattern of BP in salt sensitive hypertensive patients (defined as a $\geq 10\%$ BP difference between the high and low dietary salt states) unlike their salt resistant hypertensive counterparts.\(^5\, 6\) Similarly, intensified ultrafiltration in hemodialysis patients initially identified as risers led to a conversion to either dipper or attenuated dipper status, in the majority of cases.\(^{187}\) In addition to dietary sodium, among 58 black normotensive adolescents, a 3-week high potassium diet was able to revert salt sensitive (defined as a $\geq 5$ mmHg BP difference between the high and low dietary salt states) individuals originally deemed non-dippers to dippers, which was unlike both their salt resistant counterparts as well as the usual diet control group in which nearly all such individuals remained non-dippers.\(^{188}\) Correspondingly, among normotensive men, potassium supplementation was found to dose-dependently suppress salt
sensitivity (defined as an increase in mean BP ≥ 3 mmHg with salt loading), particularly in blacks who had a greater frequency and severity of salt sensitivity.\textsuperscript{(134)} A significant reduction of nighttime BP following a 1-year multidisciplinary weight loss program was observed among normotensive obese women.\textsuperscript{(156)} Moreover, in morbidly obese (BMI > 35 kg/m\textsuperscript{2}) hypertensive patients, bariatric surgery restored the normal circadian rhythm in non-dippers from a nocturnal systolic BP fall of 4\% to 16.4\%.\textsuperscript{(189)} In parallel, following the institution of a 20-week weight loss program, a decreased salt sensitivity of BP was observed but only in those who had successfully lost more than 1 kg of body weight.\textsuperscript{(85)}

In the area of nocturnal sympathoexcitation, a significant reduction of nocturnal BP following nighttime dosing of an alpha-1-adrenergic blocker in hypertensive patients was identified but in non-dippers only.\textsuperscript{(190)} Pharmacokinetic and pharmacodynamic differences between various antihypertensive medications and their times of administration (“chronotherapy”) may be important factors in determining nocturnal antihypertensive effects. For example, despite equivalent daytime BP control, differential antihypertensive effects on nighttime BP were demonstrated between a long-acting calcium channel blocker and a similarly long-acting angiotensin converting enzyme inhibitor in a group of elderly hypertensive patients.\textsuperscript{(191)} Nocturnal dosing of antihypertensive agents has also been associated with conversion from attenuated dipper or riser to dipper status in both controlled and refractory hypertensive patient populations.\textsuperscript{(192-196)} Uncontrolled short-term studies have revealed reductions in nighttime BP readings along with transition from non-
dipper to dipper status among normotensive and hypertensive subjects following nocturnal application of therapeutic CPAP\(^{(57, 197)}\), which has been associated with sympathoinhibition unlike nocturnal application of subtherapeutic CPAP or oxygen.\(^{(59)}\) In patients with end-stage renal disease, renal transplantation has been associated with a significant lowering of the prevalence of non-dipping from 73% within the first year after transplantation to 27% following the first year after transplantation, independent of 24-hour BP and concomitant medication changes\(^{(198)}\), with the nocturnal BP fall correlating with renal allograft function.\(^{(147)}\) Finally, in randomized parallel and crossover trials, nocturnal administration of exogenous melatonin was associated with a significant reduction of nighttime BP, especially among non-dippers, unlike daytime BP and in some cases, this could not be entirely explained on the basis of an improvement in sleep.\(^{(199-201)}\) Non-dipping in subjects with type 2 diabetes mellitus has been found to relate more closely to post-prandial glycemic excursions, rather than fasting hyperglycemia.\(^{(202)}\) However, whether improvements in glycemic control as well as sleep quality and duration translate into normalization of the circadian BP rhythm remains to be seen.
2.3 OBSTRUCTIVE SLEEP APNEA-HYPOPNEA

2.3.1 Summary

This subchapter, which has been published in part in the American Journal of Hypertension and reproduced with permission\(^{(61)}\), addresses the cardiovascular, cerebrovascular and metabolic consequences that accompany OSAH in conjunction with the mechanistic pathways implicated in mediating these effects. Particular emphasis is placed on the association with hypertension. Varying levels of evidence support a role of OSAH in perpetuating sustained hypertension, nocturnal hypertension and RH as well as in contributing to the occurrences of non-dipping of BP and increased BP variability. In this context, the emergence of matched designs, adjusted analyses, meta-analyses as well as longitudinal and interventional studies strengthens causal inferences drawn from older observational studies, which suffered from such limitations as confounding.

In summary, the probability of OSAH in a given patient can be readily estimated using a bedside prediction rule that encompasses several key patient characteristics. Clinical epidemiological evidence in support of a causal and independent association between OSAH and other disease entities has grown tremendously and is particularly robust in the case of hypertension. There remains a relative paucity of studies addressing mechanisms beyond the sympathetic nervous system by which OSAH wreaks adversity coupled with disparate results addressing the effects of CPAP on some of these purported mechanisms. However, CPAP has been demonstrated, particularly in short-term studies, to effectively, albeit modestly,
reduce BP and possibly other detriments in a mixed population of normotensive and hypertensive individuals with OSAH. The circumstances under and degree to which CPAP treatment will attenuate the long-term risks and sequelae of cardiovascular, cerebrovascular and metabolic disorders in patients with OSAH remain to be defined.
2.3.2 Definitions

Repeated apneas, hypopneas and respiratory effort related arousals occurring during sleep characterize sleep-related breathing disturbances. Whereas apnea is defined by the cessation of oronasal airflow for >10 seconds, hypopnea is defined by a diminution of oronasal airflow to <50% of normal. On the other hand, episodes of increased respiratory effort with maintained oronasal airflow, but which result in arousal from sleep, are referred to as respiratory effort related arousals. The preferred metric for reporting polysomnography results remains the AHI, the total number of apneas and hypopneas per hour of sleep. Other popularly reported parameters include the respiratory disturbance index (total number of apneas, hypopneas and respiratory effort related arousals per hour of sleep), the oxygen desaturation index (the number of times that the oxygen saturation falls by more than 3 to 4% per hour of sleep) and the minimum and mean oxygen saturation. Differentiating obstructive from central apneas or hypopneas rests on ascertaining whether ventilatory effort is present, absent, or reduced. This subchapter, however, focuses on OSAH, typically defined as either an AHI or respiratory disturbance index ≥15 in an asymptomatic patient or ≥5 in a patient with symptoms or signs of disturbed sleep\(^{(203)}\) with an emphasis on its association with hypertension.
2.3.3 Epidemiology and diagnosis

Among middle-aged adults in the general population, the prevalence of sleep-related breathing disturbances, defined as an AHI ≥5 and daytime hypersomnolence, is estimated at 2 to 4% according to data from the Wisconsin Sleep Cohort.\(^{(51)}\) Men are two to three times more likely than women to have OSAH, although the gender difference narrows after menopause. Excessive daytime sleepiness is a cardinal feature of OSAH and likely relates more to sleep fragmentation with resultant chronic sleep deprivation than nocturnal hypoxemia.\(^{(204)}\) Predictably, motor vehicle accidents are alarmingly common in patients with OSAH.\(^{(205)}\) The Epworth Sleepiness Scale is a paper and pencil scale (range, 0 to 24) that provides a rapid and validated method of screening for tiredness in both the clinical practice and research settings.\(^{(206)}\) Several patient characteristics have been associated with OSAH; a prediction model based on neck circumference, hypertension, habitual snoring and bed partner reports of nocturnal gasping or choking can be easily applied to estimate a patient’s probability of having an AHI >10.\(^{(207)}\) However, nocturnal polysomnography conducted in a sleep laboratory has remained the gold standard for diagnosing OSAH.
2.3.4 Consequences

2.3.4.1 Limitations of current literature

Much work has sought to determine whether a causal and independent association between OSAH and a wide assortment of health problems exists. Epidemiological studies implicating OSAH in their pathogenesis are limited by virtue of shared risk factors that confound interpretation such as obesity. Additionally, any causal association between OSAH and another disease may be affected by the constraints that such disease may induce or aggravate OSAH (e.g. stroke or heart failure and reduced drive to pharyngeal dilator muscles as well as peripharyngeal edema in the latter instance).\(^{31}\) Finally, methodological differences among the studies (e.g. different study populations, sample sizes, outcome measures, duration of follow-up) limit comparability. Nevertheless, longitudinal studies examining disease incidence as well as adequately powered studies evaluating the effects of CPAP provide strong support in favour of a causal relationship between OSAH and several disease entities.
2.3.4.2 Hypertension

By far, the association between OSAH and hypertension is the most robust with a reported prevalence of OSAH, using an AHI ≥10 threshold, among hypertensive patients approximating 30% to 40%, depending on the population studied.(52) Recent large-scale observational studies conducted in both general(208, 209, 48) and sleep or weight loss clinic(210, 211) populations using high quality methods to assess for OSAH consistently reveal increased prevalence and incidence of hypertension among patients with sleep apnea. The strongest evidence for an association comes from the Wisconsin Sleep Cohort Study. The odds ratio for the presence of hypertension after 4 years of follow-up was 1.42 (AHI 0.1 to 4.9), 2.03 (AHI 5.0 to 14.9) and 2.89 (AHI ≥15) compared to those with an AHI of zero, after adjustment for several covariates including BMI. Moreover, there was a significant linear trend of odds ratios across AHI quartiles.(48) As such, OSAH is accepted as a cause of hypertension by both European and American clinical practice guidelines.(212, 213)

Some studies suggest that isolated diastolic hypertension and perhaps, systolic/diastolic hypertension, are profiles more likely related to OSAH, particularly if newly diagnosed, than isolated systolic hypertension.(214, 52) In a recent cross-sectional study of 130 patients with incident OSAH, the prevalence of masked hypertension was found to be 30% as compared with sustained hypertension and white coat hypertension which were identified in 35.4% and 3.1%, respectively.(52) Moreover, the presence and/or severity of OSAH have been associated with non-
dipping and nocturnal hypertension in most studies\textsuperscript{(215, 29)}; this may be relevant given the prognostic significance of nocturnal BP and non-dipping independent of 24-hour BP.\textsuperscript{(186)} Equally, hypertensive non-dippers are more likely to have OSAH.\textsuperscript{(152)} Increased systolic BP variability, another prognostically significant parameter,\textsuperscript{(216)} with a positive correlation with AHI has been described in normotensive, otherwise healthy patients with sleep apnea (AHI >20) compared with controls matched for age, sex and BMI.\textsuperscript{(52)} Of note, nighttime awakenings,\textsuperscript{(108)} daytime naps\textsuperscript{(109)} and/or perceived sleep deprivation from repeated cuff inflations\textsuperscript{(112)} can in themselves affect the normal circadian pattern of BP, which may increase BP variability or lead to errors in categorizing individuals as non-dippers or having nocturnal hypertension. As well, the night-to-day BP ratio may be related to other indices of poor sleep quality and/or quantity independent of AHI.\textsuperscript{(217)}

OSAH is particularly prevalent among patients with persistently elevated BP despite antihypertensive drug treatment with reported values ranging from 56\% to 85\%, depending on the criteria used\textsuperscript{(218, 8)}. OSAH, defined as an AHI $\geq$10, was found to be strongly associated with RH compared with CH (adjusted odds ratio, 4.8) in a matched case-control study that corrected for age, sex, BMI and duration of hypertension.\textsuperscript{(53)}
2.3.4.3 Cardiovascular diseases

Not surprisingly, observational studies suggest that patients with OSAH have an increased risk of angina and myocardial infarction with many reports controlling for other known risk factors.\(^{211, 33}\) A case-control study using cases with angina or myocardial infarction and controls matched for age, sex and BMI demonstrated a significant occurrence of OSAH among cases (adjusted odds ratio, 3.1), even after additional adjustment.\(^{211}\) Such patients are also at increased risk of cerebrovascular disease.\(^{219}\) In a prospective cohort study, OSAH, based on an AHI $>$ 5, was found to be significantly associated with first stroke/transient ischemic attack or death (adjusted hazard ratio, 1.97), independent of other risk factors including hypertension, with incremental increases in risk across AHI quartiles.\(^{220}\) Cross-sectional data from the Sleep Heart Health Cohort revealed a significant dose-response relationship of self-reported cardiovascular and cerebrovascular disease across AHI quartiles ranging from only 1.3 to 11, even after adjustment for multiple covariates including age, sex, BMI, smoking status, self-reported diabetes mellitus, self-reported hypertension and systolic BP.\(^{221}\) In patients undergoing percutaneous coronary intervention for acute coronary syndrome, OSAH, defined as an AHI $>$ 10, was similarly found to increase the incidence of major adverse cardiac events cardiac death, reinfarction, target vessel revascularization; adjusted hazard ratio, 11.6).\(^{222}\)

The repetitively fluctuating autonomic milieu may also be associated with nocturnal bradyarrhythmias and tachyarrhythmias.\(^{223}\) Additional cross-sectional data from the Sleep Heart Health Cohort showed a significantly higher prevalence of
nocturnal atrial fibrillation (adjusted odds ratio, 4.02), nonsustained ventricular
tachycardia (adjusted odds ratio, 3.40) and complex ventricular ectopy (nonsustained
ventricular tachycardia, ventricular bigeminy / trigeminy / quadrigeminy; adjusted
odds ratio, 1.74) in patients with a respiratory disturbance index ≥30 compared to
controls with a respiratory disturbance index <5, despite controlling for potential
confounders.\(^{(30)}\) Further, OSAH is strikingly more prevalent in patients with atrial
fibrillation than in other general cardiology patients, even after adjustment for
relevant covariates\(^{(224)}\); however, this was not confirmed in a case-control study using
cases with lone atrial fibrillation and controls matched for age, sex and cardiovascular
morbidity.\(^{(225)}\) Untreated OSAH may also be associated with recurrent atrial
fibrillation following electrical cardioversion.\(^{(226)}\) Finally and consistent with many
observational studies, a high prevalence of nocturnal bradycardia including sinus
pauses in patients with OSAH has been reported as in one study which used an
implantable loop recorder.\(^{(227)}\)

In a study of men with incident OSAH (AHI ≥10 and Epworth Sleepiness
Scale score ≥10) but without hypertension, diabetes mellitus, coronary artery disease
or morbid obesity, left ventricular diastolic dysfunction was observed in 56\%\(^{(228)}\);
iminimum oxygen saturation was found to predict an abnormal relaxation pattern
(adjusted odds ratio, 4.34) independent of age and hypertension in another study.\(^{(229)}\)
Left ventricular systolic dysfunction has also been reported in patients with OSAH
without evidence of structural heart disease or silent myocardial ischemia and not
explained by a higher prevalence or degree of obesity or a higher prevalence of
hypertension.\textsuperscript{(230)} More recently, a cross-sectional study of the Sleep Heart Health Cohort showed that an increased AHI was associated with an increased left ventricular mass index and decreased ejection fraction, independent of several covariates including systolic BP and antihypertensive medication use.\textsuperscript{(231)} Sustained pulmonary hypertension with or without right ventricular involvement (hypertrophy, cor pulmonale) has been associated with OSAH even after controlling for several confounding variables although coexisting daytime hypoxemia (i.e. chronic lung disease, obesity hypoventilation syndrome) has been suggested as a prerequisite in many but not all studies.\textsuperscript{(232-235)} Finally, microalbuminuria, an independent predictor of cardiovascular events, is associated with severe OSAH, even after adjustment for hypertension and diabetes mellitus.\textsuperscript{(236)}
2.3.4.4 Insulin resistance

There is a growing body of literature in support of an association between type 2 diabetes mellitus and OSAH with a reported prevalence of OSAH of 48% in diabetics, using an AHI cutoff of >10.\textsuperscript{(237)} AHI has been shown in patients with OSAH to predict post-prandial glycemic responses and insulin sensitivity independent of age and BMI.\textsuperscript{(238)} Using data from the Wisconsin Sleep Cohort, investigators demonstrated an adjusted (for age, sex and waist circumference) odds ratio for diabetes mellitus of 2.3 in those with an AHI $\geq 15$ as compared to $<5$.\textsuperscript{(239)} This was also the first and only longitudinal study that reported an adjusted odds ratio, albeit not statistically significant, for incident diabetes mellitus at 4 years of 1.62 (95% confidence interval, 0.67-3.65) in those with an AHI $\geq 15$ as compared to $<5$.\textsuperscript{(239)}
2.3.5 Mechanisms

2.3.5.1 Pathophysiology of obstructive sleep apnea-hypopnea

The principal pathophysiological characteristic of OSAH is upper airway collapse which transpires when the normal reduction in pharyngeal dilator muscle tone at the onset of sleep is superimposed on a narrowed and/or highly compliant pharynx. Upper airway obstruction may occur at one or more levels including the oropharynx (retropalatal and retroglossal pharynx) or hypopharynx, dependent on underlying neuromuscular tone, upper airway muscle synchrony and sleep stage. Obstructive events occur more commonly during rapid-eye-movement sleep as a consequence of a reduction of drive to the upper airway dilators. A major predisposing anatomic factor contributing to upper airway narrowing is peripharyngeal adipose tissue (as characterized by magnetic resonance imaging), even in non-obese patients. Other anatomic upper airway abnormalities may also adversely affect pharyngeal size.
2.3.5.2 Pathophysiology of associated cardiovascular diseases

Throughout sleep, a self-perpetuating cycle of recurrent upper airway obstruction is set in motion as a consequence of gas exchange perturbations, increased ventilatory effort, BP surges and abrupt arousals.\(^{(244)}\) Acutely, each obstructive event is characteristically accompanied by hypoxemia, hypercapnia and generation of negative intrathoracic pressure against an occluded airway (Mueller’s maneuver) with resultant increases in right and left ventricular afterload,\(^{(245)}\) pulmonary artery pressures and myocardial oxygen demand along with decreases in left ventricular relaxation\(^{(246)}\) and a leftward shift of the interventricular septum.\(^{(247)}\) Arousal at the termination of the obstructive event heralds sleep fragmentation and sympathetic discharge.\(^{(244)}\)

Although multifactorial, the interaction between repetitive upper airway closure and vascular disease has been best studied from the standpoints of intermittent hypoxemia and the autonomic nervous system. However, many of these laboratory studies in humans suffer from many of the same limitations that apply to the epidemiological field. Whereas such studies have uncovered acute pressor mechanisms attributed to OSAH, studies addressing sustained pressor mechanisms have principally relied on animal models; further, it is often difficult, in reviewing the literature, to tease out which chemical, mechanical and/or other deleterious factors are primarily responsible. Numerous studies reveal increased sympathetic neural activity (as reflected by plasma and urine catecholamine levels and microneurography)\(^{(248, 17, 154, 59)}\) as well as heightened pressor sensitivity to catecholamines\(^{(59)}\) related to cyclical
hypoxemia, \(^{(249, 17)}\) hypercapnia, \(^{(31)}\) arousal, \(^{(59)}\) tonic activation or abnormal control of chemoreflexes, \(^{(52)}\) and impaired baroreflex sensitivity or upward resetting of the BP setpoint \(^{(250)}\). The role of sympathoactivation in OSAH-induced hypertension has been further strengthened by findings that blockade of hypoxemia-driven BP elevation can be achieved following carotid body denervation, sympathetic nerve ablation, renal sympathectomy, renal artery denervation, adrenal medullectomy and antiadrenergic drugs. \(^{(251)}\) Such sympathoexcitation may also be responsible, at least in part, for the phenomena of non-dipping and BP variability. \(^{(249, 122)}\) Other potential pathways that have been implicated in inducing hypertension include decreased vagal tone and/or parasympathetic control, \(^{(252)}\) endothelial dysfunction (decreased nitric oxide \(^{(253)}\) and therefore, endothelium-dependent vasorelaxation \(^{(78, 254, 255)}\) along with impaired endothelium-independent vasorelaxation \(^{(255)}\), systemic and vascular inflammation, \(^{(256, 31)}\) elevated endothelin-1 \(^{(257)}\) or endothelin-1 precursor \(^{(258)}\) levels along with heightened vascular sensitivity to endothelin-1 \(^{(259)}\), higher arginine vasopressin levels, \(^{(260)}\) increased arterial stiffness, \(^{(261)}\) insulin resistance and/or metabolic syndrome \(^{(262-264)}\) along with hyperleptinemia \(^{(265)}\), amplified oxidative stress, \(^{(146)}\) increased endogenous digitalis-like factor levels, \(^{(86)}\) elevated erythropoietin levels with resultant erythrocytosis \(^{(266, 267)}\) along with increased blood viscosity \(^{(268, 269)}\) and renin-angiotensin-aldosterone system activation (in part due to renal sympathetic nerve activity) \(^{(270, 271, 29)}\) with possible differences in susceptibility dependent on angiotensin converting enzyme gene polymorphisms \(^{(272, 273)}\). Of note, in patients with RH, there is a high prevalence of primary hyperaldosteronism \(^{(39)}\) with plasma
aldosterone levels and 24-hour urine aldosterone excretion positively related to AHI\(^{41}\) and symptoms of OSAH\(^{40}\), respectively.

There are other mechanisms by which OSAH may mediate its consequences beyond hypertension and those already highlighted. Not surprisingly, nocturnal myocardial ischemia as reflected by ST-segment depressive episodes\(^{274, 275}\) or angina\(^{276}\) recurs frequently in OSAH patients. Likewise, inadequate cerebral oxygenation during an obstructive event and hypoperfusion after its termination have been documented, predisposing to nocturnal cerebral ischemia.\(^{277, 278}\) Episodic hypercapnia and hypoxemia may unmask intracranial artery stenosis,\(^{279}\) particularly in territories of watershed blood supply, perhaps related to a cerebral steal phenomenon.\(^{280}\) Further, right-to-left shunting (with risk of paradoxical embolization) has been demonstrated in patients with concomitant OSAH and patent foramen ovale during obstructive apneas.\(^{281}\) Procoagulable (e.g. increased platelet activity\(^{282}\) and plasma fibrinogen levels\(^{283}\)) and proatherogenic (e.g. decreased high density lipoprotein cholesterol,\(^{284}\) increased C-reactive protein and homocysteine,\(^{285}\) and circulating leukocyte-endothelial cell adhesion molecules\(^{286}\)) states have been demonstrated in patients with OSAH unlike controls. Nocturnal bradyarrhythmias likely arise from transiently enhanced vagal tone (diving reflex) secondary to apnea and hypoxemia or Mueller’s maneuver, rather than reflecting underlying sinus node or atrioventricular conduction system disease.\(^{17, 275}\)
2.3.6 Treatment

2.3.6.1 Treatment of obstructive sleep apnea-hypopnea

Nocturnal application of CPAP has remained the treatment of choice in OSAH with the level of constant positive pressure required to sustain upper airway patency maintained throughout the respiratory cycle. Physiologically, CPAP acts as a pneumatic splint. Although CPAP represents the focus of this section, in obese patients, the salutary effects of weight loss cannot be overstated.\(^{(48, 287)}\) As well, all patients should be counselled regarding avoidance of exacerbating factors such as sleep deprivation, alcohol and sedative-hypnotic drugs.\(^{(288)}\) Finally, people who cannot tolerate CPAP treatment might consider oral appliance therapy as an effective alternative.\(^{(28)}\)
2.3.6.2 Effects of treatment of obstructive sleep apnea-hypopnea on hypertension

Most studies addressing the effects of CPAP focus on BP control. Methodological differences, small sample sizes, comorbid conditions, medication use and lack of control groups allow only a limited interpretation of the existing data. In the case of hypertension, the majority of studies usually involve fewer than 50 subjects and combine subjects with normotension, prehypertension and hypertension together with the full spectrum of OSAH duration and severity. The duration of most studies evaluating CPAP therapy is less than 3 to 6 months with few studies including a control arm (e.g. oral placebo, subtherapeutic or sham CPAP, supplemental oxygen). Concomitant antihypertensive drug and lifestyle changes are often poorly described and variably applied. Moreover, BP responses are not determined in a uniform manner. Many intervention studies report only clinical BP measurements taken manually, even though 24-h ABPM measures the BP response to treatment more accurately. Additionally 24-h ABPM provides information on BP variability, night-to-day ratio and nighttime BP levels, which are often abnormal in OSAH patients. The discontinuous measurements of 24-h ABPM, however, do not fully capture the effect CPAP bears on nightly BP variability manifested by recurrent BP surges. Finally, in studies of outcomes other than BP, the use of composite endpoints makes it difficult to determine the effects on the individual components.

CPAP appears to lower BP by eradicating OSAH. In a study of middle-aged untreated hypertensive men with undiagnosed occult OSAH (AHI ≥5) and without OSAH (AHI <5), the mean change in nocturnal BP during or following 3 weeks of
nightly CPAP was significantly greater in OSAH patients adjusted for age and BMI.\(^{(290)}\) Moreover, CPAP treatment did not affect the BP of non-apneic subjects, indicating that its use in the absence of OSAH has no harmful BP effects. Most uncontrolled studies of CPAP use applied acutely and/or over periods of day to months in normotensive and hypertensive subjects with OSAH have revealed reductions in daytime and nighttime BP readings, conversion from non-dipper to dipper status and a diminution in nocturnal BP swings.\(^{(291-293)}\) Likewise, the BP responses of subjects with uncontrolled or RH have demonstrated significant decreases in daytime, nighttime and 24-hour BP recordings as well as non-dipping in short-term uncontrolled studies.\(^{(60, 294)}\) Similarly, in uncontrolled long-term studies of 6 to 36 month duration, CPAP application results in reductions of 24-hour and daytime BP values, particularly among those with hypertension.\(^{(295-297)}\)

At least 3 meta-analyses of randomized controlled trials that addressed the impact of CPAP on BP endpoints were published in 2007. The largest of these identified 16 studies, up to July 2006, that involved at least 2 weeks (range, 2 to 24 weeks) of therapeutic CPAP.\(^{(298)}\) Although the individual trials were not significantly heterogeneous, important differences nonetheless existed including type of control, study design, method of BP measurement and diagnosing hypertension and prevalence and severity of hypertension. Of the 15 studies specifically reporting systolic and diastolic BP, the weighted reduction in BP with CPAP was modest, albeit, statistically significant at 2.46/1.83; likewise, of the 7 studies specifically reporting mean BP, the weighted reduction in BP with CPAP was significant at 2.22.
Of the 11 studies reporting outcomes from 24-h ABPM, only a fraction separately reported daytime and nighttime measurements. Results from this subset of studies revealed no differences in systolic or diastolic BP reductions with CPAP by time of day, although there was a significant effect on nighttime mean arterial pressure. Planned subgroup analyses demonstrated an increased likelihood of a BP fall in those with a BMI \( \geq 31.4 \text{ kg/m}^2 \) or baseline BP \( \geq 129.6/79.9 \). A tendency for systolic BP decline to correlate with nightly CPAP use was also identified.\(^{(298)}\) A second smaller meta-analysis identified 10 studies, again up to July 2006, in which 9 of these studies were identical to those in the largest meta-analysis.\(^{(146)}\) Findings included a trend for a BP reduction of 1.38/1.52 with CPAP with post-hoc analyses revealing trends for an association between systolic BP decline and CPAP compliance as well as for a greater BP fall in the trials involving more severe OSAH (AHI >30).\(^{(146)}\) Similarly, a third meta-analysis identified 12 studies, up to August 2006, in which 9 of these studies were identical to those in the largest meta-analysis.\(^{(299)}\) Significant BP reductions with CPAP were as follows: 1.69 (24-hour mean), 1.77/1.79 (24-hour systolic/diastolic), 1.76 (daytime) and 2.25/2.87 (daytime systolic/diastolic). Predefined meta-regression revealed falls of 24-hour mean BP by 0.89 per 10 event/hour increment in AHI and 1.38 per 1 hour/night increment in CPAP use.\(^{(299)}\) Three additional randomized controlled trials published after the inclusion period of the meta-analyses were largely confirmatory,\(^{(300-302)}\) although the study conducted in mostly untreated hypertensive sleepy patients with OSAH showed a substantial and statistically significant reduction in clinic BPs (-6.7/-4.9 mm Hg, systolic/diastolic).\(^{(301)}\) The meta-analyses, despite the
limitations of the existing trials, suggest that the BP lowering reported with CPAP may not be the only or even the most important beneficial effect on cardiovascular risk of treating OSAH.

Although predictors of a CPAP-induced hypotensive effect, such as those already cited, have not been unanimously agreed upon across all studies, a consensus tends to exist in some instances. First, observed BP reductions seem to be associated with OSAH severity as determined objectively (e.g. AHI, oxygen desaturation index) or subjectively (e.g. Epworth Sleepiness Scale). Thus, for example, CPAP may not be effective in reducing BP in nonsleepy hypertensive patients with OSAH. Second, the observed depressor response seems to relate to the presence of hypertension, possibly among non-dippers particularly and/or more RH as indicated by a higher baseline BP or a requirement for antihypertensive drug(s), although there are exceptions. Certainly, the existing literature may be underpowered to detect a significant, albeit attenuated, hypotensive effect of CPAP in those with milder forms of hypertension or normal BP. Third, adherence with CPAP therapy, a significant hurdle with this intervention, is strongly coupled with the observed BP fall; in one clinical trial, subjects who used CPAP ≥3.5 hours/night showed a greater decrease in 24-h diastolic BP. Given the association between adherence and AHI or Epworth Sleepiness Scale, it may be difficult, however, to determine the precise independent contributions of either in predicting BP response.
Reversal of many of the purported mechanisms linking OSAH to hypertension has been demonstrated in many, but not all, studies. These studies often suffer from the same limitations as those in the clinical trial arena including no control group and short duration of observations. The greatest representation of data stems from work examining the sympathetic nervous system in which improvements in baroreflex sensitivity\(^{(58, 292)}\) and chemoreflex control\(^{(314)}\), along with reductions in plasma and urine norepinephrine levels\(^{(57, 59)}\) and muscle sympathetic nerve activity\(^{(31, 52)}\) have been shown, especially among hypertensives\(^{(315)}\). Other potential and disturbed pathways that have been implicated in mediating hypertension and display a return toward normal following CPAP application include reduced vagal tone\(^{(316)}\), endothelial dysfunction (decreased nitric oxide\(^{(253, 58, 146)}\) and therefore, endothelium-dependent vasorelaxation\(^{(317, 254, 255)}\)) along with impaired endothelium-independent vasorelaxation\(^{(255)}\), systemic and vascular inflammation,\(^{(318, 256, 31)}\) elevated endothelin-1\(^{(257)}\) or endothelin-1 precursor\(^{(258)}\) levels, increased arterial stiffness\(^{(319)}\) and central aortic systolic BP (despite no parallel reduction in peripheral BP)\(^{(320)}\), insulin resistance (particularly in non-obese subjects)\(^{(321, 313)}\) along with hyperleptinemia\(^{(322)}\), amplified oxidative stress,\(^{(323)}\) increased endogenous digitalis-like factor levels\(^{(324)}\) and elevated erythropoietin levels with resultant erythrocytosis\(^{(325)}\).
2.3.6.3 Effects of antihypertensive drug therapy on obstructive sleep apnea-hypopnea

There is limited information on the effects of antihypertensive medications in hypertensive patients with OSAH on sleep architecture and BP and as yet no consistent picture has emerged. Early reports based largely on case studies observed an increase in severity of OSAH on beta blocker therapy\(^{(326, 327)}\), although a later randomized controlled trial found no adverse effects of beta blocker therapy on sleep or OSAH\(^{(211)}\). The latter study also assessed the effects on BP of 5 different classes of antihypertensive drugs (amlodipine, enalapril, atenolol, hydrochlorothiazide, losartan) in hypertensive men with OSAH and found that all drugs were equally effective in lowering daytime BP, but only atenolol and hydrochlorothiazide reduced nighttime BP.\(^{(211)}\) In an 8-day randomized clinical trial, neither the beta blocker, metoprolol, nor the ACE inhibitor, cilazapril, affected total sleep time or the proportion of rapid-eye-movement to non-rapid-eye-movement sleep, although both agents reduced nocturnal BP.\(^{(328)}\) Moreover, the alpha-2 adrenergic agonist, clonidine, was found to suppress rapid-eye-movement sleep in 8 male volunteers with OSAH, which lessened the level of nocturnal hypoxemia.\(^{(329)}\) Finally, in addition to inducing a fall in BP, a 3-day course of combination diuretic therapy, specifically furosemide and spironolactone, was also shown to significantly decrease AHI in 15 obese adults with severe OSAH (mean AHI, 74.9) and diastolic heart failure.\(^{(67)}\)
2.3.6.4 Effects of treatment of obstructive sleep apnea-hypopnea on cardiovascular diseases and insulin resistance

CPAP has been shown to reduce the occurrence of nocturnal myocardial ischemia (angina,\(^{(276)}\) ST-segment depression\(^{(274)}\)) as well as sinus pauses and other bradyarrhythmias.\(^{(227)}\) Indices of left ventricular diastolic function showed improvement following 3 months of effective CPAP, despite no changes in daytime or nighttime BP, in a randomized crossover trial using a comparator sham CPAP arm.\(^{(228)}\) CPAP has also been shown to improve left ventricular ejection fraction,\(^{(330, 230)}\) perhaps related to a lowering of left ventricular transmural pressure during systole consequent to increased intrathoracic pressure.\(^{(331)}\) Another randomized crossover trial of 3 months of therapeutic CPAP revealed a significant reduction in systolic pulmonary artery pressure from 28.9 mmHg to 24 mmHg, particularly among those with pulmonary hypertension, as compared with subtherapeutic CPAP in which the pressure remained at 28.8 mmHg.\(^{(235)}\) As touched upon briefly above, uncontrolled studies on the effects of CPAP in diabetic and non-diabetic patients have generally revealed improvements in insulin responsiveness, glycosylated hemoglobin and post-prandial glycemic responses\(^{(321, 332)}\); however, a recent double blind randomized controlled study of 3 months of CPAP in type 2 diabetic patients with newly diagnosed OSAH showed no such improvements, although the variance of the change in insulin sensitivity by euglycemic clamp was large.\(^{(333)}\)

There are several other mechanisms by which CPAP may mediate reductions in cardiovascular disease burden independent of BP lowering and the foregoing
mechanisms. Improvements in several determinants of hypercoagulability (e.g. platelet activity,\(^{(282)}\) plasma fibrinogen and factor VII concentrations\(^{(268)}\)) and atherogenesis (e.g. high density lipoprotein cholesterol levels,\(^{(284)}\) circulating leukocyte-endothelial cell adhesion molecules\(^{(334)}\)) have been shown in response to CPAP. Of course, it may be that some of the OSAH-induced mechanisms that continue to perpetuate disease as in vascular remodelling may be only partially reversed with CPAP. For example, if applicable to the case of sustained hypertension, one would predict a differential antihypertensive response according to the duration of hypertension among patients with OSAH.\(^{(335)}\) Moreover, there may be a salutary role of CPAP in preventing death and cardiovascular disease in subjects with OSAH above and beyond BP lowering as determined peripherally. Mechanistically, for example, improvements in procoagulable (e.g. platelet activity,\(^{(282)}\) plasma fibrinogen and factor VII concentrations\(^{(268)}\)) and proatherogenic (e.g. high density lipoprotein cholesterol levels,\(^{(284)}\) circulating leukocyte-endothelial cell adhesion molecules\(^{(334)}\)) states in patients with OSAH have been shown in response to CPAP. Clinically, a prospective cohort study of age- and BMI-matched male patients with untreated severe OSAH (AHI >30) identified significantly greater risks of fatal and nonfatal cardiovascular events (including stroke; adjusted [including for systolic and diastolic BP] odds ratio, 2.87 and 3.17, respectively) than healthy controls, unlike CPAP treated patients with similar AHI at baseline, untreated patients with mild-moderate OSAH (AHI 15-30) and simple snorers (AHI <5).\(^{(336)}\) Treatment of OSAH in another prospective cohort study was also associated with a significant reduction in the risk of a cardiovascular or
cerebrovascular event (adjusted hazard ratio, 0.36) after adjustment for age, sex, cardiovascular risk factors and baseline comorbidities with untreated patients having a mean AHI of only 15.3.\(^{(337)}\) However, not surprisingly, there are no longitudinal, large-scale, randomized, double-blind trials in this regard.
CHAPTER 3. HYPOTHESIS AND SPECIFIC AIMS

Accumulating evidence strongly suggests that extracellular fluid volume expansion, which is often occult, may be responsible for both the non-dipping BP pattern and for the high prevalence of OSAH in RH. With assumption of the recumbent position prior to sleep at night, the interstitial fluid volume of the lower extremities accumulated in the upright position during the day is reabsorbed from the interstitium into the intravascular compartment\(^{(338)}\) and redistributes into the upper body in relation to gravitational forces\(^{(69, 339, 340)}\). Such rostral displacement of fluid causes an increase in central blood volume\(^{(341, 342)}\) leading to an increase in nuchal and peripharyngeal fluid content, which subsequently compresses the upper airway and obstructs airflow. The objectives of this project are to describe the nocturnal BP abnormalities occurring in RH via ABPM and to evaluate the emerging role of extracellular fluid volume expansion in the pathogenesis of OSAH in RH, specifically, in the latter case, by examining both the acute effects of rostral fluid displacement via LBPP application on upper airway cross-sectional area as well as the spontaneous rostral fluid displacement occurring overnight. We anticipated that the “Nocturnal blood pressure study” would show an attenuated fall in nocturnal BP along with a high prevalence of non-dipping and nocturnal hypertension in patients with RH as compared to control subjects. We anticipated that the “Fluid shift study” would demonstrate an exaggerated reduction in upper airway cross-sectional area and greater severity and prevalence of OSAH in patients with RH as compared to those
with CH attributed to greater LBPP-induced and spontaneous overnight leg fluid volume shifts.
CHAPTER 4. STUDY I: NOCTURNAL BLOOD PRESSURE STUDY

4.1 Introduction

Resistant hypertension (RH) is emerging as a significant and growing problem in managing hypertension; indeed, 11% of patients referred for uncontrolled hypertension were found to meet its criteria.\(^{(2)}\) Not surprisingly, RH is associated with an increased risk of hypertensive target organ damage.\(^{(18)}\) Experts recommend 24-hour ambulatory blood pressure monitoring (ABPM) to differentiate true RH from white-coat hypertension.\(^{(15)}\) The test also provides information on nocturnal blood pressure (BP) and the means to assess its relationship to survival and the occurrence of cardiovascular and renal events, adjusted for daytime or 24-hour BP.\(^{(343, 174, 176, 177, 109, 182, 186)}\)

Several sleep BP pattern disturbances in normotensive and hypertensive subjects have been identified. A Japanese study found a 20% increase in cardiovascular mortality for every 5% attenuation in nocturnal BP fall, independent of overall 24-hour BP.\(^{(174)}\) A J-shaped curve of stroke incidence was found in another study among extreme dippers (subjects with a \(\geq 20\%\) fall in nocturnal BP) and non-dippers, albeit from different causes, with risers (subjects with a paradoxical increase in nocturnal BP) having the worst cardiovascular prognosis.\(^{(92)}\) Recently, nighttime BP, independent of daytime level, was found to be a predictor of death and cardiovascular disease.\(^{(186)}\) Surprisingly, little attention has thus far focused on sleep BP patterns in subjects with RH, and the results in the existing literature are conflicting, at least in part, due to the use of different definitions of the condition itself and methods to define the nighttime period.\(^{(344, 24, 25, 213)}\)
This study was undertaken to determine whether sleep BP pattern disturbances are related to the presence or severity of hypertension. We used a widely accepted definition of RH and diaries to ascertain the nighttime period to overcome shortcomings of some earlier studies.\(^{(214)}\) This study has been accepted for publication in the Canadian Journal of Cardiology and reproduced with permission.\(^{(75)}\)
4.2 Methods

Study design

We employed a cross-sectional study design to compare the sleep BP patterns of normotensive and hypertensive subjects, individually matched for age, sex and body mass index (BMI). This study was approved by the Research Ethics Board of Mount Sinai Hospital (Toronto, Canada).
Subjects

Patients with RH were previously identified through chart review in the Mount Sinai Hospital Hypertension clinic in which an evaluation to ascertain the causes of apparent resistance to medical therapy was conducted. RH was defined as a daytime BP $\geq 135/85$ on ABPM while adherent to $\geq 3$ antihypertensive medications at maximal or near-maximal doses including a diuretic, unless contraindicated or intolerant (e.g. hyponatremia). Patients were excluded if noncompliant with antihypertensive medication (self-report), underlying correctable secondary form of hypertension, chronic kidney disease defined as a body surface area-adjusted creatinine clearance $< 45$ mL/min/1.73 m$^2$, ingestion of exogenous substances that can raise BP or a history of alcohol abuse. Controlled hypertension (CH) was defined as a daytime BP $< 135/85$ on ABPM while on $\leq 3$ antihypertensive medications. Normotension (NT) was defined as a daytime BP $< 135/85$ on ABPM while on no antihypertensive medications. The latter two groups of patients were then matched for age ($\pm 10$ years), sex and BMI ($\pm 5$ kg/m$^2$) to the patients with RH in a 2:1 ratio. In determining number of antihypertensive medications, dihydropyridine and non-dihydropyridine calcium channel blockers were considered as two drugs as were diuretics with actions on different nephron sites. Diabetes status was determined via medication review (prescription for oral hypoglycemic agent or insulin).
Ambulatory blood pressure monitoring

ABPM was performed for 24 hours starting at the same time of a regular working day in all patients following their usual prescribing pattern for those on antihypertensive medications using an automated sphygmomanometer (SL-90207; SpaceLabs Medical, Redmond, WA, USA). An appropriate sized cuff was fitted to the non-dominant arm with BP measured every 20 minutes during the day and every 30 minutes during the night. Patients recorded the times when they retired at night and awoke in the morning and such times were then rounded to the nearest hour. The formula, \( \frac{2}{3} \times \text{diastolic BP} + \frac{1}{3} \times \text{systolic BP} \), was used to calculate mean arterial pressure. 24-hour BP was determined using the formula: proportion of 24 hours awake x daytime BP + proportion of 24 hours asleep x nighttime BP. The nocturnal BP fall was defined as the degree of fall (%) in nocturnal mean arterial pressure relative to the diurnal mean arterial pressure (i.e. \( 100 \times \left[ 1 - \frac{\text{nighttime mean arterial pressure}}{\text{daytime mean arterial pressure}} \right] \)). We also assessed the nocturnal BP profile in three other ways (dipping status, nocturnal BP status and nocturnal BP pattern) to reflect the various, albeit related, measures reported in the literature. Dipping status was expressed as either dipper or non-dipper, with dipping defined as \( \geq 10\% \) nighttime fall in mean arterial pressure relative to its daytime value. Nocturnal BP status was expressed as either nocturnal normotension or hypertension, with nocturnal hypertension defined as a nighttime BP \( \geq 125/75 \). Four nocturnal BP patterns were defined based on the percentage change in nocturnal mean arterial pressure: extreme
dipping (≥ 20% fall), normal dipping (≥ 10% but < 20% fall), attenuated dipping (≥ 0% but < 10% fall) or rising (< 0% fall). Analyses were then repeated using systolic and diastolic BP.
Statistical analysis

We calculated a sample size of 21 per group assuming nocturnal BP falls of 15% (NT)\(^{(347)}\), 12% (CH)\(^{(24)}\) and 9% (RH)\(^{(24)}\), a pooled standard deviation of 6% and equal group sizes (two-tailed alpha=0.05, power=0.8). Assuming non-dipper proportions of 13% (NT)\(^{(347)}\), 50% (CH)\(^{(24)}\) and 69% (RH)\(^{(24)}\) and equal group sizes (two-tailed alpha=0.05, power=0.8), we calculated sample sizes of 11 per group (NT vs. RH) and 80 per group (CH vs. RH).\(^{(348)}\) Continuous variables were expressed as means ± standard deviation while categorical variables were described as proportions. Although the groups were matched, one-way ANOVA (Neuman-Keuls post hoc test) was employed for continuous variables; the unpaired t-test was used in comparing the number of antihypertensive drugs between the two hypertensive groups. Pearson’s chi-square test or Fisher’s exact test, as appropriate, were utilized to evaluate categorical variables followed by decomposition, where indicated.\(^{(349)}\) ANCOVA (for nocturnal BP fall) and logistic regression (for non-dipping and nocturnal hypertension) were also enlisted in order to determine whether the associations between BP status and the above parenthesized outcomes were independent of diabetic status. A two-tailed p-value of < 0.05 was considered statistically significant. All analyses were conducted using SAS Version 9.1.3 (SAS Institute, Cary, NC).
4.3 Results

Baseline characteristics

We identified 46 eligible subjects with RH (age 59.7 ± 10.9 years, BMI 33.1 ± 6.3 kg/m², 60.9% men, 19.6% diabetic) of whom 26 were individually matched with 52 subjects with NT and 52 subjects with CH. The groups were therefore similar with respect to age, sex and BMI with higher daytime, nighttime and 24-hour BP values in the RH group along with a greater number of antihypertensive drugs (p<0.0001; Table 1). The mean serum creatinine among subjects with RH was 89.0 ± 21.5 umol/L.
Nocturnal BP profiles

During sleep, the percentage fall in nocturnal mean arterial pressure was 8.4 ± 7.2 and the proportions of non-dippers and nocturnal hypertension were 58.7% and 84.8%, respectively, in the RH group (n=46) as a whole. The proportions of extreme dippers, attenuated dippers and risers were 4.4%, 37.0% and 15.2%, respectively. Following matching, the percentage fall in nocturnal mean arterial pressure was 15.1 ± 6.1 in the NT group, 11.5 ± 7.0 in the CH group and 7.7 ± 7.7 in the RH group (Figure 1; p<0.0001). All pairwise comparisons were significant. The non-dipper proportions were 25.0% in the NT group, 42.3% in the CH group and 61.5% in the RH group (Figure 2; p=0.006). Deconstruction revealed significantly more non-dippers among subjects with RH compared with NT (p=0.003); other pairwise comparisons were not statistically significant. The proportions for nocturnal hypertension were 9.6% in the NT group, 23.1% in the CH group and 84.6% in the RH group (Figure 3; p<0.0001). Deconstruction revealed significantly more nocturnal hypertension among subjects with RH compared with NT and CH (p<0.0001); the remaining pairwise comparison was not statistically significant. In analyses that adjusted for diabetes status, the effect sizes observed for nocturnal BP fall, non-dipping and nocturnal hypertension remained unchanged (data not shown). The proportions of extreme dippers, attenuated dippers and risers were 19.2%, 25.0% and 0% in the NT group, 17.3%, 40.4% and 1.9% in the CH group and 3.9%, 42.3% and 19.2% in the RH group, respectively (p=0.003). Deconstruction revealed significantly more risers among subjects with RH compared with NT and CH (p=0.001); on the
other hand, the proportion of extreme dippers was less, although this did not reach statistical significance (p=0.08). Analyses using systolic and diastolic BP generated equivalent results. For systolic BP, nocturnal BP falls (%) and non-dipper proportions were 12.8 ± 5.7 and 28.8% (NT), 9.9 ± 6.0 and 51.9% (CH), 5.6 ± 8.7 and 69.2% (RH), respectively; for diastolic BP, 17.0 ± 6.8 and 13.5% (NT), 12.9 ± 8.1 and 34.6% (CH), 9.5 ± 7.4 and 42.3% (RH), respectively.
4.4 Discussion

This cross-sectional study demonstrates that patients with RH manifested a lesser fall in nocturnal mean arterial pressure and had a higher proportion of non-dippers, nocturnal hypertension and paradoxical rise in nocturnal BP compared to those with CH and NT. Not surprisingly, nocturnal hypertension was the most common abnormality, being found in 85% of refractory hypertensive patients. There is a growing body of literature indicating that nighttime ambulatory BP is a better predictor of death and cardiovascular disease than daytime ambulatory BP readings in hypertensive patients\(^{(177, 109, 186)}\) and may be a more consistent predictor of outcomes than the night-to-day BP ratio\(^{(343, 176, 109)}\).

Other studies have reported similar results although none determined and compared the nocturnal BP patterns and prevalence of nocturnal hypertension in the three different groups evaluated in this study. In a study of 62 healthy middle-aged men, the percentage fall in nocturnal mean arterial pressure was 15% and the proportion of non-dippers, 13%\(^{(347)}\). Using a standard definition of RH, Muxfeldt et al. found that the percentage fall in nocturnal systolic and diastolic BP was 7% and 10.5%, respectively and the proportion of non-dippers, 65.8%, in a study of 313 such patients\(^{(25)}\). However, 63% of the 184 white-coat hypertensive patients in this study also had a non-dipping pattern. The results of an earlier and smaller study by the same group were similar except for the proportion of non-dippers among white-coat hypertensives which was significantly lower at 49.6%\(^{(24)}\).
Findings of several other studies differed from our own. Two cross-sectional studies revealed that although the absolute nocturnal BP was higher in hypertensive than NT subjects, the magnitude of and percent decline in nocturnal BP positively correlated with daytime BP.\(^{(350, 351)}\) In another study, hyperkinetic (having a cardiac index greater than the mean + 1 standard deviation of the normotensive group) borderline hypertensive subjects were found to have a greater night-to-day BP gradient than normotensive subjects, and a similar trend was also observed in normokinetic (having a cardiac index below that level) borderline hypertensive individuals.\(^{(352)}\) Pickering et al. showed the same circadian BP profile in hypertensive and normotensive individuals.\(^{(129)}\) A study of RH patients found no differences in the night-to-day BP ratio among subjects with an office diastolic BP > 100 when stratified into tertiles according to daytime diastolic BP.\(^{(213)}\) Similarly, another RH study found an equivalent fall in nocturnal BP in patients with RH and white-coat hypertension\(^{(344)}\) and the percentage of non-dippers in the true RH group was only 27% compared to 61.5% in our study. There are several possible explanations for these disparate results including the use of fixed time intervals rather than diaries to identify the daytime and nighttime periods and different definitions of RH. White-coat hypertensives as a comparator group may not be identical to our CH subjects. Finally, we employed a matching strategy prior to data analysis to control for the potential confounding effects of age, sex and BMI. Of note, we did not include patients with a daytime BP < 135/85 on ABPM but requiring \(\geq 4\) antihypertensive medications in the present analysis. According to a recently published consensus paper, such patients
with ‘controlled RH’ are still considered resistant to treatment\(^{(14)}\); whether their nocturnal BP profiles are similar to those with RH or CH remains unknown.

The mechanism(s) accounting for the association of RH with non-dipping, nocturnal hypertension and rising of nocturnal BP remains speculative; however, several possibilities exist including an underlying abnormal nocturnal sympathovagal balance\(^{(110, 95, 122)}\) and occult fluid retention\(^{(187)}\), among others.\(^{(113)}\) Salt-sensitive hypertension is associated with the phenomenon of non-dipping\(^{(115)}\) and interventions that reduce total body sodium content in salt-sensitive individuals re-establish the normal circadian BP rhythm.\(^{(5, 6)}\) For example, in a study of Japanese patients with essential hypertension, non-dippers on a high sodium diet had a significantly higher nighttime systolic BP and mean arterial pressure and showed no nocturnal decrease in BP compared to the dippers.\(^{(5)}\) Indeed, the nocturnal fall in BP from daytime to nighttime was only significant among salt-resistant hypertensives (defined as a < 10\% BP difference between the high and low dietary salt states) unlike their salt-sensitive hypertensive counterparts; dietary sodium restriction was able to restore the normal dipping pattern of BP in the salt-sensitive hypertensives only. Similarly, diuretic therapy was also able to normalize the dipping BP pattern in non-dippers but had no effect on the nocturnal BP fall in dippers.\(^{(6)}\) Moreover, intensified ultrafiltration in hemodialysis patients initially identified as risers led to a conversion to either dipper or attenuated dipper status, in the majority of cases.\(^{(187)}\) These findings suggest that excess extracellular fluid volume may be causally related. Consistent with this suggestion are the parallel observations of greater attenuation of the normal nocturnal
dipping pattern in our study and a higher prevalence of salt sensitivity in hypertensive compared to normotensive subjects.\(^{4}\) Other observations suggest that occult volume expansion may be a dominant feature accounting for apparent treatment resistance in refractory hypertensive patients.\(^{22, 26}\) In a 3 month interventional trial of drug-resistant hypertension, patients randomized to receive antihypertensive treatment dictated by non-invasive hemodynamic measurements had significantly lower BP and better BP control than those receiving specialist care alone, and the differences were attributed to a significantly higher final diuretic dosing.\(^{26}\)

Our study is limited by its observational nature and the always-present problem of confounding. Despite matching for age, sex and BMI, there may have been an uneven distribution of other potential confounding factors associated with salt sensitivity and/or non-dipping status (e.g. African-American ethnicity, chronic kidney disease, poor sleep quality or quantity, sleep-disordered breathing, dietary potassium intake),\(^{4, 151, 188, 353, 110}\) and alterations in daytime or nighttime BP (e.g. nocturnal body position, diurnal activity).\(^{110, 111}\) Importantly herein, sleep-disordered breathing, particularly obstructive sleep apnea-hypopnea, has been strongly associated with both non-dipping and RH\(^{61}\); this raises the very real possibility that the observed association between nocturnal hypertension, the non-dipping BP pattern, and RH merely reflects recurrent upper airway obstruction and BP surges occurring throughout sleep. It is unlikely that a significant subset of subjects with RH suffered from chronic kidney disease, as the estimated mean body surface area-adjusted creatinine clearance works out to $81.5 \pm 19.6$ mL/min/1.73 m\(^2\); further, possible
inclusion of chronic kidney disease among subjects with CH and NT would bias towards a more conservative estimate. Although antihypertensive drug prescriptions were elucidated among the patients with CH and RH, timing of administration was not; conversion from non-dipper or riser to dipper status with nighttime dosing of antihypertensive agents has been shown in some studies.\(^{(190, 354, 194)}\) There are also data suggesting that, despite equivalent daytime BP control, various antihypertensive medications may exert differential effects on nighttime BP.\(^{(191)}\)

There are several shortfalls with respect to ascertainment of dipping status, particularly given the greater dependency of this measure on nocturnal BP readings.\(^{(190, 110)}\) First, we did not account for nocturnal awakenings in determining average nighttime BP (nor did we account for naps during the day in determining average daytime BP) which could result in misclassification.\(^{(109, 108)}\) Second, nighttime BP was determined using each subject’s reported bedtime as opposed to polysomnographically verified sleep time or on the basis of an arbitrarily fixed clock time; however, our operating definition was applied throughout and all definitions appear adequately reliable.\(^{(59)}\) Third, since dipping is defined by a difference score, its reproducibility falls short of the individual daytime and nighttime BP levels\(^{(18, 110, 96)}\); on repeated ABPM, agreement in diagnosing nocturnal hypertension on the basis of the two measurements was greater using an absolute nocturnal BP cutoff rather than the percentage nocturnal BP fall.\(^{(99)}\) As well, one would anticipate a regression to the mean effect had we repeated measurements.
All in all, our cross-sectional study, in agreement with the findings of some\textsuperscript{(24, 25)} but not all investigators\textsuperscript{(344, 213)}, demonstrates that non-dipping is a common finding in patients with RH. The novel aspects of our study are the extraordinarily high prevalence of nocturnal hypertension and the increased prevalence of nocturnal BP rising (and trend to less extreme dipping) in subjects with RH. While the nature of these circadian disturbances remains undetermined, we postulate that it may reflect an underlying abnormality (e.g. covert volume overload or obstructive sleep apnea-hypopnea) that also accounts for the challenges in attaining BP control in RH.
CHAPTER 5. STUDY II: FLUID SHIFT STUDY

5.1 Introduction

Resistant hypertension (RH) is emerging as a major and growing problem in managing hypertension. It accelerates the atherosclerotic burden of aging and is associated with a significant increased risk of hypertensive target organ damage.\(^{(18)}\) We were the first to report that patients with RH had a high prevalence of obstructive sleep apnea-hypopnea (OSAH)\(^{(8)}\) and subsequently demonstrated that successful treatment of OSAH with continuous positive airway pressure (CPAP) resulted in a significant fall in both nighttime and daytime blood pressure (BP), implying a possible causal role of OSAH in the pathogenesis of RH.\(^{(60)}\)

Other studies in this patient population revealed depressed plasma renin, elevated plasma aldosterone and aldosterone/renin ratio and a fall in BP with diuretic treatment.\(^{(38, 42, 39, 43, 355, 44, 41)}\) The best predictor of BP lowering following the institution of a diuretic was the depressed plasma renin level.\(^{(45)}\) These observations strongly suggested that extracellular fluid volume is expanded in RH and may be the basis for the high prevalence of OSAH in these patients. We hypothesize that with assumption of the recumbent position prior to sleep at night, the fluid volume of the legs redistributes rostrally causing an expansion of the central blood volume leading to an increase in nuchal and peripharyngeal fluid content, which subsequently compresses the upper airway and obstructs airflow. Support for this hypothesis comes from our laboratory in which we demonstrated in healthy non-obese subjects that the application of medical anti-shock trousers to increase lower body positive pressure
(LBPP) reduces leg fluid volume, increases neck circumference, upper airway resistance and upper airway collapsibility, and decreases upper airway cross-sectional area\(^{9-11}\); further, we also documented in healthy non-obese men with suspected OSAH that the overnight reduction in leg fluid volume accounted for a significant proportion of the variability in the severity of OSAH.\(^{72}\)
5.2 Methods

Study design

This is a physiologic interventional study comparing spontaneous overnight and LBPP-induced daytime fluid shifts and their relationship to the presence and severity of OSAH and pharyngeal size, respectively, between RH and CH patients. The study’s aims are to determine whether rostral fluid shift, as has been demonstrated in healthy, non-obese adults, operates in hypertensive patients and importantly, whether the magnitude of such shifts differs between subjects with CH and RH. The Human Subjects Review Committee of the University of Toronto and the Research Ethics Boards of Mount Sinai Hospital, University Health Network and Toronto Rehabilitation Institute (Toronto, Canada) approved the study. All subjects provided written informed consent prior to participation.
**Subjects**

Patients with CH and RH were recruited from the Mount Sinai Hospital Hypertension clinic. RH was defined as a clinic BP ≥ 140/90 mmHg (or ≥ 130/80 mmHg in patients with diabetes or chronic kidney disease) and a daytime BP ≥ 135/85 mmHg on 24-hour ABPM while adherent to ≥ 3 antihypertensive medications at maximal or near-maximal doses including a diuretic, unless contraindicated or intolerant or as a BP below these levels while on ≥ 4 antihypertensive medications.\(^{(14)}\) CH was defined as a clinic BP < 140/90 mmHg and a daytime BP < 135/85 mmHg on 24-hour ABPM while on ≤ 3 antihypertensive medications. Patients were excluded if they were noncompliant (self-report\(^{(345)}\)), had a correctable secondary form of hypertension, ingested exogenous substances (including alcohol) that can raise BP (current or in prior 2 weeks), had symptomatic heart failure with a left ventricular ejection fraction < 50%, were morbidly obese with a body mass index (BMI) ≥ 40 kg/m\(^2\) or were symptomatic from coronary artery disease requiring nitrates\(^{(356)}\). Otherwise, if applicable, nitrates and phosphodiesterase-5 inhibitors\(^{(223)}\) were withheld for 2 weeks prior to study enrolment.

The following sociodemographic, anthropometric and medical variables were collected on each patient: age, sex, race, diabetic status, cardiovascular disease, BMI, body surface area, weekly physical activity and class and number of antihypertensive drug(s). The race of patients was determined jointly from the information gathered on self-reported ethnicity, country of birth and surname.\(^{(357)}\) Race was then classified into
three categories according to the 2000 United States Census Bureau: White, Black or Asian (no patient belonged in the Other race category).\(^{(358)}\) BMI was calculated as weight in kg divided by the square of height in m. Body surface area was calculated using the formula of Dubois and Dubois.\(^{(359)}\) Weekly physical activity was gauged using a validated 7-day physical activity questionnaire assessment (“Stanford 7-day recall questionnaire”).\(^{(360)}\) In determining number of antihypertensive medications, dihydropyridine and non-dihydropyridine calcium channel blockers were considered as two separate drugs as were diuretics with actions on different nephron sites. Two-dimensional echocardiography was used to determine the following indices: left ventricular ejection fraction, left ventricular hypertrophy, left ventricular mass index, left atrial diameter and right ventricular systolic pressure. Left ventricular ejection fraction was classified into three categories: \(\geq 60\%\), 40-59\% or 20-39\% (no patient had an ejection fraction < 20\%). Urine was collected for 24 hours to determine the sodium, potassium and protein excretion rates along with the measured creatinine clearance.
Ambulatory blood pressure monitoring

ABPM was performed for 24 hours starting at the same time of a regular working day in all patients following their usual prescribing pattern using an automated sphygmomanometer (SL-90207; SpaceLabs Medical, Redmond, WA, USA). An appropriate sized cuff was fitted to the non-dominant arm with BP measured every 20 minutes during the day and every 30 minutes during the night. Normal daily activities were encouraged and patients were told to keep their non-dominant arm still and held relaxed to the side during measurements. Patients recorded the times when they retired at night and awoke in the morning and such times were then rounded to the nearest hour. The nocturnal BP fall was defined as the degree of fall (%) in nocturnal systolic BP relative to the diurnal systolic BP (i.e. $100 \times [1 - \text{nighttime systolic BP} / \text{daytime systolic BP}]$) such that non-dipping was defined as a $< 10\%$ nocturnal systolic BP fall. The 24-hour pulse pressure was calculated as the difference between the mean systolic BP and mean diastolic BP over the 24-hour recording period.
**Polysomnography**

Diagnostic overnight polysomnography was performed using standard techniques and scoring of sleep stages and arousals.\(^{(361, 362)}\) Sleep studies were performed by personnel who were blinded to the BP status of the subjects. Thoracoabdominal movements and tidal volume \(V_T\) were monitored by a respiratory inductance plethysmograph (Respirace; Ambulatory Monitoring Inc., White Plains, NY, USA) and airflow by nasal pressure cannulae.\(^{(363)}\) Oxyhemoglobin saturation \(\text{SaO}_2\) was continuously monitored by a pulse oximeter (Nellcor, N-200 pulse oximeter; Nellcor Inc., California, USA) and recorded on a computerized sleep scoring system (Sandman, Nellcor Puritan Bennett Ltd., Ottawa, ON, Canada). The mean and minimum \(\text{SaO}_2\) during sleep were recorded as previously described.\(^{(330)}\) Apneas were defined as an absence of \(V_T\) for \(\geq 10\) seconds and were classified as obstructive if there was out of phase rib cage and abdominal motion and as central if there was no rib cage or abdominal motion. Hypopneas were defined as a \(> 50\%\) reduction in \(V_T\) but above 0 for \(\geq 10\) seconds and were classified as obstructive if there was out of phase rib cage and abdominal motion or airflow limitation on the nasal pressure tracing and as central if there was in phase rib cage and abdominal motion and no evidence of airflow limitation on the nasal pressure tracing.\(^{(8, 60)}\) The frequency of apneas and hypopneas per hour of sleep was expressed as the AHI. Subjects who had an AHI \(\geq 10\) events/hour were classified as having a sleep-related breathing disturbance and then further sub-classified as either having OSAH if \(\geq 50\%\)
of the events were obstructive or having central sleep apnea if > 50% of the events were central.\textsuperscript{(8, 60)}
Overnight fluid shift measurements

Just before sleep studies, subjects filled in an hourly diary indicating how much time they spent in the sitting, standing and lying positions and briskly walking on the day preceding the sleep study from the time they arose in the morning until the time they laid down in the sleep laboratory. They also recorded how much time they spent upright during the course of the night while undergoing the sleep study. Body weight was measured before being instrumented prior to going to bed and within 30 minutes of awakening the next morning before urinating. With subjects instrumented for sleep studies, lying awake and supine, TBW and leg fluid volume were measured using a bioelectrical impedance spectrum analyzer (model 4200; Xitron Technologies Inc., San Diego, CA)\(^{(364)}\) with impedance to electrical current being inversely proportional to fluid content.\(^{(365, 366)}\) For TBW determination, electrodes were placed on the right wrist and ankle whereas for leg fluid volume determination, electrodes were placed on the right upper thigh and ankle. The electrodes are imbedded in an adhesive-containing pad that is fixed to the skin. Electrodes were further secured to the skin with adhesive tape and left in place throughout the night. The circumference of the neck was measured above the thyroid cartilage and of the right mid-calf at its maximum girth with a tape measure. Lines were drawn at these levels with a marker pen to ensure that measurements after sleep were made at exactly the same levels as those before sleep. Within 15 minutes of awakening the next morning and before they had arisen, urinated, eaten or drank, measurements of TBW, leg fluid volume, neck and calf circumferences were repeated.
Lower body positive pressure experiment

LBPP was applied by wrapping the deflated medical antishock trousers (MAST III – AM; David Clark Inc., Worcester, MA) around both legs from the ankles to the hips while lying supine. Leg fluid volume was measured as described above. Percentage changes in neck circumference were monitored continuously by a mercury strain gauge plethysmograph (EC6; D.E. Hokanson Inc., Bellevue, WA) whose resistance to electrical flow is altered in proportion to the change in its length. The gauge was wrapped around the neck above the thyroid cartilage and secured in place with tape. Given that a 0.1% change in neck circumference corresponds to a 20 unit change in strain gauge signal, the percentage change in neck circumference was calculated from the change in strain gauge signal using the formula: 0.1 × (change in strain gauge signal from baseline / 20). The corresponding absolute change in neck circumference was then calculated from the percentage change in neck circumference (i.e. baseline neck circumference × [percentage change in neck circumference / 100]). Upper airway cross-sectional area was determined using acoustic pharyngometry (Eccovision Acoustic pharyngometry; Sleep Group Solutions, Miami, FL) at end-expiration. Two parameters, oropharyngeal junction area and mean cross-sectional area from the velum to the glottis, were recorded via the mean of four consecutive measurements. The device was positioned in the mouth (using a modified scuba mouthpiece designed to secure the tongue in place) of subjects while they lay supine with head fixed in the neutral position by resting the head in form-shaping sand bags. The pulse emitter produced five pulses/second and
two microphones detected the amplitude and temporal changes of the reflected pulse. Acoustic pharyngometry is highly reproducible and has been validated against computed tomography and magnetic resonance imaging for assessment of upper airway cross-sectional area in non-snorers and in snorers with and without OSAH in both the supine and upright positions.\(^{(368-375)}\) Given that lung volume is a determinant of upper airway cross-sectional area, changes in end-expiratory lung volume (i.e. functional residual capacity) were assessed by respiratory inductance plethysmography calibrated against a spirometer in the DC coupled mode.\(^{(376)}\) BP and heart rate were monitored during LBPP application by an automated sphygmomanometer (Dinamap 1846SX NIBP; Critikon, Tampa, FL).

Following a 30-minute stabilization period in which subjects lay supine, baseline measurements of all variables were made. Subjects were then exposed to increasing LBPP beginning at 10 mmHg and ending at 40 mmHg, at 10 mmHg increments. Each of the 4 applied pressures was maintained for 5 minutes and measurements of all variables were made at the end of the 5-minute period.
Sample size

We determined the delta and sigma for the LBPP-induced change in mean upper airway cross-sectional area from the study by Shiota et al.\textsuperscript{(10)} We used a delta of 0.48 cm\textsuperscript{2} assuming the change in mean upper airway cross-sectional area at a LBPP of 40 mmHg would be –0.28 cm\textsuperscript{2} in the CH group (equal to the value reported by Shiota et al.\textsuperscript{(10)}) and –0.76 cm\textsuperscript{2} in the RH group as well as a sigma of 0.65 cm\textsuperscript{2} (equal to the pooled standard deviation reported by Shiota et al.\textsuperscript{(10)}). Using the unpaired t-test statistic, a beta of 0.2 and a 2-tailed alpha of 0.05, the n per group was 30.
**Statistical analysis**

Continuous variables were expressed as means ± standard error while categorical variables were expressed as proportions. Changes in fluid volume and calf circumference of the right leg were presented. Missing data values for continuous variables were handled using multiple imputation. To compare baseline differences between the CH and RH groups, the unpaired t-test, Wilcoxon rank sum test and Fisher’s exact test were used, as appropriate. For continuous variables that were not normally distributed, either a parametric analysis of transformed data or the non-parametric Wilcoxon rank sum test was employed. For the overnight fluid shift measurements, between-group comparisons were carried out using either the abovementioned tests or ANCOVA (with the pre-overnight value as a covariate), as appropriate. For the LBPP-related changes, between-group comparisons were evaluated using 2-way repeated-measures ANOVA. The impact of several covariates (age, sex, race, BMI, diabetic status, body surface area-adjusted creatinine clearance and left ventricular ejection fraction) were examined to ascertain whether they were associated with the overnight and LBPP-related changes in leg fluid volume as well as whether they confounded the observed differences between the CH and RH groups. Logistic regression was also used to assign each subject a predicted probability of RH (i.e. a propensity score) based on age, sex, race, BMI and diabetic status that was then entered as a single covariate in the between-group comparisons of the overnight and LBPP-related leg fluid volume shifts. Tests for interactions between BP status and antihypertensive drug class were also performed. The relationship between the
overnight leg fluid volume reduction and the mean LBPP-induced leg fluid volume reduction was assessed using linear regression. Scatter plots were constructed to determine the curve of best fit for the univariable relationships between the AHI and the overnight reduction in leg fluid volume as well as between the change in upper airway cross-sectional area or neck circumference at all LBPP levels and the LBPP-induced reduction in leg fluid volume using data from the entire cohort of hypertensive subjects. Residual plots were then constructed to determine the appropriateness of the fit. Given the repeated nature of the LBPP-related measurements, mixed effects regression was used to model the relationship between the change in upper airway cross-sectional area or neck circumference and the LBPP-induced leg fluid volume shift. Moreover, given the nature of the AHI and the fact that a log-linear relationship provided the best fit, Poisson regression was used to model the relationship between the number of apneas / hypopneas and the overnight leg fluid volume shift. Regression coefficients were calculated for the subjects with CH or RH and tests for interactions were performed to ascertain whether the coefficients differed according to BP status. A two-tailed p-value of < 0.05 was considered statistically significant. All analyses were conducted using SAS Version 9.1.3 (SAS Institute, Cary, NC).
5.3 Results

Baseline characteristics

We identified 40 hypertensives for our cohort with 25 of these subjects having RH and the remaining 15 subjects having CH. The characteristics of this cohort, stratified by BP status, are summarized in Tables 2 and 3. The RH group were more likely to be diabetic compared to the CH group (Table 2). By definition, subjects with RH had higher office, daytime and 24-hour systolic BP values (Table 3) despite a greater number of antihypertensive drugs (Table 2); accordingly, they also had higher 24-hour pulse pressure values (Table 3). As shown previously (75), subjects with RH also had higher nighttime BP values and were more likely to be non-dippers (Table 3). The RH group had increased cardiac target organ damage as reflected by a higher prevalence of left ventricular hypertrophy and a greater left atrial size and left ventricular mass index (Table 2). Polysomnography revealed a greater severity of OSAH in the RH group with an AHI of 43.0 events/hour compared to an AHI of 18.1 events/hour in the CH group (p=0.001 [unadjusted]; p=0.02 [adjusted for age, sex, race, BMI and diabetic status]).
**Overnight fluid shift study measurements**

Neck and calf circumferences prior to sleep and while supine were significantly greater in the RH group (41.8 ± 0.7 cm and 40.4 ± 1.0 cm, respectively) compared to the CH group (38.0 ± 1.3 cm and 36.6 ± 0.9 cm, respectively; p=0.02 and 0.01, respectively); on the other hand, although the supine leg fluid volume prior to sleep was also greater in the RH group (4634.2 ± 163.8 mL) compared to the CH group (4061.3 ± 283.5 mL), this did not achieve statistical significance (p=0.09). The overnight fluid shift measurements of the cohort, stratified by BP status, are summarized in Table 4. Insensible fluid loss led to a 0.5 L overnight reduction in TBW. The overnight reduction in calf circumference and overnight increase in neck circumference were significantly greater in the RH group compared to the CH group (p=0.001 and 0.02, respectively). Importantly, the overnight reduction in leg fluid volume was significantly greater in the RH group at 346.7 mL compared to 175.8 mL in the CH group (p=0.0002). There was no significant modification of the foregoing association between BP status and the overnight leg fluid volume reduction according to use of any of the antihypertensive drug classes (p=0.30 [BP status × antihypertensive drug class interaction term]). None of the covariates (i.e. age, sex, race, BMI, diabetic status, body surface area-adjusted creatinine clearance and left ventricular ejection fraction) were found to be associated with the overnight leg fluid volume reduction. The difference in the overnight leg fluid volume reduction remained significantly greater in the RH group despite either adjustment for age, sex,
race, BMI, diabetic status, body surface area-adjusted creatinine clearance and left ventricular ejection fraction (p=0.006) or the propensity score (p=0.01).

The relationship between the AHI and the overnight reduction in leg fluid volume was fit best using log-transformed values of the AHI (i.e. log-linear or exponential relationship as previously described for non-hypertensive subjects\(^{(72)}\); squared correlation coefficient \([R^2] = 0.56; p<0.0001\) and is depicted using the untransformed values of the AHI in Figure 4. The hazard ratio (HR) associated with each 100 mL increase in the overnight leg fluid volume reduction was 1.83 (95% CI, 1.53-2.18), implying a 1.83-fold increase in the AHI. Moreover, there was no difference between the HRs in the RH group and the CH group (p=0.49 [BP status \(\times\) overnight leg fluid volume reduction interaction term]). Similarly, using Poisson regression, the HR associated with each 100 mL increase in the overnight leg fluid volume reduction was 1.58 (95% CI, 1.38-1.84; p<0.0001), implying a 1.58-fold increase in the AHI and once again, there was no difference between the HRs in the RH group and the CH group (p=0.53 [BP status \(\times\) overnight leg fluid volume reduction interaction term]). In contrast, there was only a trend toward a significant relationship between the AHI and either the BMI (HR [associated with each 1 kg/m\(^2\) increase] = 1.03; 95% CI, 1.00-1.06; p=0.08) or baseline neck circumference (HR [associated with each 1 cm increase] = 1.06; 95% CI, 1.00-1.12; p=0.05).
Lower body positive pressure study measurements

Leg fluid volume and neck circumference prior to LBPP application were significantly greater in the RH group (4025.2 ± 161.0 mL and 42.1 ± 0.8 cm, respectively) compared to the CH group (3224.7 ± 245.0 mL and 39.0 ± 1.3 cm, respectively; p=0.01 and 0.04, respectively); on the other hand, the baseline oropharyngeal junction area and mean upper airway cross-sectional area were comparable in the RH (1.40 ± 0.1 cm² and 1.79 ± 0.1 cm², respectively) and CH (1.34 ± 0.1 cm² and 1.62 ± 0.2 cm², respectively) groups (p=0.70 and 0.46, respectively). The LBPP measurements of the cohort, stratified by BP status, are summarized in Table 5. Compared to the CH group, the LBPP-induced reductions in oropharyngeal junction area and mean upper airway cross-sectional area were significantly greater in the RH group (p<0.0001 and 0.001, respectively). Figure 5 shows tracings of upper airway cross-sectional area as a function of distance from the mouth at baseline and at a LBPP of 40 mmHg from two representative subjects, one with CH and the other with RH. The LBPP-induced increase in neck circumference was also significantly greater in the RH group (p<0.0001). Figure 6 displays the grouped data for the changes in mean upper airway cross-sectional area, oropharyngeal junction area and neck circumference in response to LBPP in the CH and RH groups. Importantly, the LBPP-induced reduction in leg fluid volume was significantly greater in the RH group (e.g. 114.7 mL at a LBPP = 40 mmHg) compared to the CH group (e.g. 52.9 mL at a LBPP = 40 mmHg; p<0.0001). There was no significant modification of the foregoing
association between BP status and the LBPP-induced leg fluid volume reduction according to use of any of the antihypertensive drug classes (p=0.14 [BP status × antihypertensive drug class interaction term]). Of the covariates, only a greater BMI was associated with increased LBPP-induced leg fluid volume reduction (p<0.0001). Further, the LBPP-induced leg fluid volume reduction remained significantly greater in the RH group despite either adjustment for age, sex, race, BMI, diabetic status, body surface area-adjusted creatinine clearance and left ventricular ejection fraction (p=0.004) or the propensity score (p=0.001). The relationship between the overnight reduction in leg fluid volume and the mean LBPP-induced reduction in leg fluid volume is depicted in Figure 7 (R² = 0.23, p=0.002); each 50 mL increase in the mean LBPP-induced reduction in leg fluid volume was associated with a 104.3 mL (95% CI, 41.1-167.5) increase in the overnight leg fluid volume reduction.

The relationship between the reduction in mean upper airway cross-sectional area and the LBPP-induced reduction in leg fluid volume was fit best using log-tranformed values of the reduction in mean upper airway cross-sectional area and is depicted in Figure 8a (R² = 0.41, p<0.0001). There was no difference between the regression coefficients in the RH group and the CH group (p=0.31 [BP status × LBPP-induced leg fluid volume reduction interaction term]). Similarly, the relationship between the reduction in oropharyngeal junction area and the LBPP-induced reduction in leg fluid volume was fit best using log-tranformed values of the reduction in oropharyngeal junction area and is depicted in Figure 8b (R² = 0.42, p<0.0001). Once again, there was no difference between the regression coefficients in
the RH group and the CH group (p=0.20 [BP status × LBPP-induced leg fluid volume reduction interaction term]). There was also a significant correlation between the increase in neck circumference (using log-transformed values) and the LBPP-induced reduction in leg fluid volume as depicted in Figure 9 ($R^2 = 0.47$, $p<0.0001$) with no difference between the regression coefficients in the RH group and the CH group (p=0.12 [BP status × LBPP-induced leg fluid volume reduction interaction term]).
5.4 Discussion

The basis for the present study originated from several important and parallel observations. First, both older and newer studies suggest that the RH state may be associated with refractory volume overload, in a significant subset of patients.\(^{19-22, 26}\) This has been further supported by ancillary studies revealing a high prevalence of non-dipping\(^ {24, 25, 75}\) and primary hyperaldosteronism\(^ {38, 7}\) and a marked hypotensive response with administration of mineralocorticoid receptor antagonists and other diuretics\(^ {42-44}\). Second, driven by the realization that increased BMI and neck girth only account for approximately one third of the variability in the AHI\(^ {62}\), a number of mechanistic and interventional studies have emerged suggesting that nuchal and peripharyngeal edema and/or rostral fluid displacement may contribute to the pathogenesis of OSAH.\(^ {63, 9, 64, 67, 10, 11, 65, 72}\) Therefore, in light of the finding of a remarkably high prevalence of OSAH in RH that has yet to be fully explained\(^ {8, 53, 41}\), we hypothesized that volume overload may contribute not only to apparent refractoriness of hypertension to drug therapy but also to the occurrence of OSAH.

This study has given rise to several novel findings and extends previous work by addressing a potential underlying basis of OSAH in a hypertensive cohort. First, we found that the AHI was strongly related to the amount of fluid spontaneously displaced rostrally from the legs during sleep. Indeed, the overnight change in leg fluid volume accounted for nearly three fifths of the variability in the AHI and this applied similarly to subjects with CH or RH. However, the spontaneous displacement of leg fluid volume was significantly greater in the RH group than in the CH group.
Among healthy, non-obese adults with suspected OSAH, Redolfi et al. also found that the relationship between the AHI and the overnight reduction in leg fluid volume was best described by an exponential curve with the latter accounting for nearly two thirds of the variability in the AHI.\(^{(72)}\) In that study, however, the mean AHI and volume of displaced leg fluid were 18 events/hour and 133 mL, respectively, compared to our values of 34 events/hour and 283 mL overall, respectively. Second, we found that the LBPP-induced reduction in upper airway cross-sectional area was strongly related to the amount of fluid displaced from the legs following LBPP application. In fact, the LBPP-induced change in leg fluid volume accounted for two fifths of its variability and applied similarly in subjects with either CH or RH. In addition, the LBPP-induced displacement of leg fluid volume was significantly greater in the RH group than in the CH group. Among healthy, non-obese adults, Shiota et al. observed a mean volume of displaced leg fluid (at a LBPP = 40 mmHg) of 140 mL, compared to our value of 92 mL overall.\(^{(10)}\) Differences in the LBPP protocol and patient characteristics may account for these disparate findings. For example, in the study by Shiota et al., LBPP was not applied using incremental pressures. Further, although extracellular fluid volume status may be a determinant of the magnitude of leg fluid volume displaced via LBPP, there are likely also other physiological factors that mediate this response (e.g. venous tone, ventricular systolic/diastolic function). Interestingly, despite a lesser displacement of leg fluid volume with LBPP in our study, we observed a greater mean reduction in upper airway cross-sectional area of 0.38 cm\(^2\) overall than the value of 0.28 cm\(^2\) reported by Shiota et al. If confirmed, then perhaps there may
be susceptibility factors (e.g. periphraryngeal fat, ventricular systolic/diastolic dysfunction) in hypertensive subjects that render their upper airways more sensitive to a given volume of displaced leg fluid or alternatively, that reduce their ability to accommodate an augmented venous return. Taken together, these data suggest that in hypertensive subjects, rostral fluid displacement may participate in the pathogenesis of OSAH. The nature of the intercompartmental body fluid redistribution, however, remains speculative given that bioelectrical impedance analysis cannot distinguish between intravascular and interstitial fluid. Possibly, there may be an initial translocation of intravascular fluid from the legs centrally followed by a flux of interstitial fluid from the legs into the blood. Subsequent accumulation of fluid in the neck, whether in the intravascular or extravascular space, may predispose to upper airway collapse during sleep, presumably by increasing extraluminal tissue pressure resulting in upper airway narrowing.

Nevertheless, given the study’s physiologic interventional study design, the potential remains for residual confounding to have accounted for the study findings. Further, despite all subjects receiving instructions to reduce dietary sodium intake to the level recommended for hypertensive patients (377), there was a greater dietary sodium intake in the RH group (Table 2); parenthetically however, the overnight and LBPP-induced leg fluid volume reductions remained significantly greater in the RH group despite adjustment for the sodium excretion rate (p=0.002 and <0.0001, respectively). Moreover, although the literature on the effect of specific non-diuretic antihypertensives on OSAH severity is limited and perhaps modest at most (378), it
remains possible that certain antihypertensive classes may be potential confounders (e.g. calcium channel blockers, especially dihydropyridines that can cause peripheral edema) despite there being no significant interactions between BP status and antihypertensive drug class.

Given that the effects of LBPP application on neck circumference and upper airway cross-sectional area were assessed during the wake period, the findings may not be applicable to those occurring during the sleep period. However, it would have been impractical for subjects to sleep uninterrupted while undergoing simultaneous LBPP application and acoustic pharyngometry since they would be unable to sleep or would move if they did fall asleep causing artifactual changes in the acoustic pharyngometry signal. Additionally, the literature would suggest that rostral fluid shift may have an even more pronounced effect on upper airway cross-sectional area during the sleep than wake cycle because of a withdrawal of neural input to the pharyngeal dilator muscles at sleep onset resulting in pharyngeal luminal narrowing with a corresponding increase in pharyngeal resistance and collapsibility.\(^{(379, 380)}\) A limitation of acoustic pharyngometry, given that transmitted sound waves travel below the soft palate, is its inability to evaluate the lumen size at the level of the retropalatal pharynx, the site at which the pharynx collapses in most patients with OSAH.\(^{(381)}\) Nonetheless, significant changes in the mean upper airway cross-sectional area and/or oropharyngeal junction area have already been documented following LBPP application and diuretic therapy.\(^{(67, 10)}\) Furthermore, several studies have shown consistently that the upper airway cross-sectional area from velum to glottis in the
awake state is narrowed in patients with OSAH, both while upright and supine.\(^{(382, 199)}\)

Change in leg fluid volume were measured and presented using only the right leg; this of course assumes that the corresponding total leg fluid shift was twice that of the one leg. Finally, there was no control period during which LBPP was not applied in the present study; however, a number of studies have already convincingly demonstrated that leg fluid volume, neck circumference, upper airway cross-sectional area, upper airway resistance and upper airway collapsibility remain unchanged during the control period.\(^{(9-11)}\)

In aggregate, the present study suggests that rostral fluid displacement from the legs may be a unifying mechanism that explains, in part, increased tendency for the upper airway to collapse both during wakefulness in response to LBPP\(^{(9-11)}\) and during sleep in response to spontaneous rostral fluid displacement that applies both to otherwise healthy subjects\(^{(72)}\) and to patients with either CH or RH. As with otherwise healthy subjects, the spontaneous overnight leg fluid shift strongly related to the severity of OSAH, as assessed by the AHI. In addition, the magnitude of fluid displacement was greater among RH subjects and largely explained the higher AHI in this group than in the CH group. In view of the existing literature, such data strongly supports the hypothesis that volume overload may be pathogenically related to the RH state and further, may contribute to the development of OSAH. However, to what degree such fluid excess is a consequence of these subjects’ greater burden of hypertensive target organ damage (with reduced glomerular filtration rate and/or ventricular systolic/diastolic dysfunction) remains unknown.
CHAPTER 6. GENERAL DISCUSSION

Accumulating evidence suggests that hypervolemia may contribute to both the non-dipping BP pattern and the high prevalence of OSAH in RH. With respect to the former, nocturnal BP abnormalities are independently associated with increased risk of death and cardiovascular disease. In the “Nocturnal blood pressure study”, we used a cross-sectional study design to assess the nocturnal BP profiles from 24-hour ABPM recorded on RH (n = 26), CH (n = 52) and NT (n = 52) subjects, matched for age, sex and BMI. We observed that during sleep, the percentage fall in mean arterial pressure was 15.1 ± 6.1 in the NT group, 11.5 ± 7.0 in the CH group and 7.7 ± 7.7 in the RH group (p<0.0001), with all pairwise comparisons of the nocturnal BP fall being significant. The corresponding proportions of non-dipping were 25.0%, 42.3% and 61.5% (p=0.006), and those of nocturnal hypertension, 9.6%, 23.1% and 84.6% (p<0.0001). The proportion of nocturnal BP rising in the RH group of 19.2% was significantly greater than that in the other two groups (p=0.001), as was the proportion of nocturnal hypertension (p<0.0001). Therefore, we demonstrated a significantly higher prevalence of non-dipping, nocturnal hypertension and nocturnal BP rising in RH. We surmised that these sleep disturbances, or independently their cause (e.g. volume expansion or OSAH), may account for the difficulties in attaining BP control in RH.

With respect to the latter, upon recumbency prior to sleep, leg fluid volume accumulated in the upright position moves rostrally causing an increase in nuchal and peripharyngeal fluid content, subsequently obstructing airflow. In the “Fluid shift
study”, we used a physiologic interventional study design in treated hypertensive subjects, consisting of both patients with CH (n = 15) and RH (n = 25), to examine the emerging role of extracellular fluid volume expansion in inducing or aggravating OSAH. Specifically, in order to assess for rostral fluid displacement, leg fluid volume shifts were examined in all subjects under the influence of LBPP application as well as occurring spontaneously overnight during sleep. Polysomnography revealed a greater severity of OSAH in the RH group with an AHI of 43.0 compared to 18.1 in the CH group (p=0.02; adjusted for age, sex, race, BMI and diabetes). In both groups, the reduction in mean upper airway cross-sectional area with LBPP strongly related to the amount of fluid displaced from the legs ($R^2 = 0.41$; p<0.0001), although its magnitude was greater in the RH group (p=0.001; adjusted for propensity score). In both groups, the AHI strongly related to the amount of fluid spontaneously displaced from the legs during sleep ($R^2 = 0.56$; p<0.0001), although its magnitude was greater in the RH group (p=0.01; adjusted for propensity score). Taken together, these data suggest that in hypertensives, rostral fluid displacement strongly relates to both the LBPP-induced upper airway response and the severity of OSAH with its magnitude being greater in RH. The findings from these two studies therefore support the claim that hypervolemia may be pathogenically related to the RH state and further, may contribute to the development of the non-dipping pattern and OSAH.
CHAPTER 7. FURTHER DIRECTIONS

CPAP is the most widely used treatment for OSAH and in patients with daytime hypersomnolence, it is very effective in reducing symptoms and is generally well tolerated. For nonhypersomnolent patients, however, such as those with hypertension or heart failure, poor adherence is a major problem and has a significant impact on its effectiveness. In this regard, if an expanded extracellular fluid volume in RH contributes to both resistance of hypertension to antihypertensive treatment and the development of OSAH via rostral fluid shift, then presumably, a reduction in extracellular fluid volume, as with intensified diuresis, may provide a two-pronged approach by which to effectively treat both RH and OSAH. We anticipate that intensified diuresis will cause reductions in the severity of OSAH and in the LBPP-induced upper airway response due to diminishations in spontaneous overnight and LBPP-induced leg fluid volume shifts as well as in a reestablishment of the dipping pattern of BP during sleep. In this latter regard, a randomized controlled trial that assessed the effects on BP of five different classes of antihypertensive drugs (amlodipine, enalapril, atenolol, hydrochlorothiazide, losartan) in hypertensive men with OSAH found that all drugs were equally effective in lowering daytime BP but only atenolol and hydrochlorothiazide reduced nighttime BP.\(^{(211)}\) Additional maneuvers to attenuate the rostral fluid shift mechanism which could be tested include restricting dietary sodium, elevating the head of the bed during sleep and physical exercise to reduce dependent fluid accumulation (e.g. improving calf muscle pump function) and its subsequent overnight deposition in the neck.
CHAPTER 8. REFERENCES


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CHAPTER 9. APPENDIX

9.1 Tables

Table 1. Baseline characteristics of cohort for the “Nocturnal blood pressure study”.*

<table>
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<tr>
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<th>Normotension</th>
<th>Controlled hypertension</th>
<th>Resistant hypertension</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>52</td>
<td>52</td>
<td>26</td>
<td>N/A</td>
</tr>
<tr>
<td>Age (years)</td>
<td>56.5 ± 11.5</td>
<td>58.3 ± 11.7</td>
<td>57.8 ± 10.1</td>
<td>0.70</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>29.0 ± 4.9</td>
<td>29.4 ± 4.9</td>
<td>30.4 ± 4.6</td>
<td>0.50</td>
</tr>
<tr>
<td>Sex (% m)</td>
<td>46.1</td>
<td>46.1</td>
<td>46.1</td>
<td>N/A</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>5.8</td>
<td>9.6</td>
<td>15.4</td>
<td>0.33</td>
</tr>
<tr>
<td>Number of drug(s)</td>
<td>N/A</td>
<td>1.7 ± 0.7</td>
<td>3.8 ± 0.9</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Proportion of subjects taking antihypertensive drug (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACEI</td>
<td>N/A</td>
<td>44.2</td>
<td>53.9</td>
<td>0.48</td>
</tr>
<tr>
<td>ARB</td>
<td>15.4</td>
<td>11.5</td>
<td></td>
<td>0.74</td>
</tr>
<tr>
<td>BB</td>
<td>36.5</td>
<td>69.2</td>
<td></td>
<td>0.008</td>
</tr>
<tr>
<td>CCB</td>
<td>32.7</td>
<td>84.6</td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Diuretic</td>
<td>42.3</td>
<td>80.8</td>
<td>0.002</td>
<td></td>
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<td>-------</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>1.9</td>
<td>23.1</td>
<td>0.005</td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>0</td>
<td>15.4</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>VD</td>
<td>0</td>
<td>3.8</td>
<td>0.33</td>
<td></td>
</tr>
<tr>
<td><strong>Daytime SBP (mmHg)</strong></td>
<td>126.5 ± 6.4</td>
<td>124.9 ± 7.0</td>
<td>151.1 ± 16.7</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>Daytime DBP (mmHg)</strong></td>
<td>78.0 ± 5.6</td>
<td>74.2 ± 7.4</td>
<td>89.5 ± 10.7</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>Daytime MAP (mmHg)</strong></td>
<td>94.2 ± 5.1</td>
<td>91.1 ± 6.0</td>
<td>110.1 ± 10.8</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>Nighttime SBP (mmHg)</strong></td>
<td>110.2 ± 8.1</td>
<td>112.6 ± 10.6</td>
<td>143.0 ± 23.7</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>Nighttime DBP (mmHg)</strong></td>
<td>64.6 ± 6.3</td>
<td>64.5 ± 8.3</td>
<td>80.9 ± 11.9</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>Nighttime MAP (mmHg)</strong></td>
<td>79.8 ± 6.2</td>
<td>80.5 ± 79</td>
<td>101.6 ± 13.9</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>24-hr mean SBP (mmHg)</strong></td>
<td>122.0 ± 6.2</td>
<td>122.0 ± 7.3</td>
<td>149.4 ± 17.8</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>24-hr mean DBP (mmHg)</strong></td>
<td>74.5 ± 5.2</td>
<td>72.1 ± 7.3</td>
<td>87.6 ± 10.8</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td><strong>24-hr MAP</strong></td>
<td>90.3 ± 4.7</td>
<td>88.7 ± 6.0</td>
<td>108.2 ± 11.1</td>
<td>&lt; 0.0001†</td>
</tr>
<tr>
<td>(mmHg)</td>
<td></td>
<td></td>
<td>0.24#</td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>

ACEI, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; BB, beta blocker; CCB, calcium channel blocker; AB, alpha blocker; CS, central sympatholytic; VD, vasodilator; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; N/A, not applicable.

* Reported as mean ± standard deviation or % of subjects.

† P-values for comparisons of normotensive or controlled hypertensive and resistant hypertensive subjects.

# P-values for comparisons of normotensive and controlled hypertensive subjects.
Table 2. Baseline characteristics of cohort for the “Fluid shift study”.*

<table>
<thead>
<tr>
<th></th>
<th>CH cohort</th>
<th>RH cohort</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>65.9 ± 3.4</td>
<td>63.3 ± 1.8</td>
<td>0.51</td>
</tr>
<tr>
<td>Sex (% m)</td>
<td>40.0</td>
<td>51.8</td>
<td>0.53</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>30.2 ± 1.3</td>
<td>33.8 ± 1.3</td>
<td>0.06</td>
</tr>
<tr>
<td>Race (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>80.0</td>
<td>77.8</td>
<td>0.62</td>
</tr>
<tr>
<td>Black</td>
<td>6.7</td>
<td>14.8</td>
<td></td>
</tr>
<tr>
<td>Asian</td>
<td>13.3</td>
<td>7.4</td>
<td></td>
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<tr>
<td>Diabetes (%)</td>
<td>13.3</td>
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<td>0.02</td>
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<tr>
<td>Coronary artery disease (%)</td>
<td></td>
<td></td>
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<tr>
<td>Peripheral vascular disease (%)</td>
<td>13.3</td>
<td>11.1</td>
<td>1.0</td>
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<td>Cerebrovascular disease (%)</td>
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<td>1.0</td>
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<tr>
<td>Physical activity (hours/week)</td>
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<tr>
<td>Moderate (3.0-5.0 METs)</td>
<td>7.3 ± 1.7</td>
<td>9.6 ± 1.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Hard (5.1-6.9 METs)</td>
<td>0.9 ± 0.7</td>
<td>1.1 ± 0.5</td>
<td>0.84</td>
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<td>Very hard (≥ 7.0 METs)</td>
<td>0.2 ± 0.1</td>
<td>0.6 ± 0.4</td>
<td>0.36</td>
</tr>
<tr>
<td>Body positions and activity (hours)</td>
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<td>Standing</td>
<td>Lying down</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---------</td>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>Sitting</td>
<td>7.6 ± 0.7</td>
<td>6.3 ± 0.5</td>
<td>0.15</td>
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<tr>
<td>Standing</td>
<td>4.7 ± 0.5</td>
<td>5.2 ± 0.6</td>
<td>0.52</td>
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<tr>
<td>Lying down</td>
<td>1.2 ± 0.3</td>
<td>1.5 ± 0.4</td>
<td>0.56</td>
</tr>
<tr>
<td>Briskly walking</td>
<td>0.3 ± 0.1</td>
<td>0.3 ± 0.1</td>
<td>0.97</td>
</tr>
</tbody>
</table>

| Number of antihypertensive drugs   | 2.7 ± 0.1 | 4.1 ± 0.1 | <0.0001    |

<table>
<thead>
<tr>
<th>Proportion of subjects taking antihypertensive drug (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitor</td>
</tr>
<tr>
<td>Angiotensin II receptor blocker</td>
</tr>
<tr>
<td>Beta blocker</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
</tr>
<tr>
<td>Diuretic</td>
</tr>
<tr>
<td>Alpha-1 blocker</td>
</tr>
<tr>
<td>Central sympatholytic</td>
</tr>
<tr>
<td>Direct vasodilator</td>
</tr>
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<td>--------------------------------</td>
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<tr>
<td></td>
</tr>
<tr>
<td><strong>Creatinine clearance</strong></td>
</tr>
<tr>
<td>(mL/min/1.73 m²)</td>
</tr>
<tr>
<td><strong>Protein excretion rate</strong></td>
</tr>
<tr>
<td>(mg/day)</td>
</tr>
<tr>
<td><strong>Urine albumin / creatinine ratio (%)</strong></td>
</tr>
<tr>
<td>&lt; 3.4 mg/mmol</td>
</tr>
<tr>
<td>3.4-34 mg/mmol</td>
</tr>
<tr>
<td>≥ 34 mg/mmol</td>
</tr>
<tr>
<td><strong>Sodium excretion rate</strong></td>
</tr>
<tr>
<td>(mmol/day)</td>
</tr>
<tr>
<td><strong>Potassium excretion rate</strong></td>
</tr>
<tr>
<td>(mmol/day)</td>
</tr>
<tr>
<td><strong>Left ventricular ejection fraction (%)</strong></td>
</tr>
<tr>
<td>≥ 60%</td>
</tr>
<tr>
<td>40-59%</td>
</tr>
<tr>
<td>20-39%</td>
</tr>
<tr>
<td><strong>Left ventricular hypertrophy (%)</strong></td>
</tr>
<tr>
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<td></td>
</tr>
</tbody>
</table>

174
<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Left ventricular mass index (g/m²)</strong></td>
<td>81.6 ± 9.3</td>
<td>116.6 ± 5.2</td>
<td>0.01</td>
</tr>
<tr>
<td><strong>Left atrial diameter (cm)</strong></td>
<td>3.7 ± 0.2</td>
<td>4.2 ± 0.1</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Right ventricular systolic pressure (mmHg)</strong></td>
<td>30.7 ± 1.8</td>
<td>33.7 ± 1.7</td>
<td>0.24</td>
</tr>
</tbody>
</table>

CH – controlled hypertension, METs – metabolic equivalents, NA – not applicable, RH – resistant hypertension.

* Reported as mean ± standard error or % of subjects.

+ P-values for comparisons of subjects with controlled hypertension versus resistant hypertension.
Table 3. Office and ambulatory BP and polysomnographic parameters of cohort for the “Fluid shift study”.*

<table>
<thead>
<tr>
<th></th>
<th>CH cohort</th>
<th>RH cohort</th>
<th>P-value+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Office systolic BP (mmHg)</td>
<td>135.1 ± 3.0</td>
<td>155.0 ± 3.9</td>
<td>0.0002</td>
</tr>
<tr>
<td>Office diastolic BP (mmHg)</td>
<td>75.3 ± 2.6</td>
<td>82.2 ± 2.8</td>
<td>0.08</td>
</tr>
<tr>
<td>Daytime systolic BP (mmHg)</td>
<td>129.8 ± 2.1</td>
<td>144.4 ± 3.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Daytime diastolic BP (mmHg)</td>
<td>74.8 ± 3.4</td>
<td>79.7 ± 2.4</td>
<td>0.26</td>
</tr>
<tr>
<td>Nighttime systolic BP (mmHg)</td>
<td>113.2 ± 3.3</td>
<td>135.5 ± 3.8</td>
<td>0.0001</td>
</tr>
<tr>
<td>Nighttime diastolic BP (mmHg)</td>
<td>61.3 ± 2.9</td>
<td>72.8 ± 2.7</td>
<td>0.008</td>
</tr>
<tr>
<td>24-hour systolic BP (mmHg)</td>
<td>124.9 ± 2.1</td>
<td>141.8 ± 3.3</td>
<td>0.0002</td>
</tr>
<tr>
<td>24-hour diastolic BP (mmHg)</td>
<td>70.8 ± 3.0</td>
<td>77.9 ± 2.4</td>
<td>0.08</td>
</tr>
<tr>
<td>24-hour pulse pressure (mmHg)</td>
<td>54.1 ± 3.4</td>
<td>63.9 ± 2.9</td>
<td>0.04</td>
</tr>
<tr>
<td>Percentage nocturnal systolic BP fall</td>
<td>12.7 ± 2.3</td>
<td>6.2 ± 1.6</td>
<td>0.03</td>
</tr>
<tr>
<td>Non-dipping (%)</td>
<td>46.7</td>
<td>85.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Apnea-hypopnea index (events/hour of sleep)</td>
<td>18.1 ± 4.2</td>
<td>43.0 ± 5.4</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>P-value</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------</td>
<td>--------</td>
<td>---------</td>
</tr>
<tr>
<td>Sleep-related breathing disturbance (%)</td>
<td>60.0</td>
<td>85.2</td>
<td>0.13</td>
</tr>
<tr>
<td>Obstructive sleep apnea-hypopnea (%)</td>
<td>60.0</td>
<td>77.8</td>
<td>0.29</td>
</tr>
<tr>
<td>Minimum oxyhemoglobin % saturation</td>
<td>83.3 ± 1.3</td>
<td>75.7 ± 2.5</td>
<td>0.01</td>
</tr>
<tr>
<td>Mean oxyhemoglobin % saturation</td>
<td>93.0 ± 0.6</td>
<td>92.7 ± 0.6</td>
<td>0.71</td>
</tr>
<tr>
<td>Arousal index (events/hour of sleep)</td>
<td>20.3 ± 3.4</td>
<td>35.0 ± 4.0</td>
<td>0.01</td>
</tr>
</tbody>
</table>

BP – blood pressure, CH – controlled hypertension, RH – resistant hypertension.

* Reported as mean ± standard error or % of subjects.

+ P-values for comparisons of subjects with controlled hypertension versus resistant hypertension.
Table 4. Overnight fluid shift study measurements of cohort for the “Fluid shift study”.*

<table>
<thead>
<tr>
<th></th>
<th>CH cohort</th>
<th>RH cohort</th>
<th>P-value+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overnight change in leg fluid volume (mL)</td>
<td>-175.8 ± 31.3</td>
<td>-346.7 ± 24.1</td>
<td>0.0002</td>
</tr>
<tr>
<td>Overnight change in neck circumference (cm)</td>
<td>+1.08 ± 0.10</td>
<td>+1.51 ± 0.14</td>
<td>0.02</td>
</tr>
<tr>
<td>Overnight change in calf circumference (cm)</td>
<td>-0.92 ± 0.18</td>
<td>-1.84 ± 0.19</td>
<td>0.001</td>
</tr>
<tr>
<td>Overnight change in body weight (kg)</td>
<td>-0.8 ± 0.1</td>
<td>-1.1 ± 0.1</td>
<td>0.12</td>
</tr>
<tr>
<td>Overnight change in total body water (L)</td>
<td>-0.6 ± 0.2</td>
<td>-0.5 ± 0.3</td>
<td>0.78</td>
</tr>
<tr>
<td>Upright at night (hours)</td>
<td>0.04 ± 0.02</td>
<td>0.10 ± 0.03</td>
<td>0.11</td>
</tr>
</tbody>
</table>

CH – controlled hypertension, RH – resistant hypertension.

* Reported as mean ± standard error.

+ P-values for comparisons of subjects with controlled hypertension versus resistant hypertension.
Table 5. Lower body positive pressure study measurements of cohort for the “Fluid shift study”.

<table>
<thead>
<tr>
<th></th>
<th>CH cohort</th>
<th>RH cohort</th>
<th>P-value+</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Change in leg fluid volume (mL)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBPP = 10 mmHg</td>
<td>-16.7 ± 2.5</td>
<td>-42.0 ± 4.6</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LBPP = 20 mmHg</td>
<td>-31.3 ± 4.1</td>
<td>-71.6 ± 6.8</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30 mmHg</td>
<td>-42.7 ± 5.0</td>
<td>-94.8 ± 8.5</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40 mmHg</td>
<td>-52.9 ± 5.4</td>
<td>-114.7 ± 9.2</td>
<td></td>
</tr>
<tr>
<td><strong>Change in mean upper airway cross-sectional area (cm²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBPP = 10 mmHg</td>
<td>-0.06 ± 0.01</td>
<td>-0.19 ± 0.03</td>
<td>0.001</td>
</tr>
<tr>
<td>LBPP = 20 mmHg</td>
<td>-0.12 ± 0.02</td>
<td>-0.35 ± 0.07</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30 mmHg</td>
<td>-0.18 ± 0.03</td>
<td>-0.39 ± 0.06</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40 mmHg</td>
<td>-0.21 ± 0.03</td>
<td>-0.49 ± 0.09</td>
<td></td>
</tr>
<tr>
<td><strong>Change in oropharyngeal junction area (cm²)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBPP = 10 mmHg</td>
<td>-0.04 ± 0.01</td>
<td>-0.13 ± 0.03</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LBPP = 20 mmHg</td>
<td>-0.08 ± 0.01</td>
<td>-0.22 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30 mmHg</td>
<td>-0.13 ± 0.01</td>
<td>-0.31 ± 0.04</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40 mmHg</td>
<td>-0.19 ± 0.02</td>
<td>-0.45 ± 0.06</td>
<td></td>
</tr>
<tr>
<td><strong>Change in neck circumference (cm)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBPP = 10 mmHg</td>
<td>+0.02 ± 0.004</td>
<td>+0.06 ± 0.008</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>LBPP = 20 mmHg</td>
<td>+0.04 ± 0.006</td>
<td>+0.10 ± 0.010</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30 mmHg</td>
<td>+0.05 ± 0.007</td>
<td>+0.14 ± 0.014</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40 mmHg</td>
<td>+0.07 ± 0.011</td>
<td>+0.20 ± 0.019</td>
<td></td>
</tr>
<tr>
<td><strong>Change in end-expiratory lung volume (L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBPP = 10 mmHg</td>
<td>+0.06 ± 0.03</td>
<td>-0.02 ± 0.02</td>
<td>0.52</td>
</tr>
<tr>
<td>LBPP = 20 mmHg</td>
<td>+0.07 ± 0.04</td>
<td>-0.01 ± 0.03</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30 mmHg</td>
<td>-0.01 ± 0.03</td>
<td>+0.05 ± 0.05</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40 mmHg</td>
<td>+0.03 ± 0.04</td>
<td>+0.05 ± 0.04</td>
<td></td>
</tr>
<tr>
<td><strong>Change in systolic BP (mmHg)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBPP = 10 mmHg</td>
<td>+0.73 ± 1.25</td>
<td>-0.89 ± 1.18</td>
<td>0.22</td>
</tr>
<tr>
<td>LBPP = 20 mmHg</td>
<td>+1.71 ± 1.36</td>
<td>-0.52 ± 1.24</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30 mmHg</td>
<td>+0.50 ± 1.29</td>
<td>+0.41 ± 1.41</td>
<td></td>
</tr>
<tr>
<td>LBPP (mmHg)</td>
<td>Change in diastolic BP (mmHg)</td>
<td>Change in heart rate (bpm)</td>
<td></td>
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<tr>
<td>---------------</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>+1.85 ± 1.15</td>
<td>-1.53 ± 2.03</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBPP = 10</td>
<td>+0.67 ± 0.83</td>
<td>-0.64 ± 0.80</td>
<td></td>
</tr>
<tr>
<td>LBPP = 20</td>
<td>+0.07 ± 0.79</td>
<td>+0.08 ± 1.10</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30</td>
<td>+1.00 ± 0.82</td>
<td>+1.04 ± 1.12</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40</td>
<td>+1.39 ± 1.31</td>
<td>+2.16 ± 1.44</td>
<td></td>
</tr>
<tr>
<td>LBPP = 10</td>
<td>+0.20 ± 0.46</td>
<td>-0.24 ± 0.48</td>
<td></td>
</tr>
<tr>
<td>LBPP = 20</td>
<td>+0.33 ± 0.69</td>
<td>+0.04 ± 0.75</td>
<td></td>
</tr>
<tr>
<td>LBPP = 30</td>
<td>-0.13 ± 0.86</td>
<td>-0.67 ± 1.46</td>
<td></td>
</tr>
<tr>
<td>LBPP = 40</td>
<td>+0.57 ± 0.60</td>
<td>-0.16 ± 0.86</td>
<td></td>
</tr>
</tbody>
</table>

BP – blood pressure, CH – controlled hypertension, RH – resistant hypertension.

* Reported as mean ± standard error.

+ P-values for comparisons of subjects with controlled hypertension versus resistant hypertension (i.e. BP status × LBPP interaction term).
9.2 Figures

Figure 1. Nocturnal mean arterial pressure falls in subjects with normotension, controlled hypertension and resistant hypertension for the "Nocturnal blood pressure study".*

* 95% confidence bars depicted; p-value<0.0001 (overall).
Figure 2. Non-dipper proportions in subjects with normotension, controlled hypertension and resistant hypertension for the “Nocturnal blood pressure study”.

* 95% confidence bars depicted; p-value=0.006 (overall).
Figure 3. Nocturnal hypertensive proportions in subjects with normotension, controlled hypertension and resistant hypertension for the “Nocturnal blood pressure study”.*

* 95% confidence bars depicted; p-value<0.0001 (overall).
Figure 4. Relationship between the apnea-hypopnea index (AHI, events/hour) and the overnight reduction in leg fluid volume (LFV, mL) for the “Fluid shift study”.

AHI versus overnight reduction in LFV

BP status CH RH
Figure 5. Tracings of upper airway cross-sectional area (UAXSA, cm$^2$) as a function of distance from the mouth (cm) at baseline (top) and at a lower body positive pressure (LBPP) of 40 mmHg (bottom) in two representative subjects, one with controlled hypertension (left) and the other with resistant hypertension (right) for “Fluid shift study”. Oropharyngeal junction (OPJ) area is the UAXSA at the level of the velum (V, first arrowhead) and mean UAXSA is the mean area between the V and glottis (G, second arrowhead).
Reconstructed 3-dimensional representations of the upper airway in the two representative subjects are displayed below (from left to right: CH subject at baseline, CH subject at LBPP of 40 mmHg, RH subject at baseline, RH subject at LBPP of 40 mmHg).
Figure 6. Grouped data showing changes in mean upper airway cross-sectional area (UAXSA, cm$^2$; A), oropharyngeal junction area (OPJ, cm$^2$; B) and neck circumference (NC, cm; C) in response to lower body positive pressure (LBPP, mmHg) for the “Fluid shift study” (all p-values for BP status × LBPP interaction term ≤ 0.001).

A. UAXSA versus LBPP
B. OPJ versus LBPP

C. NC versus LBPP
Figure 7. Relationship between the overnight reduction in leg fluid volume (LFV, mL) and the mean lower body positive pressure-induced reduction in LFV (mL) for the “Fluid shift study”.

Overnight reduction in LFV versus mean LBPP-induced reduction in LFV
Figure 8a. Relationship between the reduction in mean upper airway cross-sectional area (UAXSA, cm$^2$) at all lower body positive pressure (LBPP) levels and the LBPP-induced reduction in leg fluid volume (LFV, mL) for the “Fluid shift study”.

Reduction in UAXSA versus LBPP-induced reduction in LFV
Figure 8b. Relationship between the reduction in oropharyngeal junction area (OPJ, cm$^2$) at all lower body positive pressure (LBPP) levels and the LBPP-induced reduction in leg fluid volume (LFV, mL) for the “Fluid shift study”.

Reduction in OPJ versus LBPP-induced reduction in LFV

Log(Reduction in OPJ + 0.05) vs. LBPP-induced reduction in LFV
Figure 9. Relationship between the increase in neck circumference (NC, cm) at all lower body positive pressure (LBPP) levels and the LBPP-induced reduction in leg fluid volume (LFV, mL) for the “Fluid shift study”.

Increase in NC versus LBPP-induced reduction in LFV

Log(Increase in NC + 0.01)

LBPP-induced reduction in LFV

BP status  
CH — RH
9.3 Supplementary figures

Supplementary figure 1. Lower body positive pressure experiment.

**Experimental Set-up**
Supplementary figure 2. Overnight fluid shift study.

**Measurements**

- Overnight polysomnography (e.g. AHI)
- Leg fluid volume (LFV) in the right leg
  - Before and after sleep
- Circumferences (Calf and Neck)
  - Before and after sleep