Sources Of Variation In Human Blood Pressure Control

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Abstract

The control of blood pressure plays a vital part in homeostasis in humans. Poor regulation of blood pressure has been associated with an increased risk of events such as myocardial infarction, sudden cardiac death, and stroke. The studies in this thesis are designed to explore sources of variation in human BP control, and in particular to examine the interactions between BP status, activity and circadian variation.

In study 1 the association between BP status and the acute exercise-mediated change in BP was investigated. A total of 32 participants, with pre-exercise MAP of 65–110mmHg, cycled for 30 min at 70% peak oxygen uptake. Systolic and diastolic BP were measured (Portapres) before exercise and for 20 min after exercise. Changes in BP were regressed against pre-exercise values, and against the mean of pre- and post-exercise BP, an index known not to be prone to the influence of mathematical coupling and regression-to-the-mean artefacts. Correlations between pre-exercise BP and the exercise-mediated reductions were typical of those previously reported ($r = 0.37–0.62$, $P<0.05$). Artefact-free indices of BP status (pre- and post-exercise mean) did not correlate with reductions in BP ($P>0.05$), which were moderated more by maximal oxygen uptake and time of day ($P<0.05$). These data indicate that, if statistical artefacts are not controlled for, the influence of BP status on the degree of PEH can be spuriously exaggerated to the extent that other more important moderators of BP change are masked.

In study 2 meta-analytical methods were used enhance the statistical power and precision with which to explore the association between BP status and exercise-mediated changes in ambulatory BP. Studies entered into the meta-analysis were required to meet inclusion criteria of ambulatory monitoring following exercise and comparisons to a control condition to minimize regression-to-the-mean artefacts. Blood pressure status was a significant moderator of PEH indicating that hypertensive patients will benefit from greater reductions in BP. Age, BMI and $\dot{V}O_{2\,max}$ were also identified as significant moderators PEH, indicating that older individuals with larger BMIs and lower fitness levels will benefit most from exercise. Pooled mean changes (95%CI) in daytime and nocturnal SBP were -3.8 (-5.4 to -2.3) and -3.0 (-4.7 to -1.3), respectively, and may be deemed as clinically significant reductions. Future meta-analyses should investigate the effects of chronic exercise on ambulatory BP and its cardioprotective effects.

In study 3 the acute effects of PA on BP and symptoms of OSA were examined using blood pressure reactivity profiles during sleep and following waking. Ambulatory BP and actigraphy data were collected between 20:00-10:00h in 11 OSA patients and 18 healthy controls. Blood pressure reactivity indices were calculated (Jones et al., 2009) and compared between groups and over time using general linear models. The greatest mean (SD) systolic BP reactivity in the healthy controls was 15.4 (42.7) mmHg/activity count, occurring 0-2 hours after waking, whereas the peak systolic BP reactivity of 12.7(14.4) mmHg/activity count occurred during sleep in OSA patients ($P<0.05$). This evidence of diminished nocturnal blood pressure control in response to activity

may be associated with the peak incidence of MI in OSA, which occurs between 00:00 and 06:00 h (Kuniyoshi et al., 2008).

In study 4 the focus moved from acute activity to chronic, with an investigation of leisure-time physical activity in OSA patients, in which the relationships with BP, OSA severity and daytime sleepiness were examined. Levels of leisure-time physical activity, estimated with self-reported activity questionnaires, were not significantly different between OSA patients (n=96) and a healthy control group (n=118). Compared with healthy controls, OSA patients displayed higher SBP, DBP and MAP ($P<0.05$), but physical activity had no effect on BP in either group when adjusted for age and gender ($P>0.05$). However, leisure-time physical activity was associated with reduced ODI and daytime sleepiness (Epworth Sleepiness Scale) in OSA patients ($P<0.05$). The differences in daytime sleepiness between the lowest and highest activity groups were comparable to the reductions found with CPAP treatment. Physical activity would provide a useful treatment for OSA patients, potentially as an adjunct to traditional CPAP therapy.

In study 5 the contribution of the mechanical and neural components of the cardiac baroreflex to diurnal variation in BP control were investigated. In 12 healthy participants, the modified Oxford method was used to quantify baroreflex gain for rising (G_{up}) and falling (G_{down}) pressures in the morning (0700h) and afternoon (1600h). A novel analysis method based on linear mixed models (Atkinson et al., 2010) was employed to compare the integrated, mechanical and neural gains between the two times of day. There was significant diurnal variation in integrated gain, with an attenuated response in the morning (G_{up}= 13.0 ±0.6; G_{down}= 6.3 ±0.4 ms/mm Hg) when compared with the afternoon (G_{up}= 15.1 ±0.6; G_{down}= 12.6 ±0.4 ms/mm Hg). For rising pressures the diminished integrated gain in the morning was caused by a reduction in mechanical gain, whereas for falling pressures it was caused by a reduction in neural gain. It is proposed that the high prevalence of cardiovascular events in the morning is due to diminished mechanical transduction of pressure into arterial distension at this time.

In study 6 postural influences on diurnal variation in cardiac baroreflex sensitivity were investigated, and the contribution of mechanical and neural baroreflex components were determined. Integrated baroreflex sensitivity was reduced in the morning and afternoon when an upright posture was assumed, and was primarily attributed to decreases in neural gain. Although observed at both times of day, reductions in baroreflex sensitivity due to the change in posture occurred to a greater extent in the afternoon. This caused the diurnal variation that was reported in the supine position to be attenuated for rising BP, and eliminated entirely for falling BP when participants changed to a standing position.

The studies in this thesis have provided further knowledge and understanding of sources of variation in human BP control, including the effects of BP status, health status, fitness, physical activity, diurnal variation and postural changes. Methodological issues in BP research, clinical applications, and mechanisms responsible for BP regulation have also been addressed.

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Declaration

I declare that the work contained in this thesis is entirely my own. Individuals acknowledged above were involved in the data collection.

Publications directly associated with this thesis

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Publications completed by the candidate during PhD tenure

Jones H, **Taylor CE**, Lewis NCS, George K, Atkinson G. Post-exercise blood pressure reduction is greater following intermittent than continuous exercise and is influenced less by diurnal variation. *Chronobiology International* 2009; 26: 293-306

Atkinson G, **Taylor CE**, Jones H. Inter-individual variability in the improvement of physiological risk factors for disease: gene polymorphisms or simply regression to the mean? *Journal of Physiology* 2010; 588: 1023-1024

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Oral communications

British Association of Sport and Exercise Sciences (BASES) Annual Student Conference, University of Bedford, April 2008; **Taylor CE**, Jones H, Lewis NCS, Atkinson G. Diurnal variation the blood pressure responses to intermittent exercise. **Awarded best oral presentation.**

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European College of Sport Science (ECSS) Annual Congress, Antalya, Turkey, June 2010; **Taylor CE,** Cable NT, Hadcroft J, Jones H, Lamb K, Atkinson G. Dying to sleep: Effects of physical activity on blood pressure and symptoms of obstructive sleep apnoea.

Poster communications

Faculty of Science Research Seminar Day, Liverpool John Moores University (LJMU), May 2009; **Taylor CE**, Jones H, Zaregarizi M, Cable NT, George K, Atkinson. Using meta-analytical and experimental approaches to explore whether blood pressure status really is the most important determinant of post-exercise hypotension.

LJMU Institute of Health Research Annual Conference, Liverpool, May 2009; **Taylor CE**, Jones H, Zaregarizi M, Cable NT, George K, Atkinson. Using meta-analytical and experimental approaches to explore whether blood pressure status really is the most important determinant of post-exercise hypotension.

European College of Sport Science (ECSS) Annual Congress, Oslo, Norway, June 2009; **Taylor CE**, Jones H, Zaregarizi M, Cable NT, George K, Atkinson. Using meta-analytical and experimental approaches to explore whether blood pressure status really is the most important determinant of post-exercise hypotension.

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European College of Sport Science (ECSS) Annual Congress, Antalya, Turkey, June 2010; **Taylor CE**, Atkinson G, Morgan N, Wallis GA. Does 'the wall' coincide with the greatest slowdown in sub-elite marathon running pace?

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BMI	Body mass index
BP	Blood pressure
CI	Confidence intervals
CPAP	Continuous positive airway pressure
DBP	Diastolic blood pressure
ECG	Electrocardiogram
FSS	Fatigue Severity Scale
G_{down}	Baroreflex gain for falling pressures
G_{up}	Baroreflex gain for rising pressures
HR	Heart rate
LBNP	Lower body negative pressure
MAP	Mean arterial pressure
MI	Myocardial infarction
MSLT	Multiple Sleep Latency Test
MSNA	Muscle sympathetic nerve activity
NTS	Nucleus tractus solitarius
OSA	Obstructive sleep apnoea
PE	Phenylephrine
$P_{ET}CO_2$	End-tidal partial pressure of carbon dioxide
PEH	Post-exercise hypotension
RDI	Respiratory Disturbance Index
RPP	Rate pressure product
SBP	Systolic blood pressure
SCN	Suprachiasmatic nuclei
SD	Standard deviation
SNP	Sodium nitroprusside
SE	Standard error
$\dot{V}O_{2\,max}$	Maximal oxygen consumption

Chapter 1

Literature Review

1.1 Introduction

Human health relies upon the maintenance of a constant internal environment within the body despite changes to the external environment, a concept termed homeostasis (Cannon, 1929). This requires the strict regulation of physiological variables within certain limits. Blood pressure is one such variable and the consequences of poor regulation include increased risk of myocardial infarction, sudden cardiac death and stroke (Muller et al., 1987, Elliott, 1998). Individuals with high BP status (hypertension) are at greater risk of such events compared with normotensives (Kannel, 1996), and therefore the management of both acute and chronic BP is vital. The importance of maintaining BP above lower limits should also be stressed, since falls in BP can have negative implications, such as syncope. Orthostatic hypotension, for example, refers to large reductions in BP due to poor control when an upright posture is maintained.

The aim of this review chapter is to synthesise and critically analyse the current literature regarding BP control in humans. First, the reader is introduced to the endogenous and exogenous components of blood pressure, with reference to circadian variation in cardiovascular risk. Secondly, the BP responses to physical activity are explored, with a specific focus on post-exercise hypotension and influential factors such as participant characteristics and exercise protocol. These responses are then discussed in the context of diurnal variation. Thirdly, BP control, circadian variation and physical activity are discussed with relation to obstructive sleep apnoea, a particularly relevant clinical population due to the independent risk of hypertension and disturbances to sleep associated with this condition. The final focus of this review is the cardiac baroreflex, a key mechanism of blood pressure control and one previously found to exhibit diurnal variation.

1.2 Blood pressure and health status

1.2.1 Circadian rhythm of blood pressure

At rest, BP displays a circadian rhythm, with a typical peak to trough difference in systolic BP of 20-30 mmHg (Pickering et al., 1982). The lowest pressures tend to be during sleep and the highest after a person wakes up and becomes active. This period is known as the "morning surge" in blood pressure and is associated with a sudden activation of the sympathetic nervous system (Kaplan,

2003). A secondary peak can occur in the early evening. This pattern is essentially the same in hypertensive patients, although the profile is shifted upwards (Pickering et al., 1982). However, some hypertensives exhibit a diminished nocturnal fall in BP with less than a 10% reduction compared with daytime BP, and are classified as 'non-dippers' (Verdecchia, 2000).

The circadian rhythm of BP has an endogenous component, meaning that it is regulated, in part, by the body clock. Research suggests that the site of the body clock is the suprachiasmatic nuclei (SCN), which consists of two clusters of cells at the base of the anterior hypothalamus (Reilly et al., 1997). Circadian rhythms in humans serve an environmental anticipatory purpose to ensure that the body is in the optimum physiological state for tasks associated with certain times of day, such as sleep, eating, and physical activity. For example, after peaking in the afternoon core body temperature begins to fall in preparation for nocturnal sleep (Reilly et al., 1997). Environmental rhythms such as the light/dark cycle are important for ensuring synchronisation of the body clock to a 24-hr period. Some physiological variables, such as core body temperature, have relatively large endogenous components. However, BP is heavily influenced by the environment and behaviour, and therefore has a substantial exogenous component.

Factors such as the sleep-wake cycle, posture, ingestion of food (Pickering, 1988) and physical activity (Leary et al., 2002, Kario et al., 1999) can cause marked changes in BP to the extent that the existence of an endogenous component has been questioned (Kerkhof et al., 1998, Van Dongen et al., 2001, Millar-Craig et al., 1978). The morning surge in BP, for example, is strongly influenced by the levels of physical activity in the hours after waking (Leary et al., 2002). Khoury et al., (1992) conducted a study specifically examining whether the rise in BP began prior to or following waking. In this study, participants either rose immediately or remained supine after waking and BP was measured 1 h prior to and 60 to 90 min after waking. The BP changes observed were 1/3 mm Hg upon waking compared with 7/5 mm Hg following getting out of bed, suggesting that the rise in morning BP occurs mainly after getting up and may be due to postural changes and/or an increase in activity. Pickering (1988) examined changes in BP compared to rest and found on

average a 10/7 mm Hg (SBP/DBP) reduction during sleep, a 9/10 mm Hg increase during eating, and a 12/6 mm Hg increase during walking.

Further evidence of a large exogenous component includes research involving travel across time zones (Fogari et al., 1997) and shiftwork (Baumgart et al., 1989) in which participants' BP adjusted rapidly to the new rhythm. However, work by Kitamura et al. (2000) indicated that the endogenous component of BP is present, although small, when they found that shiftworkers exhibiting a dipping BP profile became non-dippers on the first night shift, before adjusting to a normal dipping BP profile. This initial resistance to rapid changes in lifestyle suggests that the endogenous component exerted some influence on the BP rhythm before adjusting to the new pattern. Constant routine studies, in which environmental cues or 'zeitgebers' such as the light-dark cycle are removed, provide further support for the existence of an endogenous component. Cosinor analysis revealed amplitudes of between 5 and 15% (Shea et al., 2005) or 5-10 mm Hg (Minors and Waterhouse, 1981), representing a small influence relative to the exogenous component.

1.2.2 Incidence of cardio- and cerebro-vascular events

The incidence of cardiovascular events shows circadian variation similar to that of BP at rest. Peak incidences of sudden cardiac death and myocardial infarction occur between 06:00-12:00 hours (Figure 1.1), during the morning surge of blood pressure (Muller et al., 1987, Muller et al., 1989, Willich et al., 1989). It has been suggested that the steep increase in blood pressure after waking may contribute to the triggering of atherosclerotic plaque rupture in the arteries (Muller et al., 1989), although there are other circadian-related factors that may increase the likelihood of plaque rupture in the morning, for example, platelete aggregation, catecholamine release and cortisol levels (Muller, 1999a). It is speculated that the rupture of vulnerable atherosclerotic plaques leads to thrombosis and to cardio- or cerebro-vascular events (Millar-Craig et al., 1978, Johnstone et al., 1996). This hypothesis is consistent with findings from a meta-analysis of the timing of stroke onset (Elliott, 1998), in which it was reported that the greatest risk was between 06:00 and 12:00 h. According to the study findings, which included a total of 11,816 strokes, there was a 49% increased relative risk at this time compared with the number that would be expected if no

circadian variation were present. According to Elliot (1998), 1 in 8 strokes are caused by the increase in BP or 'morning excess'.

Bursztyn et al. (1999) suggested that taking a siesta is related to the increased incidence of cardiovascular events in the afternoon, which is supported by a study by (Muller, 1999b) in which a secondary peak in sudden cardiac death and onset of angina pain was reported in the evening between 18:00 and 19:00 h. In an earlier study it was found that BP during afternoon sleep is reduced to a similar level as during night sleep which may lead to similar effects when arising (Bursztyn et al., 1994). However, Naska et al. (2007) reported a 37% lower coronary mortality in people who regularly take a siesta compared to those people who do not, although there may be other lifestyle factors associated with this population, such as diet and exercise, which had a greater influence. Much of the research regarding circadian variation in BP is related to the increased risks due to the morning BP surge, and therefore the control of rising pressures. However, it has been hypothesised that BP control when pressures are falling may also be attenuated at this time. Poor regulation of BP can affect cerebral blood flow if mechanisms of cerebral autoregulation are unable to respond adequately to changes in BP. Therefore there may be links with increased orthostatic intolerance (Lewis et al., 2010) and risk of vasovagal syncope (Mineda et al., 2000, Zoghi et al., 2008) reported in the morning.

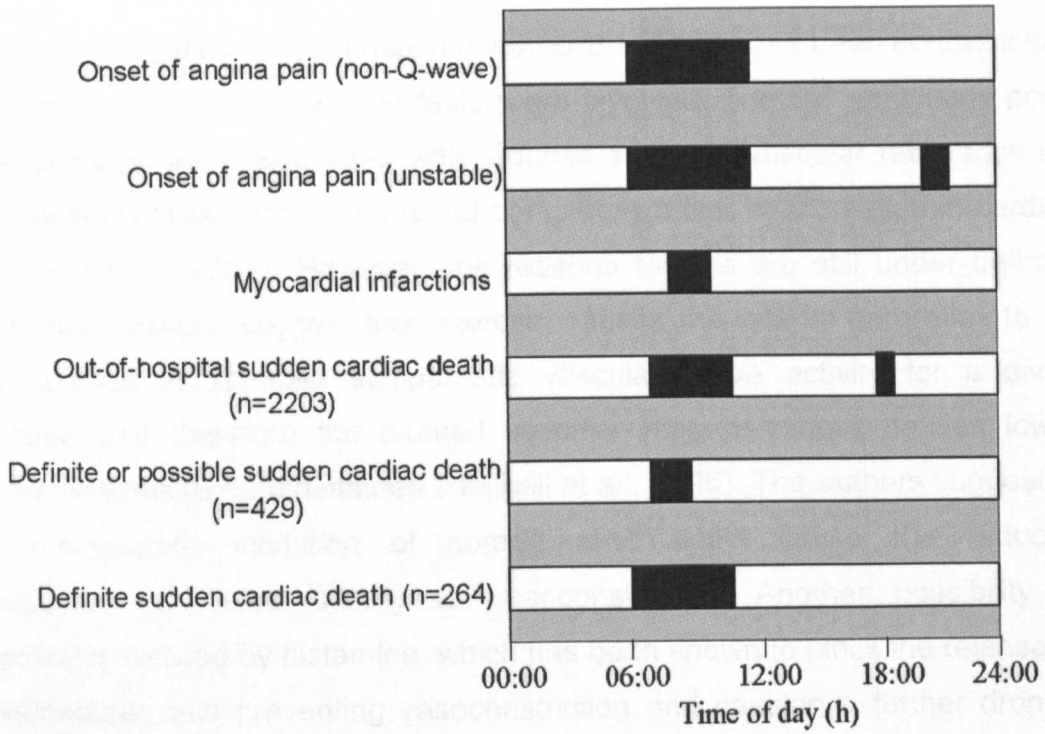


Figure 1.1: Peak times of incidence (indicated by black bars) for a number of acute cardiac events. Taken from Atkinson et al. (2006).

1.3 Blood pressure responses to physical activity

1.3.1 Post-exercise hypotension

Rapid reductions in arterial BP following exercise are now well-documented. This 'post-exercise hypotension' (PEH) can occur within a few minutes following the termination of exercise (Kraul et al., 1996), and may persist for 24 hours or more during subsequent everyday activities and sleep (Jones et al., 2009a, Fullick et al., 2009). However, conflicting findings exist regarding the duration and magnitude of the pressure decrement post-exercise. In many studies in normotensives BP has been measured for 1-2 h following exercise and initial falls followed by a rise to baseline have begun after 5-8 minutes, usually with a return to baseline at the cessation of measurement (MacDonald et al., 2000a). Somers et al. (1991) found PEH to last for up to 2 h in normotensive individuals and Pescatello et al. (2004b) reported PEH for up to 22 h in hypertensive individuals. In a review by MacDonald (2002) the pooled average decrement in pressure in studies reporting PEH, was approximately 8/9 (SBP/DBP) mm Hg in the normotensive population, 14/9 mm Hg in the borderline hypertensive population and 10/7 mm Hg in the hypertensive population.

Studies investigating the potential mechanisms of PEH have been contradictory, which may suggest that several factors are involved. The BP reductions post-exercise have been associated with reduced systemic vascular resistance (or total peripheral resistance) that is not completely offset by increases in cardiac output (Halliwill, 2001). However, the reasons for this are still under debate. Some study results suggest that exercise causes the arterial baroreflex to be reset leading to reduced sympathetic vascular nerve activity for a given pressure, and therefore the blunted vascular responsiveness causes lower blood pressures to be maintained (Halliwill et al., 1996). The authors suggested that pre-synaptic inhibition of noradrenaline could cause the reduced transduction of nerve activity to vasoconstriction. Another possibility is vasodilation caused by histamine, which has been shown to block the release of noradrenaline thus preventing vasoconstriction and causing a further drop in vascular resistance (Lockwood et al., 2005). Further research is required to unravel the complex mechanisms involved in the BP responses following exercise.

1.3.2 Participant characteristics affecting post-exercise hypotension

There is large between-study variability with regards to investigations of PEH. This may be due to differences in participant groups, exercise protocols or methods of BP measurement. Some investigators have specifically examined the time course of PEH using ambulatory monitoring (Brownley et al., 1996, Forjaz et al., 2000), although findings are contradictory and do not emulate the immediate response of BP. These confounding results may be due to the fact that post-exercise activity was not controlled because participants were monitored during their everyday routines. Pescatello and Kulikowich (2001) suggest that the most important predictor of BP differences post-exercise is directly related to baseline values and thus individuals with the highest baseline BP status will exhibit a greater magnitude of PEH. They reported that baseline SBP accounted for 37% (P = 0.001) and 26% (P = 0.018) of the variance in day and night SBP post-exercise, respectively. These findings were averaged over 23 dynamic exercise studies consisting of 12 normotensive and 22 hypertensive mixed gender groups. However, there are important methodological issues relating to the use of initial BP to predict changes in BP. Previous findings, from

both experimental studies and meta-analyses, may be questioned due to potentially spurious correlations caused by mathematical coupling and regression-to-the-mean statistical artefacts. These artefacts can compromise the relationship when pre-exercise BP is used as the measure of BP status, and can cause other potential moderators of BP change to be masked when pre-exercise BP is entered as a predictor into multiple regression models. The results of previous studies have been used to support claims made in position stands regarding the most important moderators of exercise-mediated changes in BP (Pescatello et al., 2004a). Given the importance of the relationship between BP status and BP changes for the support of exercise as an anti-hypertensive therapy, it should be explored whilst controlling for statistical artefacts.

Despite risking spurious correlations caused by statistical artefacts there are other studies using similar methods to come to the same conclusion that BP status predicts PEH (Kenney and Seals, 1993, Thompson et al., 2001, Pescatello et al., 2004b) and these findings have influenced position statements on hypertension (Pescatello et al., 2004a). Some studies have reported no reduction in BP following exercise in normotensive groups. Cléroux et al. (1992) reported a significant decrement in SBP (-11 ± 2 mm Hg) and DBP (-4 ± 1 mm Hg) in hypertensive individuals following 30 minutes of cycle ergometry at 50% $\dot{V}O_{2\max}$ compared to 30 minutes of control period of rest, but the exercise protocol had no significant effect on BP in normotensive subjects. Pescatello et al. (1991) reported that post exercise DBP and MAP were lower by 8 ± 1 (P < 0.001) and 7 ± 1 mm Hg (P < 0.05), respectively, than the baseline values for 12.7 hours in mildly hypertensive subjects. However, MAP remained unchanged in normotensive subjects and SBP increased by 5 ± 1 mm Hg (P < 0.001). On the other hand, MacDonald *et al.* (1999) found that PEH could be elicited in a normotensive population with both mild (50% $\dot{V}O_{2\max}$) and moderate (70% $\dot{V}O_{2\max}$) cycle ergometry. Reductions in systolic and diastolic blood pressure of 8/5 mmHg were reported. MacDonald *et al.* (1999) stated that although PEH can be detected in normotensive individuals it was found to be less consistent and smaller magnitude. The differences in BP responses associated with BP status may be due to mechanisms of BP control, such as the baroreflex, that

are more efficient in normotensives compared with hypertensives, therefore preventing large and prolonged reductions in BP (MacDonald, 2002).

Studies of PEH have tended to be based upon the male population. However, there have been some investigations into the effect of sex on BP responses to exercise. Senitko *et al.* (2002) reported similar magnitudes of PEH in both untrained and endurance trained men and women after a single 60 minute bout of upright cycling at 60% $\dot{V}O_{2\,max}$ (~4 - 5 mm Hg; $P < 0.05$ vs. pre-exercise). Brown et al. (1994) and Deschenes et al. (2006) also found no differences in PEH between genders. However, Kenny et al. (2006) reported a significantly greater decrease in MAP ($P<0.05$) in females (-14mmHg) compared to males (-9mmHg). It would seem possible that these differences in gender may be associated with varying effects of exercise during stages of the menstrual cycle. However, Lynn et al. (2007) investigated the vascular responses of 14 males and 14 females following 60 minutes of cycling at 60% $\dot{V}O_{2\,peak}$. Women were tested in the mid follicular, ovulatory and luteal phase. They reported that menstrual phase and gender had no effect on the magnitude of PEH and that the pattern of haemodynamic responses did not differ. The majority of studies seem to suggest that gender and the menstrual cycle have little effect on PEH, although there is currently limited research in this area. In terms of training status, study findings are contradictory. Senitko et al. (2002) found no effects of training status on PEH. However, in a study on professional footballers, those players with the lowest maximal oxygen uptakes ($\dot{V}O_{2\,max}$) showed the greatest reductions in DBP 60 minutes after a short maximal field exercise than those with higher values of $\dot{V}O_{2\,max}$. These findings were in DBP alone, as there was no significant correlation between $\dot{V}O_{2\,max}$ and change in SBP. The discrepancies between study results may be teased out with the use of meta-analytical techniques. Meta-analyses provide greater statistical power and precision by examining the effect size for a group of studies sharing the same outcome measure. For example, a meta-analysis on acute PEH may include a set of studies involving different subject cohorts with a range of ages, resting BPs, BMIs and training status. This may provide a clearer overall picture of the effects of these participant characteristics on BP responses following exercise.

1.3.3. Effects of exercise protocol on post-exercise hypotension

Given the range of exercise protocols used in studies of PEH it is possible that some of the variance in results may be related to exercise mode, duration or intensity. Significant BP reductions have been reported following a variety of aerobic exercise including; walking (Kaufman et al., 1987), running (Rueckert et al., 1996), and both leg and arm ergometry (MacDonald et al., 2000b). However, comparisons are complicated by a large range of intensities and durations. However, MacDonald *et al.* (2000a) stated that the duration of exercise does not directly influence the duration of PEH. They reported no significant difference between post-exercise SBP or DBP following 15, 30 and 45 minutes of cycle ergometry at 70% $\dot{V}O_{2\,max}$. Similarly, MacDougall (1994) found a similar magnitude of PEH following 10, 15, 30 and 45 min of exercise at 70% $\dot{V}O_{2\,max}$ in a normotensive and borderline hypertensive population. Conversely, Bennett et al. (1984) found that the PEH in hypertensives increased with duration of exercise. However, the protocol consisted of 10-min bouts of exercise separated by 3-min rest periods during which BP was measured. A brief reduction in BP immediately following exercise is often associated with pooling of the blood in the vasodilated muscle beds; therefore these mechanisms may be significantly different from those that cause PEH. However, Forjaz et al (1998b) reported greater reductions in both SBP and DBP and a longer duration of PEH in SBP following 45 minutes of exercise compared to 25 minutes of exercise.

Many researchers have investigated the effect of exercise intensity on subsequent PEH (Forjaz et al., 1998b, Forjaz et al., 2004, MacDonald et al., 1999). When examining the effects of exercise intensity on PEH, the majority of studies have employed sub-maximal cycle ergometry protocols at intensities ranging between 40 and 100%, as indicated by measurements of $\dot{V}O_{2\,max}$, heart rate reserve or predicted maximal heart rate (Pescatello et al., 1991). However, the effect of such characteristics on PEH is still not clear even in healthy normotensive individuals. Forjaz *et al.*, (1998a) found similar PEH following 45 min of exercise at intensities of 30, 50 and 80% of $\dot{V}O_{2\,max}$ in normotensive subjects. Similarly, Pescatello *et al.* (1991) found no difference in the magnitude

of PEH observed following 30-min bouts of cycle ergometry at 40 and 70% $\dot{V}O_{2\,max}$ in a normotensive population. However, contrasting evidence does exist. Forjaz *et al.* (2004) utilized the same protocol as their earlier study but altered exercise intensities to 30, 50 and 75% $\dot{V}O_{2\,max}$. They subsequently reported a greater reduction in BP following more intense exercise. Additionally, Piepoli *et al.* (1994) found a decrement only after maximal cycle exercise when compared with moderate and low intensity exercise in normotensive sedentary volunteers. According to Jones et al. (2007) the acute PEH response in normotensive men varies following exercise bouts of varying intensities and durations, but the response is consistent when the protocols are matched for total work done. The researchers reported significant reductions in MAP during 20 minutes post exercise following semi-recumbent cycling for 30 minutes at 70% $\dot{V}O_{2\,max}$ (-2 ± 4 mm Hg, P = 0.04) and 40% $\dot{V}O_{2\,max}$ for a time which corresponded to equal total work done (-1 ± 6 mm Hg, P = 0.019). This suggests the magnitude of PEH elicited is dependent upon total work done as opposed to exercise intensity or duration.

The majority of researchers who have investigated the effect of both intensity and duration on PEH have utilised continuous exercise protocols. However, it has been found that the insertion of rest periods into an exercise bout mediates a greater reduction in post exercise BP. Park *et al.* (2006) examined the BP of prehypertensive adults for 12 h following accumulated physical activity, continuous activity and control periods. It was reported that both SBP and DBP were reduced for a longer duration following accumulated physical activity and that SBP reduced by a greater magnitude (P = 0.045). Jones *et al.*, (2009b) provided further evidence for this claim with their findings that intermittent exercise mediates a greater reduction in MAP compared with continuous exercise, particularly when performed in the afternoon. Interestingly, it has been proposed that these chronic benefits of exercise are explained by repeated occurrences of acute PEH, and that the incremental capacity for greater exercise intensity and duration as a training programme progresses merely leads to more pronounced and prolonged acute PEH occurring over time (Thompson et al., 2001). If exercise is to be used as a non-pharmacological intervention in the management of BP, it is necessary to investigate the optimal

characteristics of the exercise required to induce PEH. Meta-analyses can be used to combine previous study results and determine more precisely the effects of certain exercise protocol characteristics, such as intensity, duration and exercise mode, on BP responses.

1.3.4 Circadian variation in blood pressure responses to physical activity

Circadian variation in the response of blood pressure (BP) following continuous exercise has been described by Jones et al. (2008b). Post-exercise hypotension was absent or reversed when exercise was performed between 04:00–08:00 h. This BP response remained even after tight control of posture and amount of sleep prior to exercise (Jones et al., 2008a). These studies support the findings of previous investigations suggesting that the morning surge in BP is, at least in part, explained by increases in physical activity at this time of day (Millar-Craig et al., 1978, Leary et al., 2002). Given the popularity of intermittent activities, Jones et al. (2009b) compared the post-exercise BP reductions following a continuous and an intermittent protocol performed in both the morning (08:00 h) and afternoon (16:00 h) on separate days. The continuous protocol consisted of 30 min of continuous cycling and the intermittent protocol of three 10-min bouts of cycling separated by 10-min of rest. The exercise intensity was set at 70% $\dot{V}O_{2\,max}$ during both protocols to match them for work done. Blood pressure was measured for 5-min before and 20-min following exercise. Following the intermittent protocol MAP was 8+1 mm Hg lower compared with continuous exercise. There was significant diurnal variation in MAP, with attenuated hypotension after morning exercise, although this diurnal variation was less marked following intermittent compared with continuous exercise. This study demonstrates variation in BP control caused by both time of day and exercise protocol, and highlights the importance of identifying the most beneficial exercise programs for anti-hypertensive therapy. From the results of this study, a bout of afternoon exercise that is occasionally interrupted with short rest periods would be recommended for lowering BP acutely.

According to Leary et al. (2002) the morning surge in blood pressure is strongly influenced by the levels of physical activity in the hours after waking. Kario et al.

(1999) introduced a statistical index of BP reactivity, where physical activity in individual participants (measured via actigraphy) is regressed against BP and the slope calculated is the BP reactivity (to activity). Kario et al. (1999) reported significant correlations between physical activity and BP in a sample of 160 adults. Jones et al. (2006) used this blood pressure reactivity index to investigate the BP responses to everyday physical activity over a 24-hour period in a clinical population of hypertensives (n=440). The highest reactivity of SBP was observed between 8:00 AM and 10:00 AM indicating reduced BP control coinciding with the time of day for peak incidence of cardiovascular events (Muller et al., 1987). Systolic BP reactivity still showed statistically significant 24-hour variation when the reactivity index time periods were ordered in terms of time after waking. A secondary rise in SBP reactivity was reported in the early afternoon, around the time when there is a secondary peak in the onset of angina and out-of-hospital sudden cardiac death (Atkinson et al., 2006). The predictive value of the BP reactivity index in terms of cardiovascular events remains to be verified. This relatively straightforward analysis does not take into account other variables linked with circadian variation in cardiovascular events, such as platelet hyperactivity, hypercoagulability and hypofibrinolysis, blood viscosity and increased vascular spasm (Muller et al., 1987, Willich et al., 1993, Feng et al., 1999, Andreotti et al., 1988). However, it may potentially be a simple tool for providing useful information within clinical populations associated with hypertension and cardiovascular disease, such as obstructive sleep apnoea (OSA).

1.4 Blood pressure and physical activity in the OSA population

1.4.1 Obstructive sleep apnoea

Obstructive sleep apnoea syndrome is a condition characterised by frequent episodes of upper airway collapse during sleep, causing arousal from sleep in order to re-establish airway patency and resume breathing. This leads to sleep fragmentation and recurrent hypoxaemia and hypercapnea due to the respiratory pause (Silverberg et al., 2002). Apnoea is defined as a complete or almost complete cessation of airflow and hypopnoea as a ≥30% reduction in airflow accompanied by a ≥4% drop in oxygen saturation. The apnoea-hypopnoea index (AHI) represents the average number of these episodes per

hour of sleep. A value of 5 or more indicates the presence of mild OSA; 15-30 is moderate; and above 30 is termed severe OSA. Another index generally used alongside AHI in determining the severity of OSA is the oxygen desaturation index (ODI). This indicates the average number of oxygen desaturations ≥4% per hour of sleep, and is typically correlated with AHI (Svanborg et al., 1990). Affecting up to 17% of the adult population, the prevalence of OSA is similar to that of diabetes and asthma. However, the prevalence in obese patients exceeds 30% (40-90% in morbidly obese) and up to 24% of middle-aged males may show sub-clinical symptoms (Silverberg et al., 2002). According to a study by Young et al. (1997) it was estimated that as many as 80-90% of OSA cases are undiagnosed. Current treatment is continuous positive airway pressure (CPAP), which provides a continuous flow of air via a mask to splint the upper airway open during sleep. It has reported that CPAP is a successful treatment for the reduction of AHI (Giles et al., 2006). However, compliance with this treatment is often poor (Engleman et al., 1994), and it is therefore important to explore alternative therapies for treatment of OSA.

1.4.2 Circadian variation of blood pressure in OSA

Obstructive sleep apnoea syndrome is an independent risk factor for hypertension (Lavie et al., 2000, Nieto et al., 2000), with over half of patients with essential hypertension also suffering from OSA (Silverberg et al., 2002). Obstructive sleep apnoea has also been associated with increased risk of stroke, cardiac arrhythmias, and heart failure (Parish and Somers, 2004). Nagata et al. (2008) investigated the diurnal blood pressure variation in OSA patients with a comparison of 24-hr ambulatory BP profiles. Patients with OSA had higher mean 24-hr and night-time BP values compared to control patients suffering from daytime sleepiness only. Further increases in BP were found as the severity of OSA increased. These findings concur with those of Noda et al. (Noda et al., 1993) who found that patients with the greatest severity of OSA had the highest night-time BP. The difference in systolic BP during waking and sleep was correlated with AHI ($r = -0.47$, $p<0.05$), lowest O_2saturation ($r = 0.63$, $p<0.005$) and time spent below 90%O_2saturation ($r = -0.47$, $P<0.05$). This confirms the importance of OSA severity in the elevation of nocturnal BP and the potential for ambulatory BP monitoring in the diagnosis of severe OSA.

It has been suggested previously that hypoxemia and hypercapnia caused by the cessation of airflow activates the chemoreflex and leads to increased sympathetic nerve activity (Somers et al., 1995). This causes peripheral vasoconstriction which, alongside increases in cardiac output caused by changes in cardioac transmural pressures, leads to surges in BP (Kuniyoshi et al., 2008). However, it is currently unknown whether the high night-time BP in OSA patients is also associated with greater activity at this time. It could be speculated that individuals with OSA exhibit greater movement during the night as a consequence of frequent sleep disturbances. Alternatively, BP responses to a given level of physical activity may be accentuated during the night in OSA patients. The high night-time BP found in OSA patients coincides with the peak incidence for cardiac events in this population. Kuniyoshi *et al.* (2008) examined the day-night variation of acute myocardial infarction (MI) in 92 patients where a clear onset of chest pain had been identified. The frequency of MI was compared between OSA and non-OSA groups for different times of day (Figure 1.2). In agreement with previous literature (Muller et al., 1987) the greatest percentage of MI in the non-OSAS group (47%) occurred in the morning between 06:00 and 12:00h. However, in the OSA group the peak incidence of MI occurred between 00:00 and 06:00h (32%). Of all those having MI at this time 91% had OSA. Although this study was limited due to the uncertainty of the exact timing of MI, the data suggest that OSA patients are more likely to have MI at night than any other time of day, which may be associated with night-time surges in BP that lead to plaque rupture and cardiac thrombosis (Kuniyoshi et al., 2008).

There is strong evidence in the literature indicating that BP responses to exercise are subject to circadian variation. It has been hypothesised that diminished BP control in the morning, during everyday physical activities for example, may be associated with increased prevalence of cardiovascular events. However, it remains unclear whether populations suffering from sleep disturbances and/or disruptions to circadian rhythms, such as patients with OSA, exhibit different 24-hr profiles of BP control and altered responses to physical activity. Given the proposed link between BP responses to activity and the occurrence of cardiovascular events, studies of BP reactivity in OSA patients

may provide further insight into the altered circadian variation of BP control and cardiovascular risk in this clinical population.

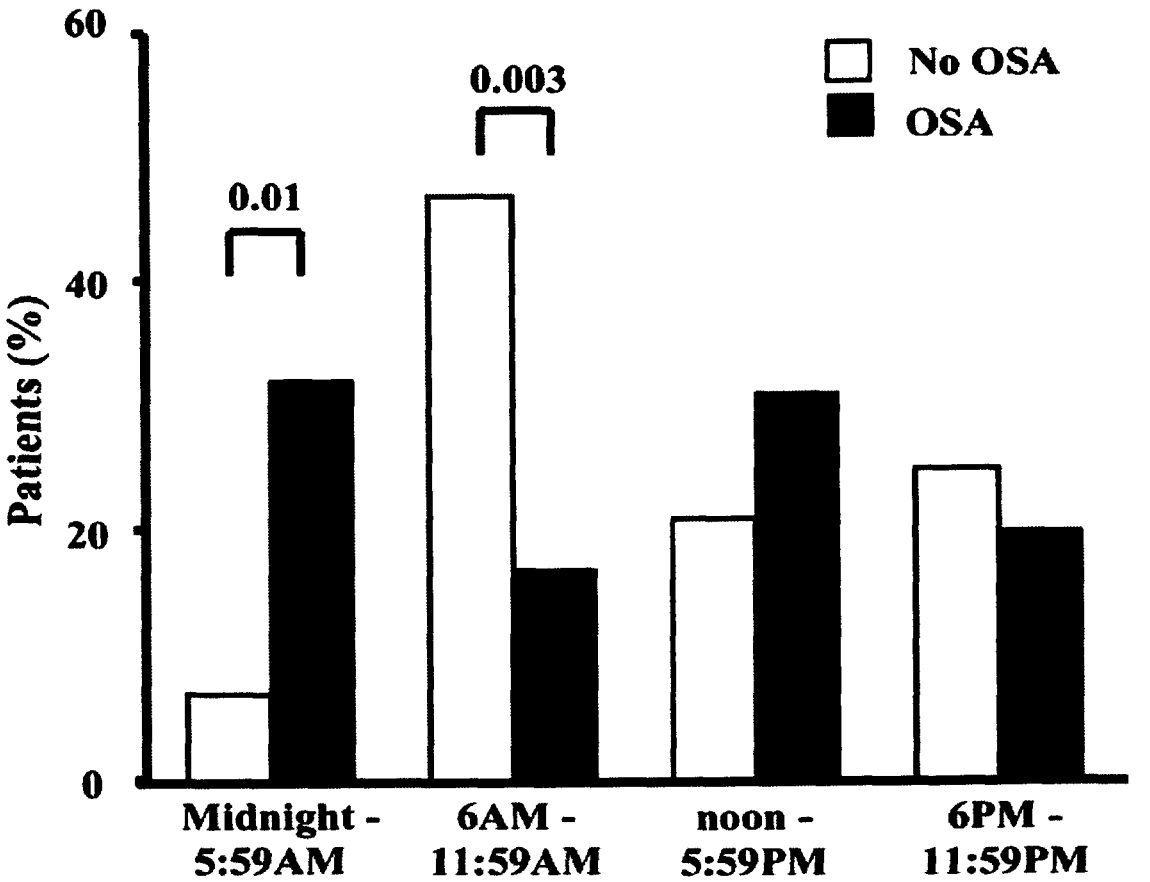


Figure 1.2: Day-night pattern of myocardial infarction based on 4 6-hr time intervals in OSA (n=64) and non-OSA (n=28) patients (Kuniyoshi et al., 2008).

There is evidence for improvement of BP control and diurnal BP profiles with CPAP (Wilcox et al., 1993, Akashiba et al., 1999, Logan et al., 2003, Haentjens et al., 2007). In particular, Logan et al. (2003) found that during a single night's application, CPAP abolished OSA and reduced systolic BP (±SD) in stage 2 sleep from 138.3 ± 6.8 to 126.0 ± 6.3 mm Hg. Long-term use was successful in improving 24-h SBP with a reduction of 11.0±4.4 mmHg after CPAP was used for 2 months. Despite positive findings of attenuations in BP, research is still ongoing to establish to what extent CPAP is effective in reducing cardiovascular-related morbidity and whether there are useful adjunct or alternative therapies, such as physical activity, that will provide an option for those patients for whom compliance with CPAP is poor.

1.4.3 Physical activity and OSA severity and symptoms

Sleep disturbance due to frequent apnoeic and hypopneic episodes often causes severe sleepiness and fatigue during the day, thus reducing quality of life (Silverberg et al., 2002). These symptoms also increase the probability of long-term sick leave (Sivertsen et al., 2008) and the risk of motor vehicle accidents (Barbe et al., 1998, Teran-Santos et al., 1999). Previously researchers have found that OSA symptoms, in particular daytime fatigue, are not correlated with OSA severity. Aguillard *et al.* (1998) recorded daytime sleepiness in 32 OSA patients using the Multiple Sleep Latency Test (MSLT). Each participant performed a maximal exercise test to assess physical fatigue whilst a fatigue severity scale (FSS) was used as a subjective measure. Results showed high levels of self-reported fatigue and low physical work capacity. Objective and subjective measures of fatigue were not correlated with OSA severity as has been found since by Hong and Dimsdale (2003). However, MSLT was not significantly correlated with the FSS or exercise test suggesting that daytime physical fatigue and sleepiness are independent OSA problems.

Hong and Dimsdale (2003) used measures of self-reported habitual physical activity in 38 OSA patients to investigate the correlations with sleep architecture, subjective well-being and OSA severity (respiratory disturbance index). Regular physical activity was significantly correlated with higher SF-36 vitality (r =.54), higher Profile of Mood States vigor (r =.41), and less fatigue (r =.46). These correlations remained even after controlling for RDI and BMI. There was no correlation between OSA severity and physical activity, suggesting that physical activity is better than OSA severity in predicting perceptions of energy and fatigue. Peppard and Young (2004) reported that the odds of having an AHI of over 15 significantly decreased with an increasing level of exercise. It was found that individuals who exercise 1-2 hours every week had 0.62 times the odds of having moderate or worse sleep disordered breathing compared to those who did no exercise. More than 7 hours per week reduced the odds further to 0.31 compared to the non-exercisers.

The findings of these previous studies have been inconsistent, with some researchers suggesting that daytime sleepiness is correlated with the AHI, and

others suggesting that it is related to fitness and physical activity rather than OSA severity. These discrepancies may be due to the definitions used to describe daytime sleepiness and fatigue and the techniques employed to measure them. It is likely that results of multiple sleep latency tests, sleepiness questionnaires and measures of physical and subjective fatigue during exercise describe distinctively different problems, all of which may be associated with OSA. Although study results suggest that chronic physical activity may have an important part to play in reducing the symptoms of OSA, there are certain limitations to previous investigations, including sample size and the lack of control for confounding factors, which may have led to important relationships going undetected. A recent study by Basta et al. (2008) addressed this problem and found significant effects of regular exercise on daytime sleepiness in OSA whilst controlling for a range of patient characteristics. However the study lacked comparisons of energy expenditure between OSA and the general population and did not report the effects of physical activity on OSA severity (AHI) or BP. The consequences of severe daytime sleepiness are very serious and therefore optimizing treatment is extremely important. Results of previous studies also suggest that those OSA patients with severe sleepiness have an increased risk of developing hypertension (Kapur et al., 2008).

Successful interventions for reducing OSA severity and improving sleep quality have typically included dietary interventions (Harman et al., 1982, Sampol et al., 1998, Kajaste et al., 2004), soft tissue surgery (such as uvulopalatopharyngoplasty) (Riley et al., 2000), and weight-loss surgery (Charuzi et al., 1992, Pillar et al., 1994). Sampol et al. (1998) found that weight loss through a dietary intervention caused substantial reductions in OSA severity, sometimes reducing AHI to normal levels of <5events/hr. Weight-loss surgery has also been found to elicit large initial reductions in AHI in OSA patients following surgery, however Pillar et al. (1994) found significant increases in AHI after only 5 to 10-yr follow-ups. It would seem that surgical treatments are not only costly, but they often provide only short-term solutions. Exercise training has also been proven to be beneficial, both as a separate treatment (Norman et al., 2000) and as an adjunct therapy to CPAP (Giebelhaus et al., 2000). Lifestyle interventions such as physical activity may be crucial in providing more long-term reductions in OSA severity and

symptoms. However, the efficacy of exercise as a treatment for hypertension and cardiovascular risk in the OSA population warrants further investigation. Physical activity interventions may have the potential for reducing BP as well as daytime sleepiness, thereby reducing the risk of cardiovascular events and road traffic accidents, and enhancing quality of life.

1.5 Blood pressure control via the cardiac baroreflex

1.5.1 Cardiac baroreflex

The arterial baroreflex is the key mechanism for the control of acute BP (Benarroch, 2008) functioning via a negative feedback system. Low baroreflex sensitivity has been reported to be a significant marker of cardiovascular disease (La Rovere et al., 1988) and is associated with myocardial electrical instability, increased susceptibility to ventricular fibrillation and cardiac sudden death (Billman et al., 1982). It is also associated with increased long-term mortality following acute myocardial infarction (La Rovere et al., 1988) and acute ischemic stroke (Robinson et al., 2003), indicating that appropriate baroreflex regulation of autonomic outflow is crucial to maintenance of cardiovascular health and homeostasis. The arterial baroreceptors are mechanosensitive afferent nerve terminals located in the adventitia of the carotid sinuses (innervated by the glossopharyngeal nerve) and aortic arch (innervated by the vagus nerve). Increases in arterial BP cause mechanical deformation of the vessel wall, which stimulates baroreceptor afferents to provide excitatory input to neurons located in the nucleus tractus solitarius (NTS) (Blessing, 2003). The cardiac baroreflex involves a direct input from the NTS to a group of vagal preganglionic neurons located in the nucleus ambiguous, which project to the cardiac ganglion neurons that inhibit the automatism of the sinus (Spyer, 1994). This provides beat-to-beat control of heart rate, and therefore serves to maintain BP within certain limits. The sympathetic baroreflex involves a sympathoinhibitory pathway initiated by the NTS neurons that mainly controls peripheral resistance (Dampney et al., 2003). The cardiac and sympathetic baroreflexes appear to be regulated independently; the focus within this thesis will be the cardiac baroreflex. This 'integrated' cardiac baroreflex response of changes in heart rate to changes in BP can be separated into two

components: the transduction of pressure into barosensory vessel stretch (mechanical component), and the transduction of barosensory vessel stretch into efferent autonomic outflow (neural component) (Hunt et al., 2001).

1.5.2 Assessment of baroreflex sensitivity

Cardiac baroreflex sensitivity or 'gain' is typically quantified by the responses in R-R interval to changes in SBP (Hunt et al., 2001). This provides an estimation of integrated baroreflex function. Many researchers use spontaneous methods of assessing baroreflex gain, such as the sequence method, α-index, and transfer function. These methods make use of naturally occurring oscillations in SBP and R-R interval, which appear to be causally related. Spontaneous methods are commonly used as they provide a non-invasive alternative to pharmacological techniques. However, the accuracy of spontaneous methods for determining baroreflex gain has been under question. Much of the variability with the spontaneous methods can be explained by respiratory sinus arrhythmia (Lipman et al., 2003), findings that have been confirmed by Tzeng et al. (2009) who reported significant increases in the α-index and spontaneous up-sequences with slow-breathing compared with fast-breathing. Lipman et al. (2003) investigated the consistency in results between five spontaneous methods (sequence method, α-index, transfer function, low-frequency transfer function, and impulse response function) and pharmacologically-derived baroreflex gain (modified Oxford method). The modified Oxford method involves sequential injections of sodium nitroprusside (SNP) followed by phenylephrine hydrochloride (PE) in order to characterise falling and rising pressures, respectively (Hunt et al., 2001). The original Oxford method involves PE injections only, and therefore only rising pressures. Although spontaneous indices were found to correlate with the modified Oxford method, the Bland-Altman method revealed limits of agreement as large as the baroreflex gain itself, indicating weak agreement. Unlike baroreflex gain determined by the modified Oxford method, none of the indices were related to barosensory vessel distensibility, suggesting that findings from spontaneous methods predominantly reflect vagally mediated heart rate variability (Lipman et al., 2003).

Unlike the Oxford method (phenylephrine alone), the modified Oxford method enables separate analysis of falling and rising pressures, whilst allowing the evaluation of dynamic cardiac responses across a wide range of blood pressures. Sodium nitroprusside is a vasodilator and therefore causes BP to fall, whereas PE is an alpha-1 agonist and therefore causes a rise in BP via vasoconstriction. According to Eckberg and Sleight (1992) cardiac baroreflex gain is smaller for falls in pressure than rising pressure, a concept that was termed hysteresis. This means that at identical pressures R-R interval is longer with falling than rising pressure and therefore there is a change in set-point of the SBP/R-R interval relation (Studinger et al., 2007). In the same way that integrated gain can be quantified by the responses in R-R interval to changes in SBP, the mechanical and neural components of the cardiac baroreflex may also be determined with the additional measurement of carotid diameter using ultrasound techniques. For mechanical gain changes in carotid diameter are plotted against SBP, and for neural gain changes in R-R interval are plotted against carotid diameter (Hunt et al., 2001). Linear regression of these pairs yields a slope which is taken as baroreflex gain. Studinger et al. (2007) applied these methods in 14 young healthy participants and found that hysteresis was not derived solely from the mechanical component, but from interactions between both mechanical and neural. The two components tended to act in opposition to determine differences in set point between falling and rising pressures, but acted in conjunction to determine differences in integrated baroreflex gain.

Many other methods of assessing baroreflex function exist, such as neck suction for example, which is a technique that directly stimulates the carotid baroreceptors. However, this method would not allow for carotid artery imaging to enable the separation of the mechanical and neural components. Also, the two sites for baroreceptors causes the central nervous system to receive conflicting information because when carotid baroreceptor activity is stimulated, aortic baroreceptor activity is reduced to oppose it (Eckberg and Fritsch, 1993). The modified Oxford method has an advantage since the baroreceptors respond to actual physiological stimuli, i.e. falling and rising SBP. This method may be questioned regarding direct drug effects on the barosensory vessels. However PE, for example, causes vasoconstriction, which leads to an increase

in SBP and increases in carotid artery diameter, thus any direct effects of the vasoconstrictor on this vessel are overridden.

1.5.3 Diurnal variation in baroreflex sensitivity

Many previous studies have identified diurnal variation in baroreflex gain, with reduced gains following waking compared with during sleep (Pickering et al., 1968, Smyth et al., 1969, Conway et al., 1983, Parati et al., 1988, Parati et al., 1995). Conway et al. (1983) used the Oxford method (PE only) in 13 participants to compare baroreflex gain during sleep with 3 stages of progressive mental arousal: awake with eyes closed, reading the newspaper, and performing mental arithmetic. Mean (±SD) baroreflex gain was highest during sleep (17.3 ± 2.4 ms/mm Hg) and was significantly and progressively lower in each of the 3 stages of mental arousal ($P<0.05$). Baroreflex gain fell to 10.0 (± 2.7 ms/mm Hg) during mental arithmetic at the same time as BP rose approximately 14.8 mm Hg above baseline daytime levels. These results suggest that baroreflex gain, and therefore BP control, continues to fall after waking as individuals become more alert. Bristow et al. (1969) investigated baroreflex gain during sleep and waking using injections of angiotensin or phenylephrine. Differences in baroreflex gain between sleep and waking were not consistent between participants with some exhibiting higher gains during sleep and other during waking. However, mean (±SD) baroreflex gain for normotensives following waking was 14.8 (± 9.2 ms/mm Hg) compared to 3.0 (± 0.9 ms/mm Hg) for hypertensives. This poor BP control after waking in hypertensives is consistent with the proposed mechanisms associated with the high risk of cardiovascular events at this time.

Hossman et al. (1980) explored baroreflex sensitivity over a 24-hr period. In 5 healthy males noradrenaline was infused for a 15-min period every 3 hours beginning at 09:00 h. Baroreflex gain was determined by regressing R-R interval on SBP for each infusion. The highest gains were reported at 0300 and 1200h, and the lowest at 1500 and 0900h, which is consistent with previous findings of high baroreflex gain at night and low gain in the morning after waking. However, it is important to acknowledge that the use of steady state l-noradrenaline infusions as a means to assess dynamic baroreflex sensitivity has been questioned (Diaz and Taylor, 2006). Infusions over 15-min periods

allow the body time to recruit mechanisms to avoid large perturbations in BP. It can be difficult to interpret the responses because the system has time to adjust, and it is therefore important within the study to have a clear input in order to examine the responses accurately. It could be argued that studies using drug infusions are predominantly focused on steady state control of BP rather than dynamic. Therefore, future studies of circadian variation in baroreflex gain ought to involve bolus drug injections in order to decipher the dynamic responses of the baroreflex.

Studies using spontaneous baroreflex indices have generally shown that the highest gains occur during the night with reduced gains in the morning following waking (Parati et al., 1988, Tochikubo et al., 1997, Nakazato et al., 1998). However, the variations during the daytime have not been consistent between studies. For example, Tochikubo et al. (1997) found the circadian variation to be bimodal with a secondary peak in baroreflex gain at 19:00 and trough at 23:00, whereas earlier research reported a peak at 23:00h. Nakazato et al. (1998) reported no difference between morning (0700h) and evening (2300h) in baroreflex gain determined by the sequence method. This technique involves estimating gain non-invasively from spontaneous changes in BP by extracting sequences of 3 or more beats where BP and R-R interval are continuously changing in the same direction (Bertinieri et al., 1985). Although baroreflex gain had a tendency to increase during sleep at night, this was not significantly different from during wakefulness. Although this study involved naturally occurring changes in BP, it may have been limited by the small ranges of BP associated with spontaneous fluctuations, and by the relatively small sample size (n=8). Nakazato et al. (1998) did, however, find significantly lower sympathetic baroreflex sensitivity during sleep compared to wakefulness in the evening before and following morning, which is the reverse of cardiac baroreflex sensitivity.

Much of the previous research has focused on sleep/wakefulness differences (Pickering et al., 1968, Smyth et al., 1969, Conway et al., 1983, Parati et al., 1988) or involved spontaneous methods of determining baroreflex sensitivity, (Parati et al., 1988, Tochikubo et al., 1997, Nakazato et al., 1998, Pagani et al., 1988), the limitations of which have been discussed. These studies also only

document diurnal changes in integrated baroreflex gain; it remains unknown whether the differences in integrated gain are due to altered mechanical transduction of pressure into barosensory vessel stretch and/or the neural transduction of barosensory stretch into efferent autonomic outflow (Hunt et al., 2001). It is repeatedly proposed in the literature that the high prevalence of cerebro- and cardio-vascular events is related to the 'morning surge' in blood pressure (Elliott, 1998, Muller et al., 1987). Investigations of the mechanisms behind the diminished BP control in the morning may help to explain the increased risk. Previous studies would suggest that circadian variation in the cardiac baroreflex response plays a role in this, and therefore it is important to examine the separate components of this mechanism to determine the site(s) responsible for diminished control of BP. Given that diurnal variation has previously been reported in carotid artery distensibility (Kool et al., 1991; 1992), it may be speculated that vessel stretch responses to changes in BP may be altered with time of day, therefore affecting the overall baroreflex gain. Clear delineation of this mechanisms may allow clinical interventions to specifically target these sites in order to enhance BP control and reduce the risk of cardiovascular events.

1.5.4 Postural influences on baroreflex sensitivity

In many of the previous studies reporting diurnal variation in cardiac baroreflex gain participants have been in the supine position (Hossmann et al., 1980, Conway et al., 1983, Parati et al., 1988). However, this does not accurately represent posture during everyday living. It would be more likely that individuals are seated or standing in the first few hours after waking, when the peak occurs in the incidence of cardiovascular events. A number of studies have been performed to investigate the effects of orthostatic stress on baroreflex function. The current consensus is that orthostatic stress reduces cardiac baroreflex sensitivity (O'Leary et al., 2003, Hughson et al., 1994, Taylor and Eckberg, 1996, Jasson et al., 1997, Iellamo et al., 1996, Pickering et al., 1971, Bahjaoui-Bouhaddi et al., 1998, Westerhof et al., 2006, Steinback et al., 2005, Steptoe and Vogele, 1990, Saeed et al., 2009). However, other studies report either an increase (Pawelczyk and Raven, 1989), or no change in the cardio-vagal arm of the baroreflex (Cooper and Hainsworth, 2002). These discrepant findings may be attributed to methodological differences such as the use of spontaneous

techniques versus more invasive neck suction and pharmacological methods (O'Leary et al., 2003).

Cooper & Hainsworth (2002) compared supine posture with 60 degree head upward tilt using the neck chamber method. This technique involves the loading and unloading of carotid baroreceptors by application of pressures of -30 and +30 mmHg to a chamber fitted over the neck. The responses of R-R interval were determined, as well as forearm vascular resistance (mean arterial pressure/brachial artery velocity by Doppler ultrasonography). The authors found that responses of R-R interval (i.e. baroreflex function) was not affected by the postural change. However, forearm vascular responses to neck suction and pressure during head upward tilt were enhanced in healthy patients. However, there was no vascular effect of tilt in patients with orthostatic intolerance, which may suggest that the sympathetic baroreflex controlling peripheral vascular resistance is key to maintaining BP during orthostatic stress.

A large number of studies have reported significant reductions in cardiac baroreflex gain, many of which have involved using spontaneous techniques. Bahjaoui-Bouhaddi et al. (1998) used the spontaneous sequence method to explore the effects of active standing and passive tilting on baroreflex gain in 13 healthy individuals. A significant reduction in cardiac baroreflex gain was found following a postural change from supine (14.6 ± 2 ms/mm Hg) to active standing (7.8 ± 1.2 ms/mmHg). Similar effects of 60 degree head upward tilt were observed (8.8 ± 1.2 ms/mmHg). The number of spontaneous sequences observed during active stand and passive tilt also increased, which reiterates the changes in BP caused by postural stress and the ability of the baroreflex to control them. Steinback et al. (2005) also used the sequence method to investigate baroreflex gain between supine and 60 degree head upward tilt. Similar to the previous study by Bahjaoui-Bouhaddi et al. (1998), the authors found a diminished baroreflex gain caused by orthstatic stress. Additionally Steinback et al. (2005) reported that carotid distensibility was significantly lower with the tilt compared with when supine, with a positive correlation between changes in distensibility and baroreflex gain between subjects (r^2=0.75, P<0.05). The authors therefore concluded that orthostatic stress, induced via head

upward tilt, altered carotid artery mechanics, which contributed to diminished BP control via the cardiac baroreflex.

Steptoe and Vogele (1990) reported reductions in baroreflex gain from sitting to active standing using the sequence method. No differences were found between participants with high-normal BP and low-normal BP, suggesting that BP status was not influential on BP control at least while values remain in within the normal limits. Westerhof et al. (2006) found a linear relationship ($r = 0.99$, $P<0.05$) between reductions in baroreflex gain and the degree of othostatic stress, induced by tilting, using spontaneous methods in both the frequency and time domains. This suggests that baroreflex control of BP is progressively attenuated as orthostatic stress increases.

The findings of Steinback et al. (2005) suggest that altered carotid artery mechanics contributes to diminished BP control via the cardiac baroreflex during orthostatic stress. Mattace-Raso et al. (2006) investigated the risk of orthostatic hypotension associated with age-induced arterial stiffness in a cross-sectional study of 3362 elderly men and women. Arterial stiffness, adjusted for age, gender and MAP, was associated with orthostatic hypotension and greater reductions in BP without significant differences in heart rate. These findings suggest that arterial stiffness associated with aging may explain the reduced baroreflex gain observed in older adults studied previously (Monahan, 2007). Saeed et al. (2009) used transfer function analysis to investigate the mechanical and neural components of the cardiac baroreflex in supine and upright seated positions. A significant reduction in mean (±SD) integrated gain was reported following a postural change from supine (17.1 ± 4.3 ms/mm Hg) to upright sitting (9.8 ± 3.3 ms/mm Hg). The reduction in integrated gain was associated with significant reductions in mechanical gain, which supports the findings of Steinback et al. (2005) and Mattace-Raso et al. (2006) suggesting that reductions in baroreflex function are vascular-related. However, the accuracy of spontaneous techniques, such as transfer function analysis, has recently been questioned due to the inconsistent results compared with standard techniques where the system is actively engaged (Lipman et al., 2003). Classic assessment of baroreflex gain using bolus injections if angiotensin introduced by Smyth et al. (1969), and more recent techniques, such as the

Oxford method, are based on the fact that baroreceptor responses are greatest and most apparent in reponse to rapid changes in BP as opposed to stationary or minimally changing pressures (Chapleau and Abboud, 1987). There is a lack of studies investigating cardiac baroreflex responses to postural changes using pharmacological techniques, such as the modified Oxford method, which in contrast to spontaneous baroreflex indices, enables the robust assessment of cardiac baroreflex function under near open-loop conditions. The separate components of the cardiac baroreflex have also yet to be explored directly during standing, which is clinically relevant given the well-documented effects of upright posture on vasovagal syncope. The combined effects of time of day and postural changes are also unknown. Investigations of these sources of variation in BP control may help to explain previous study findings, such as the increased orthostatic intolerance (Lewis et al., 2010) and risk of vasovagal syncope (Mineda et al., 2000, Zoghi et al., 2008) reported in the morning.

1.6 Aims and objectives

The specific aims of this thesis are:

1. To investigate sources of variation in human blood pressure control, with particular reference to the effects of blood pressure status, physical activity and time of day.
2. To explore and apply the investigations above to a clinical population of patients with obstructive sleep apnoea, who suffer from both circadian-related issues and generally high blood pressure status.
3. To explore mechanisms responsible for diminished blood pressure control in the morning in the general population.

The above aims will be achieved through the following objectives accompanied by their respective hypotheses:

1. To investigate the importance of blood pressure status, amongst other variables, in predicting post-exercise hypotension using both experimental (chapter 4) and meta-analytical (chapter 5) approaches.
   Hypotheses: Blood pressure status is not as important in predicting PEH as has previously been suggested; There are other important moderators of PEH that have previously been overlooked.

2. To examine blood pressure reactivity profiles during sleep and following waking in obstructive sleep apnoea patients (chapter 6).
   Hypothesis: Blood pressure reactivity in OSA patients is greatest during sleep at night.

3. To investigate the relationships between leisure-time physical activity, blood pressure, OSA severity and daytime sleepiness (chapter 7).
   Hypothesis: Greater leisure-time physical activity is associated with lower BP and less daytime sleepiness in OSA, independent of confounding factors of BMI, age and gender.

4. To investigate the influences of time of day and posture on cardiac baroreflex sensitivity, and to determine the relative contribution of the mechanical and neural components (chapters 8 and 9).
   Hypotheses: Integrated baroreflex gain is reduced in the morning due to time of day effects on the mechanical component; Integrated baroreflex gain is reduced with standing compared with supine due to changes in the neural component, and that these posture-induced reductions are greater in the morning than the afternoon.

Chapter 2

General Methods

2.1 Participants

All participants were informed of the details of the studies both verbally and in writing prior to giving their written consent. All studies were approved by Liverpool John Moores University Ethics Committee, except studies 5 and 6 which were approved by New Zealand Central Regional Ethics Committee and took place at the University of Otago, Wellington, New Zealand. All studies conformed to the Declaration of Helsinki. For laboratory-based experiments participants were instructed to refrain from exercise and the consumption of alcohol for 24 h prior to testing, as well as caffeine on the day of the study.

2.1.1 Healthy participants

Healthy participants were non-smokers and recreationally active, typically engaging in low-to-moderate intensity activity on 2-3 days/week. Individuals on regular medication or with a known history of respiratory, cardiovascular, or endocrine disease were excluded from participating.

2.1.2 Obstructive Sleep Apnoea patients

Obstructive sleep apnoea patients were recruited from Liverpool Sleep Clinic at the Liverpool Heart and Chest Hospital. All had an apnoea-hypopnoea index of >5 and were diagnosed using the gold standard technique of polysomnography. Patients were excluded from participation if they had a history of other respiratory or cardiovascular diseases, were receiving treatment for hypertension, or were aged <18 or >65 yrs.

2.2 Physiological measurements

2.2.1 Continuous beat-to-beat arterial blood pressure

Beat-to-beat arterial BP was measured continuously and non-invasively via finger photoplethysmography (Portapres Model 2 [Study 1, Fig 2.1A] or Finometer MIDI [Studies 5 & 6, Fig 2.1B], Finapres Medical Systems, Arnhem, Netherlands). The 'Finapres' and 'Portapres' were developed by Wesseling et al. (1993). The Portapres is suitable for exercise experiments provided that the hand can be kept in a stable condition, such as when using a cycle ergometer.

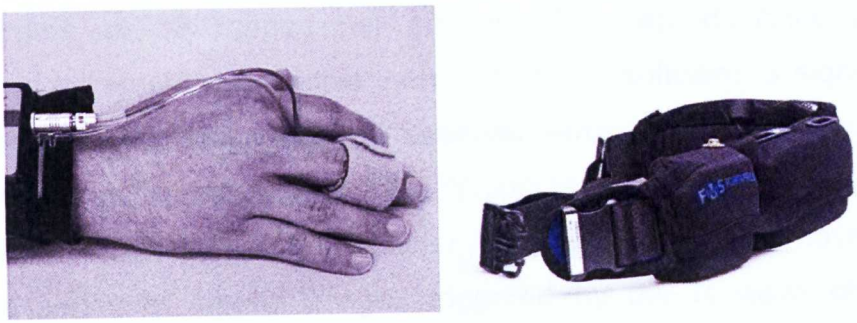
The Finometer MIDI has since been developed which is designed for optimal performance when monitoring changes in haemodynamic variables.

Both the Portapres and Finometer are based on the volume-clamp method introduced by the Czech physiologist Jan Penaz (1954). Firstly, the pressure in the finger cuff is increased until it equals the pressure within the artery. At this point the transmural pressure across the arterial walls is zero, meaning that the artery is not collapsed but is at its unstressed (or unloaded) diameter (Wesseling et al., 1993). The pressure in the cuff is then adjusted as changes in the artery diameter are detected by an infrared photo-plethysmograph, thus 'clamping' the artery at the unloaded diameter. The cuff pressure therefore provides an indirect measure of intra-arterial pressure. The vital aspect of this method is the accuracy in determining the unloaded pressure, which is variable due to affects of haematocrit, stress and smooth muscle tone on artery diameter. Therefore this must be corrected at regular intervals with periods of constant cuff pressure, referred to as 'physiocal'. By applying a range of pressures the cuff can be calibrated to equal the intra-arterial pressure once again, before measurement continues. The physiocal automatic algorithm is built into both the Portapres and Finometer.

A key limitation with finger photoplethysmography is that as the pulse pressure is transmitted along the arteries of the arm the pulse waveform becomes distorted. This variation in arterial waveform through the arterial tree can cause discrepancies between brachial and finger BP. However, finger photoplethysmography has been evaluated against simultaneous intra-arterial monitoring (Parati et al., 1989) at rest and during laboratory tests involving rapid changes in BP, including intravenous injections of phenylephrine. During 30 minutes rest the average difference between finger and intra-arterial BP was 6.5±2.6 mm Hg and 5.4±2.9 mm Hg for systolic and diastolic BP, respectively. A high correlation was reported between the two methods for systolic ($r = 0.98$) and diastolic ($r = 0.93$) BP. With regards to changes in BP during laboratory tests, the average difference between the two methods did not rise above 4.2 and 1.9 mm Hg for systolic and diastolic BP, respectively. Assessment of baroreflex function was also similar when using the two methods of BP measurement. This ability to accurately track rapid increases and decreases in

BP make this approach superior to manual or automatic sphygmomanometric methods, with which the accuracy is reduced when the measurements are not performed at rest (Mancia, 1983).

A.



B.

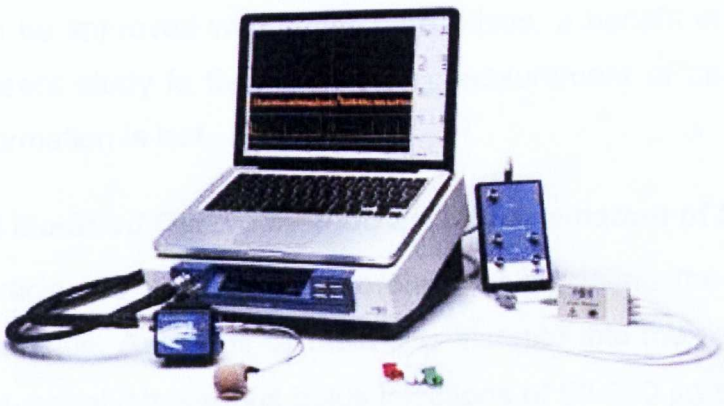


Figure 2.1: Finger cuff and Portapres (Part A) and Finometer MIDI (Part B) for continuous measurement of beat-to-beat arterial pressure via finger photoplethsmography (www.finapres.com/site)

2.2.2 Carotid artery diameter

Ultrasound imaging (Terason t3000, Burlington, MA, USA) was used to measure beat-to-beat carotid artery diameter. A longitudinal section of the left carotid artery < 2cm proximal to the bifurcation was imaged and recorded (Camtasia Studio, TechSmith Co., Ltd, Okemos, MI, USA) for offline analysis using custom edge tracking software. This method, described in detail by Black et al. (2008), involves the identification of a B-mode user-selected region of

interest (ROI) on the first frame of each recording. A pixel-density algorithm automatically identifies the angle-corrected near and far wall e-lines for every pixel column within the ROI. The position of the edge is established by determining the point where the pixel intensity changes most rapidly. Typically B-mode ROIs contain approximately 200–300 diameter measurements per frame at 30 frames per second. The reproducibility of these diameter measurements using this semi-automated software is significantly better than manual methods, reduces observer error, and possesses an intra-observer coefficient of variation of 6.7% (Woodman et al., 2001). Previous methods used for determining carotid diameter in baroreflex research have involved acquiring a series of carotid images triggered by the R wave of the ECG to gain approximately a third of the cardiac cycle, encompassed end-diastolic and peak systolic diameters. Using this technique researchers have previously had problems with data acquisition, capturing images for approximately every other cardiac cycle (Hunt et al., 2001). Although the portion of cardiac cycles captured can be improved with faster hard drives, a benefit of the methods used in the present study is the continuous measurement of carotid diameter so that no information is lost.

2.3 Modified Oxford method and determination of baroreflex gain

Participants underwent baroreflex testing using the modified Oxford method technique. A venous cannula was inserted into the right anticubita vein to allow sequential intravenous bolus injections of 50-250 μg sodium nitroprusside (SNP) followed 60 s later by 150-300 μg phenylephrine hydrochloride (PE). Doses given for SNP and PE were typically 150 and 250μg, respectively, although this was adjusted depending on the subject's mass and their previous responses to the drugs. Oxford tests were repeated until a valid trial was completed, i.e. the drop and rise in systolic blood pressure were >15 mmHg relative to baseline levels. All off-line data processing was performed using custom written software in LabView 8.2 (National Instruments, Texas, USA) on a Macintosh 2.26 GHz MacBook Pro computer. Systolic BP values were matched to either the concurrent heartbeat for R-R intervals >800ms, or a one beat delay for shorter heart periods (typically between 500 and 800ms). Baroreflex gains were calculated separately for SNP and PE injections to identify the gain against falling (G_{down}) and rising (G_{up}) blood pressures. A principal advantage of the

modified Oxford method is that it enables separate analysis of falling and rising pressures, whilst allowing the evaluation of dynamic cardiac responses across a wide range of blood pressures. Such ranges cannot be achieved with spontaneous methods of assessing baroreflex gain.

Integrated gain was determined by plotting the R-R interval – systolic BP relationship, which for G_{down} began at the onset of the systolic BP decrease following the SNP bolus injection and ended when systolic BP reached its nadir. For G_{up} the section of data selected began at the nadir in systolic BP and ended when pressure peaked following the bolus injection of PE. To identify and remove the saturation and threshold regions, a piecewise linear regression algorithm was applied to the raw data points to statistically identify breakpoints that occur at the upper and lower ends of the data set (Figure 2.2). Following this, respiratory related fluctuations in R-R interval and systolic BP were accounted for by averaging R-R intervals across 2 mm Hg bins. The mechanical and neural components of the baroreflex gain were calculated for both G_{up} and G_{down}, with exclusion of the same threshold and saturation regions removed for integrated gain. For the mechanical component carotid diameter measurements were plotted against systolic BP and for the neural component R-R intervals were plotted against carotid diameter.

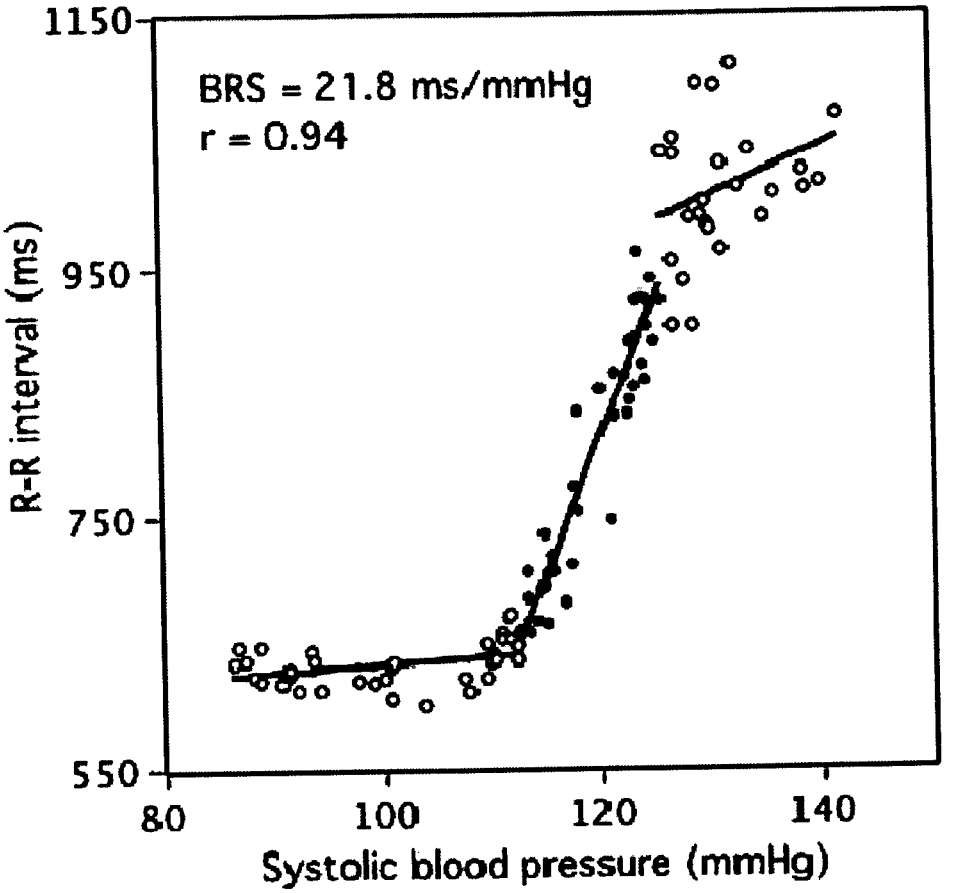


Figure 2.2: Piecewise regression model for elimination of threshold and saturation regions of the integrated baroreflex response to rising pressures. Open circles (○) represent the threshold and saturation regions and the closed circles (●) represent the linear portion of the baroreflex gain.

2.4 Linear mixed modelling

In contrast to previous studies of baroreflex function (Studinger et al., 2007, Halliwill and Minson, 2002), we applied a linear mixed model to compare baroreflex gains between two conditions (Atkinson et al., 2011). This method controls for the fact that systolic BP and R-R interval data are collected using a within-subjects design over time and are therefore correlated in nature. The 'subjects' factor is entered into the linear mixed model as a random effect, 'R-R interval' is the dependent variable and 'systolic BP' is entered as a covariate. To investigate the effect of a condition this was added as a fixed effect and a condition x systolic BP interaction term was added as another covariate to allow baroreflex gains to be compared. This approach improves precision by ensuring

all data points are entered into the analysis in a single step (Lazic, 2010). The linear regression method commonly used in the determination of baroreflex gains is based on the assumption that x-y cases are mutually exclusive, i.e. data pairs have been collected from independent participants. However, the slope of the baroreflex response is made up of data pairs from one individual, which clearly violates the assumption of case-independence. By controlling for the correlated nature of the data, the linear mixed model not only reduces the susceptibility to outliers in small samples, but the statistical power is greatly improved compared with conventional summary measure analyses (Atkinson et al., 2011).

Chapter 3

Study 1

Acute variation in blood pressure following exercise: an experimental approach

This work has been published in the Journal of Human Hypertension, 2010.

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Chapter 4

Study 2

Acute variation in blood pressure following exercise: a meta-analytical approach

4.1 Introduction

The relationship between an individual's general blood pressure status and the magnitude of the acute exercise-mediated change in blood pressure has been investigated in several past studies (Kenney and Seals, 1993, Thompson et al., 2001, Pescatello et al., 2004b). The results of these studies have been used to support claims made in position stands about the most important moderators of the exercise-mediated change in BP (Pescatello et al., 2004a). Using the experimental approach in chapter 3, it has already been shown that mathematical coupling and regression-to-the-mean statistical artefacts can compromise this relationship when pre-exercise BP is used as the measure of BP status. Specifically, when appropriate controlling procedures were employed in Chapter 4 (Oldham, 1962, Tu and Gilthorpe, 2007), the strength of the association between BP status and changes in BP is over-estimated when pre-exercise BP is designated as the indicator of BP status. The findings of chapter 3 also indicated that explorations into the influence of other potential moderators of PEH may be compromised because the spuriousness of the initial value - change correlation can mask the results of multiple regression analyses when pre-exercise BP is included as a potential predictor of BP change.

It is important to note that findings from published meta-analyses have also been cited to support the claim that the magnitude of exercise-mediated BP change depends on initial BP status. In a previous meta-analysis on the effects of exercise on BP, baseline BP was included as a predictor and found to be a strong correlate of PEH magnitude (Pescatello and Kulikowich, 2001). Following a multiple regression analysis, these authors reported that exercise intensity was not a significant predictor of the degree of PEH, concluding that change in BP after exercise is a function of initial BP, with the groups with highest baseline BP experiencing the greatest reductions post-exercise. Nevertheless, mathematical coupling and regression to the mean would also be predicted to exert spurious influences in such analyses.

It has been reported that PEH can persist for 24 hours or more during subsequent everyday activities and sleep (Fullick et al., 2009, Jones et al., 2009a), suggesting that exercise interventions are effective as anti-hypertensive

therapies. Therefore, the aim of present study is to apply the approach adopted in Chapter 3 to a meta-analysis, in order to examine in more detail what the true relationship is between BP status and the acute exercise-mediated changes in ambulatory BP over 24 hours.

It can be speculated that post-exercise measurements of BP which are further apart in time from the pre-exercise measurements (e.g. a number of hours) may result in smaller correlations between pre- and post-exercise values, and therefore greater potential spuriousness between initial BP and change in BP (Bartko and Pettigrew, 1968). Therefore, it is hypothesised that ambulatory BP measurements taken on separate days leads to a greater attenuation of the correlation between pre- and post-exercise BP compared with those seen in the experimental study (chapter 3). However, limiting the meta-analysis to studies that include a control ambulatory BP monitoring day is expected to reduce the effects of regression to the mean to a certain extent because the initial BP data is based on multiple readings. There are currently inconsistencies in the literature regarding predictors of the magnitude of PEH, potentially due to inadequate sample sizes. Therefore, the secondary aim of this meta-analysis is to provide adequate statistical power to examine the importance of factors such as age, BMI and maximal oxygen uptake in predicting acute changes in BP post-exercise.

4.2 Methods

4.2.1 Data Sources

A literature search of peer-reviewed studies examining the acute effects of exercise on ambulatory BP was conducted using MEDLINE and PubMed electronic databases. The keywords and phrases employed in the online search included 'post-exercise hypotension', 'blood pressure', 'ambulatory', 'exercise' and 'physical activity'. Reference lists from published papers were examined in order to identify any other relevant studies not cited in the online databases.

4.2.2 Study selection and data extraction

For inclusion in the meta-analyses studies had to meet the following criteria: 1) study design included a randomised controlled trial or cross-over trial with control phase; 2) participants were healthy normotensive or hypertensive adults;

3) ambulatory blood pressure monitoring for a minimum of 5 consecutive hours following exercise 4) acute aerobic exercise; 5) data available included pre and post and/or change in mean SBP and/or DBP values with standard deviation(SD) or another statistic allowing the calculation of SD.

Sixty-seven potential studies were initially identified via the literature search. Fifty-three studies were eliminated according to the following exclusion criteria: 1) outcome measures of changes in BP were not separated into daytime and night-time when ambulatory monitoring included night-time sleep; 2) diet, medication or another influential factor was included in the intervention; 3) the inclusion of populations with disease. The following data were extracted from the 14 studies that were found to be eligible for meta-analyses: primary author's name; year of publication; sample size; characteristics of participants (age, BMI and $\dot{V}O_{2\,max}$ if available); characteristics of the exercise protocol (mode, duration, intensity). The primary outcome measures were the mean changes (and SD of the change) in SBP and DBP following acute exercise compared with control. Study selection and data extraction was verified independently by a reviewer. Due to the experimental designs most studies were split, providing data for changes in daytime SBP from a total of 24 participant groups (total participants, n=451) from the 14 peer-reviewed studies. However, due to the availability of data these numbers were reduced for night-time SBP changes (11 participant groups, total n=238), daytime DBP changes (22 participant groups, total n= 437), and night-time DBP changes (8 participant groups, total n=174).

4.2.3 Statistical Analyses

Pooling of results

Random-effects meta-analyses of the mean difference in BP following exercise were performed using Comprehensive Meta-Analysis (Version 2, Biostat, Englewood, NJ). Four separate meta-analyses were performed for daytime and nocturnal changes in both systolic and diastolic BP. Ninety-five percent confidence intervals (95% CI) were calculated for the mean changes in BP for each study. All of the studies included in the analyses comprised of a repeated measures design with an experimental (post-exercise) and control condition (i.e. ambulatory BP monitoring of everyday living following no exercise). Therefore, the standard error calculated from the standard error of the differences between

conditions was meta-analysed. Each study was assigned a weighting according to sample size and between-subjects standard error.

Exploration of heterogeneity and publication bias

Heterogeneity of mean changes was examined with the Q-test and I^2 value associated with the fixed-effects model (Higgins et al., 2003). A Q statistic was deemed statistically significant if $P < 0.10$ and I^2 values of 25, 50, and 75% were used to indicate small, medium, and large heterogeneity, respectively. The presence of publication bias was explored using Egger's regression intercept and funnel plots.

Sensitivity Analysis

In comparison to the majority of the studies included in the meta-analyses, the sample sizes were relatively large for studies by Ciolac et al. (2008) (n=50) and Pescatello et al. (2004b) (n=49). Therefore, sensitivity analyses were performed so that pooled mean changes in BP were re-calculated with the exclusion of results from these studies.

Meta-regression analyses

Weighted meta-regression methods were used to examine the effects of continuous variables on changes in ambulatory BP following exercise. The reported exercise-mediated changes in daytime and nocturnal BP (i.e. differences between exercise and control day) were meta-regressed against control day and night BP (BP status), respectively. Despite the use of ambulatory monitoring and control conditions in the meta-analysed studies, mean BP (the mean of control and exercise-day BPs) was used as a measure of BP status to ensure that statistical artefacts were minimised. Changes in BP were also meta-regressed against participant characteristics of $\dot{V}O_{2\,max}$ (data unavailable for 5 participant groups), age and BMI.

Subgroup analyses

A subgroup analysis was performed to investigate the effects of gender, with studies split into 3 groups (males only, females only, and mixed). Subgroup analyses were also performed to explore the influences of exercise mode (cycle versus treadmill), duration (≤30 versus >30 mins), and intensity (low versus

moderate). Moderate intensity exercise was defined as >58% $\dot{V}O_{2max}$ or >70% maximum HR (McArdle et al., 2001). None of the studies included exercise protocols that exceeded the set upper limit of moderate intensity exercise (80% $\dot{V}O_{2max}$), and therefore analyses were limited to low and moderate intensity. Mean differences between subgroups were determined with 95% confidence limits. Alpha was 0.05 for all analyses.

4.3 Results

4.3.1 Overall changes in daytime BP

The overall weighted mean (95% CIs) changes in daytime SBP and DBP following exercise were -3.84 mm Hg (-5.40 to -2.27, Fig. 4.1) and -1.81 mm Hg (-2.58 to -1.04), respectively. Following a sensitivity analysis with the removal of data from studies with large sample sizes (Ciolac et al., 2008, Pescatello et al., 2004b), the overall weighted mean changes increased only very slightly to -4.14 mm Hg (-5.88 to -2.39) for SBP and -1.84 mm Hg (-2.69 to -1.00) for DBP.

The investigation of potential moderating factors on changes in BP is especially relevant since large and statistically significant heterogeneity between studies was revealed by the Q-test on SBP data (Q_{23} = 265.7, P<0.0005, I_2 = 91%) and DBP data (Q_{21} = 62.0, P<0.0005, I_2 = 66%). Funnel plots revealed publication bias for both SBP (Fig. 4.2) and DBP, indicating the possibility that studies with relatively small sample sizes reporting no PEH (i.e. increases in daytime systolic and diastolic BP following exercise) were not published. Eggers regression intercept was -3.00 (-3.93 to -2.06, P<0.0005) for SBP and 1.09 (0.24 to 1.94, P=0.04) for DBP, confirming that the publication bias was significant.

Study name	Total	Mean	Standard error	Variance	Lower limit	Upper limit	Z-Value	p-Value
Brownley et al. 1996(a)	11	-6.320	2.000	4.000	-10.240	-2.400	-3.160	0.002
Brownley et al. 1996(b)	20	-0.500	1.400	1.960	-3.244	2.244	-0.357	0.721
Taylor-Tolbert et al. 2000	11	-9.000	2.600	6.760	-14.096	-3.904	-3.462	0.001
Wallace et al. 1997(a)	36	0.500	0.125	0.016	0.256	0.744	4.009	0.000
Wallace et al. 1997(b)	25	-5.500	0.748	0.560	-6.967	-4.033	-7.350	0.000
Wallace et al. 1999(a)	25	-1.000	1.497	2.240	-3.933	1.933	-0.668	0.504
Wallace et al. 1999(b)	21	-7.000	2.245	5.040	-11.400	-2.600	-3.118	0.002
Ciolac et al. 2008	50	-2.000	0.990	0.979	-3.939	-0.061	-2.021	0.043
Park et al. 2005(a)	9	-5.670	2.590	6.708	-10.746	-0.594	-2.189	0.029
Park et al. 2005(b)	5	-6.000	1.230	1.513	-8.411	-3.589	-4.878	0.000
Pescatello et al. 1991(a)	6	-10.000	1.122	1.260	-12.200	-7.800	-8.909	0.000
Pescatello et al. 1991(b)	6	-2.000	1.122	1.260	-4.200	0.200	-1.782	0.075
Pescatello et al. 1999(a)	11	-1.600	1.500	2.250	-4.540	1.340	-1.067	0.286
Pescatello et al. 1999(b)	7	-4.600	2.800	7.840	-10.088	0.888	-1.643	0.100
Park et al. 2006	21	-5.600	1.600	2.560	-8.736	-2.464	-3.500	0.000
Pescatello et al. 2003(a)	7	-8.800	3.592	12.902	-15.840	-1.760	-2.450	0.014
Pescatello et al. 2003(b)	11	0.200	2.320	5.382	-4.347	4.747	0.086	0.931
Pescatello et al. 2004	49	-0.300	1.235	1.525	-2.720	2.120	-0.243	0.808
Guidry et al. 2006(a)	23	-4.300	1.600	2.560	-7.436	-1.164	-2.688	0.007
Guidry et al. 2006(b)	22	-4.900	1.900	3.610	-8.624	-1.176	-2.579	0.010
Syme et al. 2006(a)	17	-6.300	2.300	5.290	-10.808	-1.792	-2.739	0.006
Syme et al. 2006(b)	16	-0.700	2.000	4.000	-4.620	3.220	-0.350	0.726
Syme et al. 2006(c)	17	-5.000	2.300	5.290	-9.508	-0.492	-2.174	0.030
Bermudes et al. 2003	25	-1.000	1.010	1.021	-2.980	0.980	-0.990	0.322
		-3.835	0.796	0.634	-5.396	-2.274	-4.816	0.000

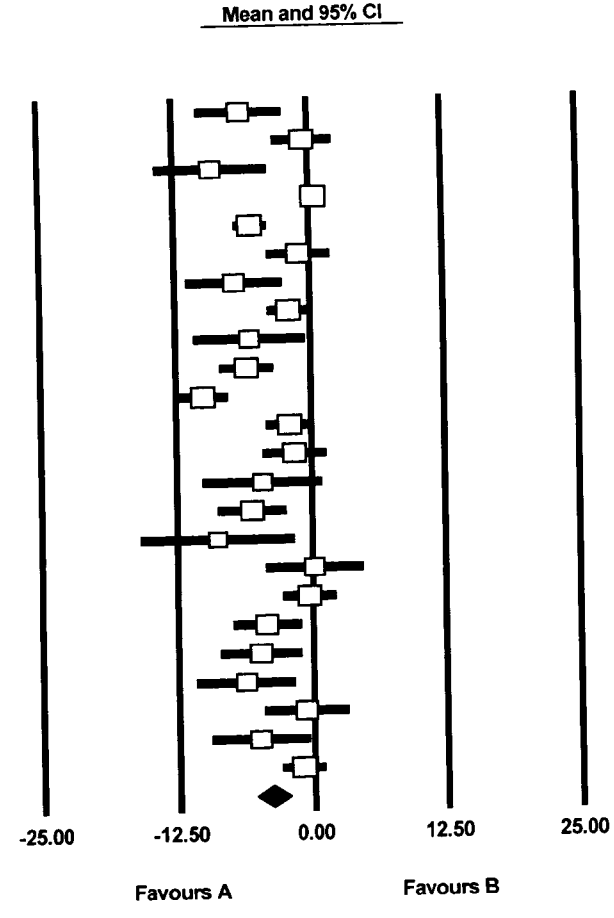

Figure 4.1: Forest plot showing mean change in daytime SBP following exercise, and 95% confidence limits for each study. The overall random-effects mean change in daytime SBP for the 437 participants is shown at the bottom of the raw plot.

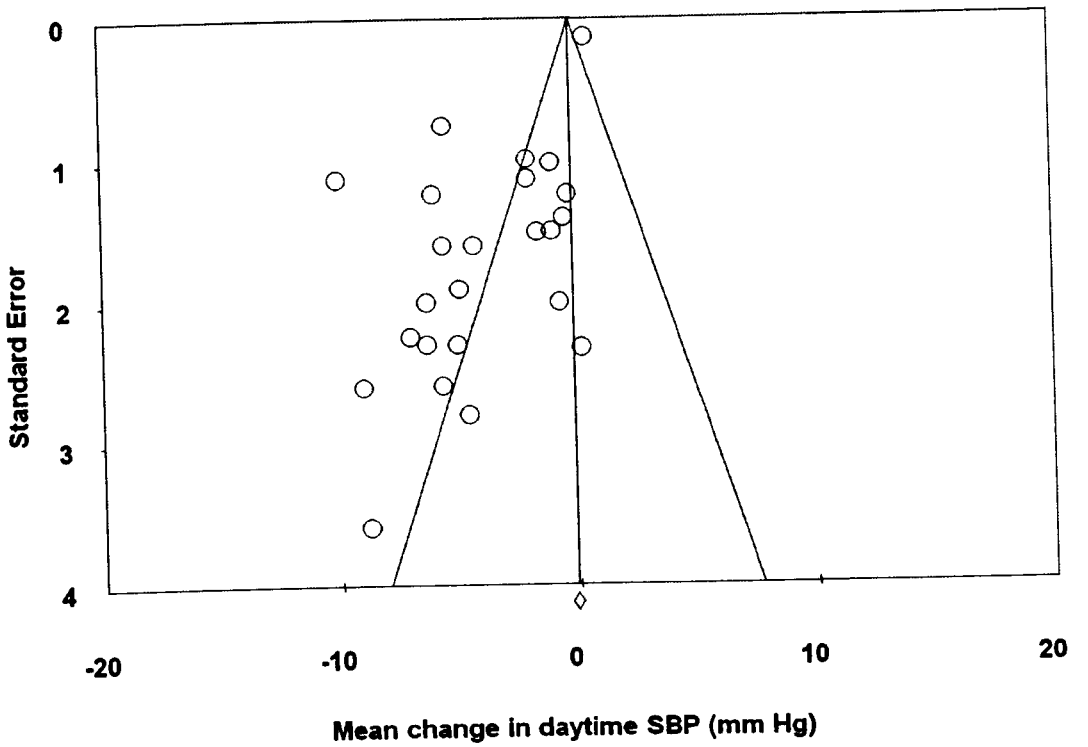

Figure 4.2: Funnel plot of mean changes in daytime SBP vs standard error. Large standard errors are indicative of a small sample size.

4.3.2 Overall changes in nocturnal BP

The overall weighted mean (95% CI) change in nocturnal SBP following exercise was -3.01 mm Hg (-4.69 to -1.50). However, the overall weighted mean change for nocturnal DBP was very small (-0.61 mm Hg) with 95% CIs overlapping zero (-3.04 to 1.81), and was therefore not significant (P=0.62). A sensitivity analysis was performed with the removal of the study by Ciolac et al. (2008). This analysis was for SBP only, as this study was not included in the original analysis of nocturnal DBP. The overall weighted mean change in SBP was reduced very slightly to -2.95 mm Hg (-4.68 to -1.21). Large, statistically significant heterogeneity between studies was found for both SBP (Q_{10} = 32.0, P<0.0005, I_2 = 69%) and DBP (Q_7 = 65.5, P<0.0005, I_2 = 89%). Funnel plots demonstrated no publication bias, confirmed quantitatively by Egger's regression intercept, which was -1.99 (-4.14 to 0.17, P=0.13) for SBP and -2.51 (-7.92 to 2.91, P=0.40) for DBP.

4.3.3 Meta-regression analyses

BP status

Daytime SBP and DBP status obtained from control periods of ambulatory monitoring, were significant moderators of the respective changes in daytime SBP and DBP following exercise (P<0.05). Mean BP (the mean of control and exercise-day BPs) was then used instead of control BP as BP status. Mean SBP and mean DBP were also found to be significant moderators of changes in BP (Table 4.1). Higher daytime mean SBP was associated with greater reductions in daytime SBP post-exercise (mean slope of meta-regression line = -0.25, P<0.0005, Fig 4.3). This equates to a 1 mm Hg fall in post-exercise SBP for every 4 mm Hg increase in SBP status. Nocturnal BP status was also a significant moderator of nocturnal changes in SBP and DBP post-exercise, when either control BP or mean BP was used (Table 4.1).

Table 4.1: Regression slopes (95% CI) for moderators of changes in ambulatory BP following exercise

Moderator variable	Daytime SBP (95% CI)	Daytime DBP (95% CI)	Nocturnal SBP (95% CI)	Nocturnal DBP (95% CI)
BP status (Control BP)	-0.24 (-0.27 to -0.20)*	-0.14 (-0.21 to -0.07)*	-0.12 (-0.18 to -0.05)*	-0.25 (-0.32 to -0.19)*
BP status (Mean BP)	-0.25 (-0.29 to -0.22)*	-0.11 (-0.19 to -0.04)*	-0.11 (-0.18 to -0.04)*	-0.29 (-0.36 to -0.22)*
Mean age	0.06 (-0.03 to 0.16)	-0.12 (-0.21 to -0.03)*	-0.08 (-0.24 to 0.07)	-0.27 (-0.42 to -0.16)*
Mean BMI	-1.34 (-1.61 to -1.07)*	-0.09 (-0.34 to 0.16)	-0.55 (-1.00 to -0.10)*	-1.21 (-1.59 to -0.83)*
Mean $\dot{V}O_{2max}$	0.35 (0.22 to 0.47)*	-0.10 (-0.19 to 0.00)*	0.10 (-0.07 to 0.28)	0.45 (0.31 to 0.60)*

*Significant moderator (P<0.05)

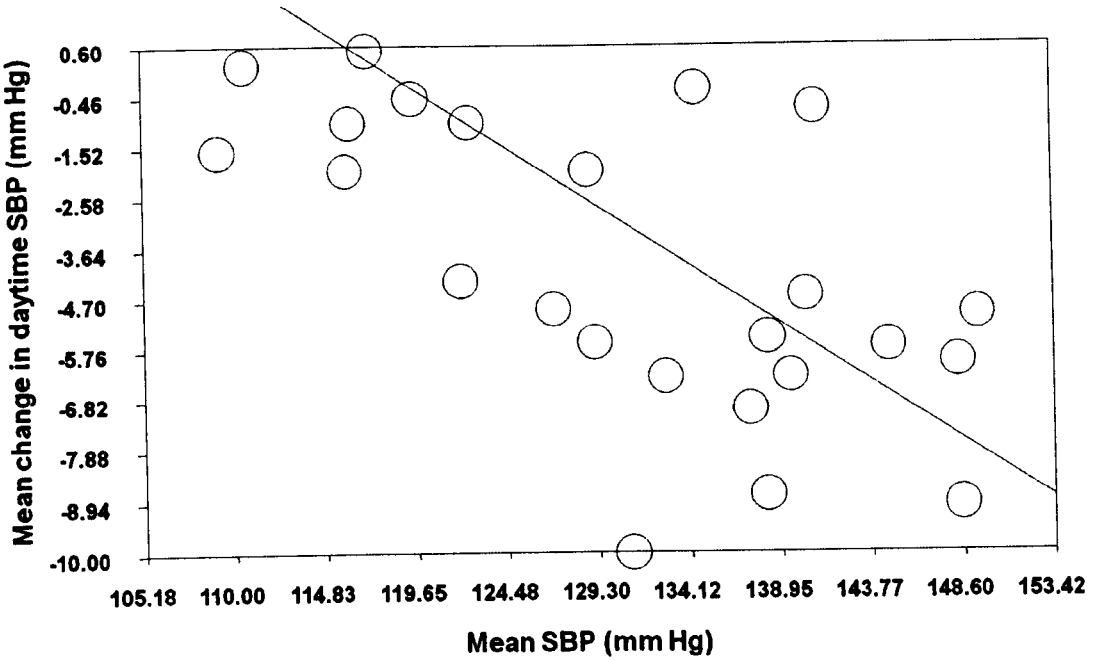

Figure 4.3: Scatterplot showing the relationship between blood pressure status (mean of control-day and exercise-day SBP) and mean changes in daytime SBP. The slope of the regression line is -0.25 (95% CI = -0.29 to -0.22).

Participant characteristics

Slopes and 95% confidence limits for all continuous variables are reported in Table 5.1. Mean age was a significant moderator of changes in daytime DBP (slope = -0.12, P=0.01) and nocturnal DBP (slope = -0.27, P<0.0005), but not for changes in daytime or nocturnal SBP (>0.05). Increasing age was associated with greater reductions in DBP post-exercise. Body mass index (BMI) was a significant moderator of changes in daytime SBP (slope = -1.34, P<0.0005, Fig 4.4), nocturnal SBP (slope = -0.55, P=0.02) and nocturnal DBP (slope = -1.21, P<0.0005). Increases in BMI were associated with greater reductions in BP post-exercise. However, mean BMI was not found to be a significant moderator of changes in daytime DBP (slope = -0.09, P=0.47). Finally, mean $\dot{V}O_{2\,max}$ was a significant moderator of changes in daytime SBP (slope = 0.35, P<0.0005) and nocturnal DBP (slope = 0.45, P<0.0005), suggesting that lower mean $\dot{V}O_{2\,max}$ is associated with greater PEH. However, $\dot{V}O_{2\,max}$ was also found to be a significant moderator of daytime DBP (slope = -0.10, P=0.04) but, unexpectedly, the direction of the relationship was reversed. Given the extremely small slope it is unlikely that this result would be of any

clinical significance, as will be discussed later. Mean $\dot{V}O_{2\,max}$ was not a significant moderator of changes in nocturnal SBP (slope = 0.10, P=0.25).

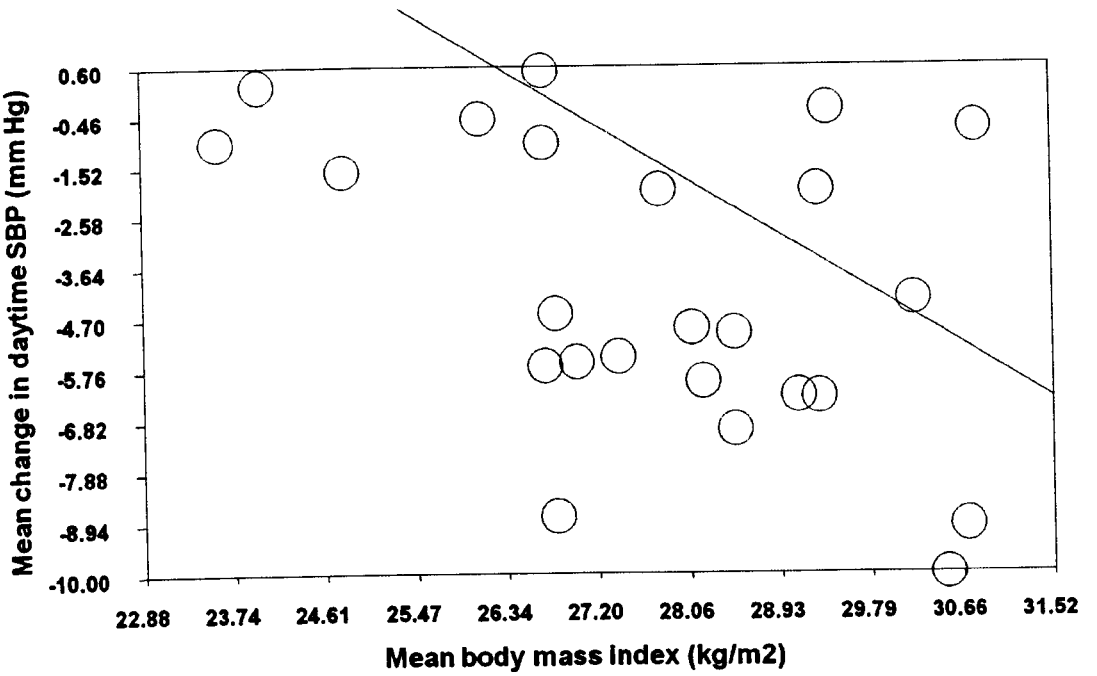

Figure 4.4: Scatterplot showing the relationship between mean body mass index of participants and mean changes in daytime SBP. The slope of the regression line is -1.34 (95% CI = -1.61 to -1.07).

4.3.4 Subgroup analyses

The subgroup analysis for gender revealed no significant differences between studies of males, females or mixed gender groups for changes in daytime or nocturnal BP (P<0.05). Subgroup analyses also revealed that exercise protocol characteristics were not significant moderators of the changes in BP. The changes in daytime and nocturnal SBP and DBP following exercise were not significantly different between low and moderate intensity exercise, between cycling and treadmill exercise, or between exercise protocols of ≤ and >30 minutes in duration (P>0.05).

4.4 Discussion

The results demonstrate that, across all the relevant studies, exercise mediated mean reductions in daytime ambulatory SBP and DBP of approximately 4 and 2 mm Hg, respectively. Similar reductions were also observed in post-exercise ambulatory nocturnal SBP (approx. 3 mm Hg), but the pooled mean reduction

for post-exercise nocturnal DBP was not statistically significant. Using other meta-analytical techniques, Staessen et al. (2001) found that a substantial part of cardiovascular protection was gained from modest reductions of 5 mm Hg in SBP via drug therapy. Although the pooled mean SBP reductions in the present meta-analysis are marginally below this level and are acute in nature it is possible that they may still be of clinical value. A reduction of 4 mm Hg in hypertensive patients receiving felodipine resulted in a reduction in the incidence of fatal and non-fatal strokes by 27% (95% CI, 11 to 40%) over a 40-month follow-up (Liu et al., 2005). Future studies should examine the chronic effects of exercise training on blood pressure and its cardio-protective effects. Although meta-analyses have been used to investigate the effects of aerobic training (Fagard, 2001, Kelley et al., 2001), the main outcome measures were changes in resting SBP and DBP, as opposed to ambulatory measurements which are more relevant to the control of BP and related cardiovascular events.

The pooled mean changes in BP might mask particular studies which are associated with larger reductions in BP. This notion can also be explored with meta-analytical techniques. First, the association between BP status and exercise-mediated changes in ambulatory BP were examined. Whether control BP or mean BP was used, BP status was a significant moderator of SBP and DBP during both day and night. Higher BP status was associated with greater PEH, which provides support for exercise as an anti-hypertensive treatment. In the experimental study (Chapter 4) changes in BP post-exercise were regressed against pre-exercise BP determined from a 5-min baseline measuring period. The strength of this association was over-estimated compared with methods where statistical artefacts of regression to the mean and mathematical coupling were controlled for. In the current study regression-to-the-mean artefacts were reduced because exercise-induced changes in BP were compared with a control condition, and BP status was determined using multiple measurements (i.e. ambulatory monitoring). It was hypothesised that this type of experimental design in the meta-analysed studies would lead to reduced spuriousness of correlations between BP status and changes in BP. The results showed that the importance of BP status as a predictor of PEH was not attenuated when mean BP was used. In fact, the gradients of the regression slopes for daytime SBP and nocturnal DBP increased slightly when control BP

was replaced with mean BP. This suggests that the measures taken to control for statistical artefacts were successful in the current study. All studies included in the meta-analysis involved two periods of ambulatory BP monitoring (control and exercise). The control day provided the pre-exercise values, which were divided into daytime and nocturnal periods where monitoring extended into sleep at night. By determining pre-exercise BP over multiple readings the influence of regression to the mean was reduced compared with the experimental data (chapter 4) where pre-exercise BP was determined over a short baseline period.

The minimisation of statistical artefacts with control conditions should encourage the use of control trials in study designs when investigating the anti-hypertensive effects of exercise and other therapies. However, the studies in the present meta-analysis involved relatively short delays of approximately 2-3 days between ambulatory monitoring sessions. The greater spuriousness associated with tests that are further apart in time (Bartko and Pettigrew, 1968) means that experiments that include measurements performed weeks apart, such as exercise training studies, may be more susceptible to statistical artefacts. It is important in future investigations of the effects of exercise on ambulatory BP that comparisons of post-exercise measurements are made against a control period of ambulatory monitoring, or at least to a control group. Large exercise-induced reductions in ambulatory SBP (>9 mm Hg) were reported in a study that compared these post-exercise measurements to those taken over a 15-min baseline period (Pescatello et al., 1999). The changes in SBP were attributed to the elevated post-exercise response suggested to be present in hypertensive populations. This was despite falls of 4.9 mm Hg in the same participants following a sham exercise condition. Changes of this magnitude were not seen in normotensives for the sham condition, and therefore the conclusions should be drawn with caution.

Assessment of publication bias using funnel plots and Egger's regression intercept revealed that studies with relatively small sample sizes reporting no reductions in SBP or DBP were not published. However, publication bias was not present for changes in nocturnal SBP and DBP. It is suspected that this is because the studies included in the meta-analysis that reported nocturnal BP

also reported daytime values (often as the primary outcome) and therefore publication was not biased by the significance of findings within the nocturnal data. The current meta-analysis is limited by the publication bias present for daytime BP changes, and future meta-analyses should aim to include data from unpublished studies of post-exercise hypotension.

An advantage of meta-analytical methods is the ability to explore heterogeneity in study results due to moderating factors. It was found that $\dot{V}O_{2\,max}$ was a significant moderator of changes in daytime SBP and nocturnal DBP. According to the regression slopes a decrease in $\dot{V}O_{2\,max}$ of 10 ml/kg/min was associated with larger reductions in daytime SBP and nocturnal DBP of approximately 3.5 and 4.5 mm Hg, respectively. These findings concur with those of the experimental data (chapter 4) in which a multiple regression model revealed $\dot{V}O_{2\,max}$ to be a significant predictor of PEH, although in MAP and DBP only. As the current results suggest, individuals with a lower $\dot{V}O_{2\,max}$ tended to experience greater reductions in BP following exercise. It is worth noting that although these results agree with previous findings (chapter 4), the regression slope for daytime DBP was reversed indicating greater reductions in BP with increasing $\dot{V}O_{2\,max}$. Meta-analytical techniques vastly improve precision and power, however it is important to interpret the results with caution and consider the clinical significance of the findings. Although this result was statistically significant the upper confidence interval was extremely close to zero (-0.00413 mm Hg) so it remains possible that $\dot{V}O_{2\,max}$ has very little effect on daytime DBP changes.

Age did not significantly influence SBP, but was found to be a significant moderator of daytime and nocturnal DBP, with greater BP reductions associated with increasing age. An increase in age of 10 years was associated with reductions in daytime and nocturnal DBP of 1.2 and 2.7 mm Hg, respectively. This relationship with age may be interpreted as a positive outcome as it indicates that exercise is a useful tool for BP control amongst the older population for whom hypertension is most prevalent. The nocturnal reduction in particular may provide support for exercise interventions in aging

non-dipping populations, although further research is warranted to confirm that these effects would apply to this group of hypertensives. Park et al. (2005) found that acute bouts of exercise in the morning and evening caused greater nocturnal BP reductions in non-dippers than dippers. In the current study BMI significantly moderated reductions in daytime SBP and DBP, and nocturnal DBP. In the experimental data (chapter 4) neither age nor BMI were not found to be significant predictors of PEH, although the study was limited to a younger and healthier participant group with a mean ±SD age of 32 ±7.4 yrs and BMI of 24.7 ±2.9 kg/m^2. A strength of the current study was the ability to investigate the influences of these moderators with improved power and precision. However, no significant differences were found between studies using males, females or mixed gender participant groups. This finding concurs with previous reports of similar magnitudes and durations of PEH in women to those found in men (Birch et al., 2002, Lynn et al., 2007), although the nadir in BP may occur earlier in females (Birch et al., 2002) and the mechanisms behind the reductions in BP may also differ between genders (Senitko et al., 2002).

Despite being unable to identify age and BMI as predictors of PEH, the use of mean BP as a predictor variable in the analysis of the experimental data meant that other predictors of PEH were not masked by statistical artefacts. Time of day for exercise was found to be a significant predictor of changes in SBP, when previously it was masked by spurious correlations between pre-exercise SBP and SBP changes. Due to missing information regarding time for exercise in the meta-analysed studies time of day was not included as a potential moderator in the present study. Of those studies where details of time of day were given, the majority of experimental designs involved morning exercise to allow for ambulatory BP monitoring throughout the rest of the day, thus limiting the usefulness of time of day as a moderator in meta-regression analyses. However, this would be an interesting aspect of exercise protocols to investigate given the evidence in the literature for diurnal variation in acute BP responses to exercise (Jones et al., 2008a, Jones et al., 2008b).

Exercise protocol characteristics did not influence the degree of PEH. There was no significant difference in mean BP changes between studies involving low intensity exercise versus those involving moderate intensities. This finding

reflects those of Jones et al. (2007), who found that PEH is not dependent upon exercise intensity but on the total amount of work done. Pescatello et al. (2004b) also found that even though moderate intensity exercise evoked greater PEH after 5 hours of ambulatory BP monitoring, low intensity exercise was equally as effective over the course of 9 hours. In the current study there was no significant effect of exercise duration. This may be attributed to the lack of range in durations (20-50 mins) and very little spread within this range, leading us to perform a subgroup analysis based upon protocols of ≤ and >30 minutes. It would therefore be instructive for future meta-analyses to include a computed variable that takes into account both exercise intensity and duration in order to assess the influences of the two components combined. There was also no effect of exercise mode, with no significant difference between cycling and treadmill exercise. Although this finding is positive for the flexibility of anti-hypertensive treatment with exercise, the meta-analysis was limited because treadmill protocols included both running and walking due to a lack of studies. It is possible that this may have masked potential differences between cycling, running and walking had they been analysed as three separate subgroups. More studies are required to examine the effects of exercise mode on PEH, which is currently biased towards cycle ergometry in the literature, perhaps due to the relative ease with which physiological measurements can be taken during this mode of exercise. Ambulatory monitoring provides a simple method of recording BP measurements for any mode of exercise since equipment can be fitted after the completion of the exercise protocol.

4.5 Conclusion

The current study demonstrates that exercise causes reductions in daytime and nocturnal ambulatory SBP and DBP that are likely to be of clinical significance. Meta-analytical methods were used to explore the association between BP status and exercise-mediated changes in ambulatory BP. Blood pressure status was a significant moderator of PEH indicating that hypertensive patients will benefit from greater reductions in BP. The current findings highlight the importance of including ambulatory monitoring and control conditions within study designs to reduce the effects of regression to the mean. This is important if study results are to influence position statements and the treatment of hypertension. Age, BMI and $\dot{V}O_{2\,max}$ were also identified as significant

moderators of reductions in BP, indicating that older individuals with larger BMIs and lower fitness levels will benefit most from exercise. These populations are amongst those where hypertension is most prevalent and therefore these results support the use of exercise as an anti-hypertensive therapy. There were no effects of exercise protocol characteristics on the degree of PEH. However, it would be of value for future studies and meta-analyses to investigate the combined effects of exercise duration, intensity and mode to determine the most beneficial protocols. The current study focuses on the BP responses to acute exercise. However, for full investigations of the use of exercise as a treatment for hypertension, future studies should examine the chronic effects of exercise training on ambulatory BP and its cardio-protective effects.

Chapter 5

Study 3

Circadian variation of blood pressure reactivity in obstructive sleep apnoea

5.1 Introduction

An interesting population model in which patients suffer from both circadian-related issues and a generally higher BP status is people with obstructive sleep apnoea (OSA). The condition is characterised by frequent episodes of upper airway collapse causing arousal from sleep and is an independent risk factor for hypertension (Lavie et al., 2000, Nieto et al., 2000). In this chapter, the diurnal variation in the blood pressure responses to activity are explored and applied to a clinical population of patients with OSA.

In the general population, BP exhibits a circadian rhythm with the lowest pressures occurring during sleep at night and a rapid 'morning surge' following waking (Kaplan, 2003). This diurnal variation in BP control coincides with the incidence of cardiovascular events (Muller et al., 1987) and stroke onset (Elliott, 1998), which peak between 06:00 and 12:00 h. The morning surge in blood pressure is strongly influenced by the levels of physical activity in the hours after waking (Leary et al., 2002). In hypertensive patients, the reactivity of BP to activity is greatest in the morning between 08:00 and 10:00 h (Jones et al., 2006), potentially amplifying the risk of cardiovascular events at this time. In OSA patients investigations have shown that night-time BP is elevated above that of control patients suffering from daytime sleepiness only (Nagata et al., 2008). Further increases in night-time BP are observed as OSA severity increases (Nagata et al., 2008, Noda et al., 1993). The high night-time BP found in OSA patients coincides with the incidence for myocardial infarction in this population, which peaks between 00:00 and 06:00 h (Kuniyoshi et al., 2008). It is possible that BP reactivity may also be greater during sleep in OSA further increasing the risk of cardiovascular events.

The aim of this study is to determine whether the time of day for peak BP reactivity differs in OSA patients compared to healthy controls. Given the disturbances to sleep and circadian rhythms of BP associated with OSA, it is hypothesised that BP reactivity in OSA patients is greatest during sleep at night. Such a different circadian profile may partly explain the increased risk of cardiovascular events at this time. A secondary aim of this study is to assess the use of clinic measurements of BP in diagnosing hypertension in OSA, and in predicting day and night ambulatory BP.

5.2 Methods

5.2.1 Participants

Eleven male OSA patients (aged 48.5 ± 13.2) were recruited from the Liverpool Sleep Clinic and 18 healthy controls (13 males, 5 females, aged 28.1 + 7.3) were recruited from Liverpool John Moores University, to take part in the study.

5.2.2 Measurements

Participants underwent simultaneous ambulatory BP and activity monitoring on a single day from 20:00 to 10:00 h. A TM-2421 ambulatory BP monitor (A&D, Tokyo, Japan) was fitted to each participant's non-dominant arm following calibration with a mercury sphygmomanometer. The monitor took readings of SBP, DBP and HR every 15 minutes, except between 23:00 and 06:00 h where recordings were reduced to one per hour in order to minimise sleep disturbance. Mean arterial pressure (MAP) and rate pressure product (RPP) were calculated following data collection. An actigraphy device was attached to each participant's dominant wrist to monitor physical activity via accelerometry (CamNtech Ltd, Cambridge, UK). Participants were asked to record the time they went to bed and time of getting up. In the event that participants got up after 08:00 h they were asked to continue with the ambulatory monitoring until 12:00h to ensure sufficient data was collected following waking. All OSA patients completed at least 14hrs ambulatory monitoring, 7 of which completed a full 24-hrs. In OSA patients measurements were also taken of height, mass, clinic SBP and DBP, and OSA severity (AHI and ODI via polysomnography).

5.2.3 Data reduction

Mean activity data were calculated for every minute, and the score was logarithmically transformed to correct for positive skew (Kario et al., 1999). Activity data were averaged over the 15-minute period preceding each BP measurement (Leary et al., 2002). The BP and activity data were divided into seven 2-hour time periods relative to waking time. For each individual participant and time period, a least squares regression slope was calculated for the relationship between activity and SBP, DBP, MAP, and RPP (Kario et al., 1999). For a separate analysis, ambulatory BP data for the 7 OSA patients who completed 24-hr monitoring were divided into daytime (10:00-20:00h) and night-

time (00:00-06:00h) and means were calculated for day, night and 24-hr SBP and DBP.

5.2.4 Statistical analysis

Two-way mixed general linear models (GLM) were be used to examine differences between OSA patients and healthy controls, firstly in the absolute BP and then in mean reactivity indices over 2-hour time periods relative to time of waking. The actigraphy data were also analysed independently of BP. A two-way mixed GLM was used to compare 15-min mean activity (logged) over 14 hrs between OSA and healthy controls, relative to the time of getting up (10 hrs pre- and 4 hrs post-getting up).

One-way repeated measures GLMs were used to compare ambulatory SBP and DBP measurements (day, night, and 24-hr) to clinic SBP and DBP measurements in 7 male OSA patients who completed 24-hr ambulatory monitoring. Linear regression analyses were used to investigate the use of clinic BP in predicting ambulatory BP. Finally, multiple regression analyses were used to identify predictors of clinic SBP and DBP in all 11 OSA patients. Independent variables entered into the regression model were age, BMI, collar size, AHI and ODI.

5.3 Results

5.3.1 Participants

The OSA patients were significantly older (48.5 ± 13.2 yrs) than the healthy controls (28.1 ± 7.3 yrs, $P<0.05$). Additional information about the OSA patients is contained in Table 5.1.

Table 5.1: Participant characteristics for OSA patients

Variable	Mean ± SD
Age (yrs)	48.5 ± 13.2
Height (m)	1.78 ± 0.06
Mass (kg)	114.1 ± 19.2
Body Mass Index (kg/m^2)	35.8 ± 5.4
Collar size (inches)	18.1 ± 1.3
Apnoea Hypopnoea Index (events/hr)	44.0 ± 30.2
Oxygen Desaturation Index (events/hr)	35.5 ± 33.1
Clinic systolic blood pressure (mm Hg)	153.3 ± 16.5
Clinic diastolic blood pressure (mm Hg)	90.9 ± 5.3
Clinic mean arterial pressure (mm Hg)	111.7 ± 6.4

5.3.2 Blood pressure

Ambulatory BP measurements were compared between OSA patients and healthy controls. All participants completed ambulatory monitoring for a minimum of 14 hrs, incorporating measurements prior to and during sleep, and following waking. Data was analysed relative to the time of getting up reported by each individual, which was confirmed with the actigraphy data. There was a significant effect of health status ($P<0.0005$) with significantly greater mean (±SD) SBP over 14hrs in OSA (131.3 ± 11.9 mm Hg) compared with controls (111.7 ± 11.9 mm Hg). There was a significant effect of time on SBP ($P=0.019$), with lower SBP during sleep than following waking. Despite trends for greater dipping followed by a morning surge in SBP in controls compared with OSA

patients (Fig 5.1), there was no significant interaction between time and health status (P=0.145).

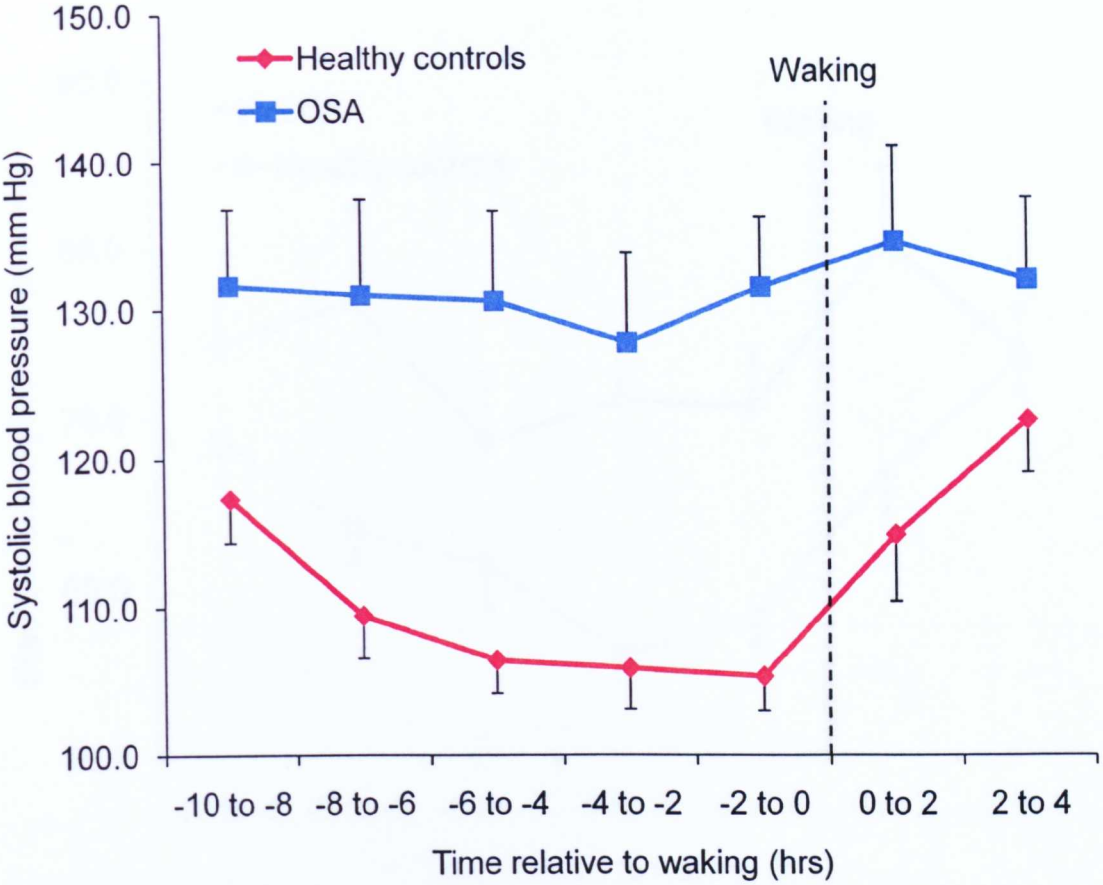

Figure 5.1: Systolic blood pressure relative to waking in OSA patients and healthy controls.

Mean (±SD) DBP was significantly greater over 14hrs in OSA patients (73.5 ± 2.0 mm Hg) compared with controls (63.9 ± 1.6 mm Hg, P<0.001). There was a significant effect of time (P<0.0005), with lower DBP during sleep than following waking, and a significant interaction between time and health status (P=0.042, Fig 5.2). Mean arterial pressure was also significantly greater in OSA patients (92.8 ± 2.4 mm Hg) compared with healthy controls (79.5 ± 1.9 mm Hg, P<0.0005), and there was a significant effect of time (P<0.001), but no significant interaction (P=0.099). Rate-pressure product was significantly greater in OSA patients (8910 ± 1266) compared with healthy controls (6915 ± 1266, P<0.0005). There was a significant effect of time (P<0.0005), with

reduced RPP at night, but no significant interaction between time and health status (P=0.96).

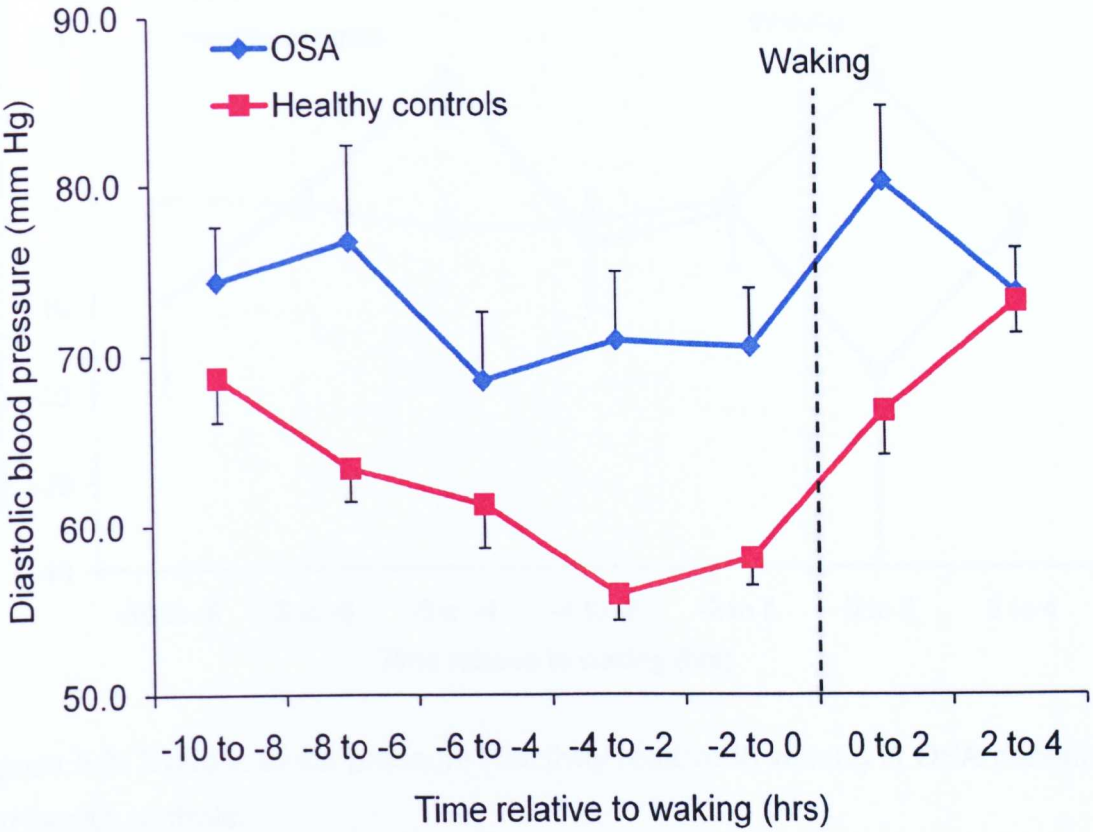

Figure 5.2: Diastolic blood pressure relative to waking in OSA patients and healthy controls.

5.3.3 Blood pressure reactivity to activity

There was no significant difference in mean SBP, DBP or MAP reactivity between healthy controls and OSA patients (P>0.05). There was, however, a significant interaction between time and health status for SBP reactivity (P<0.05). Greatest SBP reactivity in healthy controls occurred 0-2 hrs after waking, whereas peak SBP reactivity in OSA patients occurred during sleep (4-6 hrs prior to waking). Lowest SBP reactivity in OSA patients was during the first 2 hours following waking (Fig 5.3). There was a trend for greater MAP reactivity after waking in healthy controls compared to OSAS patients, but the interaction between time and health status was not significant (P=0.18) Both groups displayed similar patterns of DBP reactivity, with peak reactivity

occurring 0-2 hrs after waking. There were no significant effects of time or health status, nor interactions between them, on RPP reactivity (P<0.05).

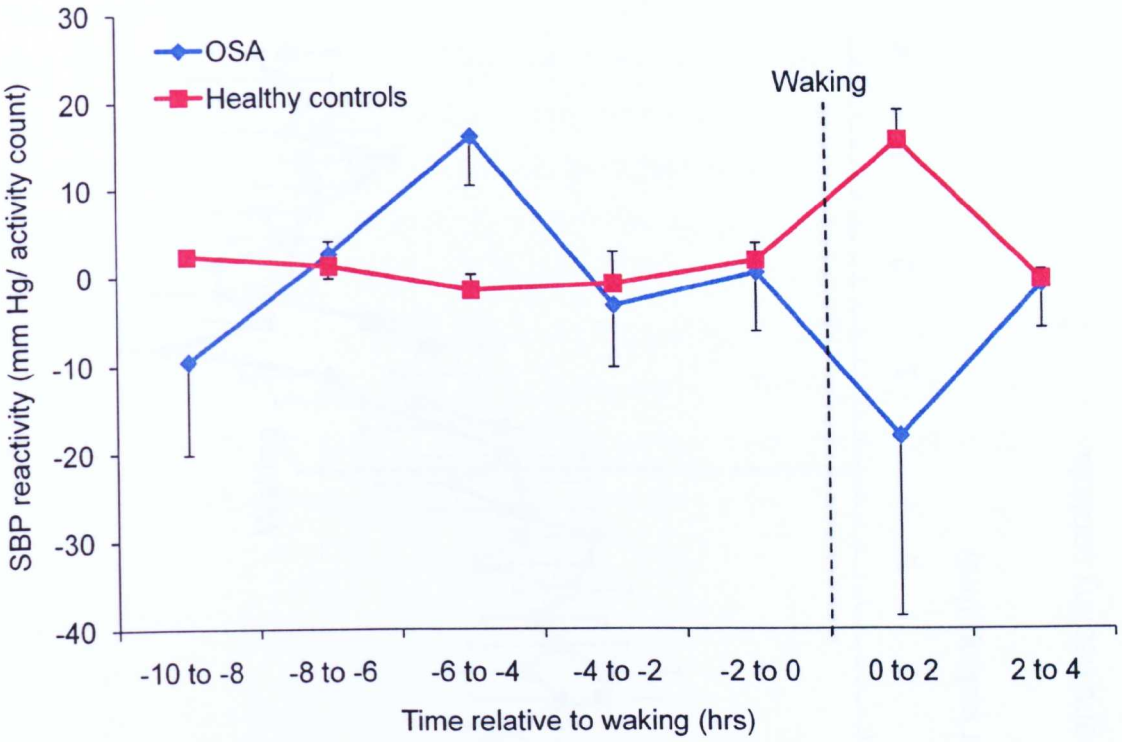

Figure 5.3: Systolic blood pressure reactivity relative to waking in OSA patients and health controls.

5.3.4 Activity monitoring in OSA and healthy controls

Actigraphy data, measured over 14 hrs (relative to time of getting up), were compared between OSA patients and healthy controls. There was no significant difference in mean activity (logged ± SD) between OSA (3.2 ± 1.0) and controls (2.8 ± 0.8, P=0.202). There was a significant effect of time (P<0.0005), with reduced activity during sleep but no significant interaction between time and health status (P=0.30, Fig 5.4).

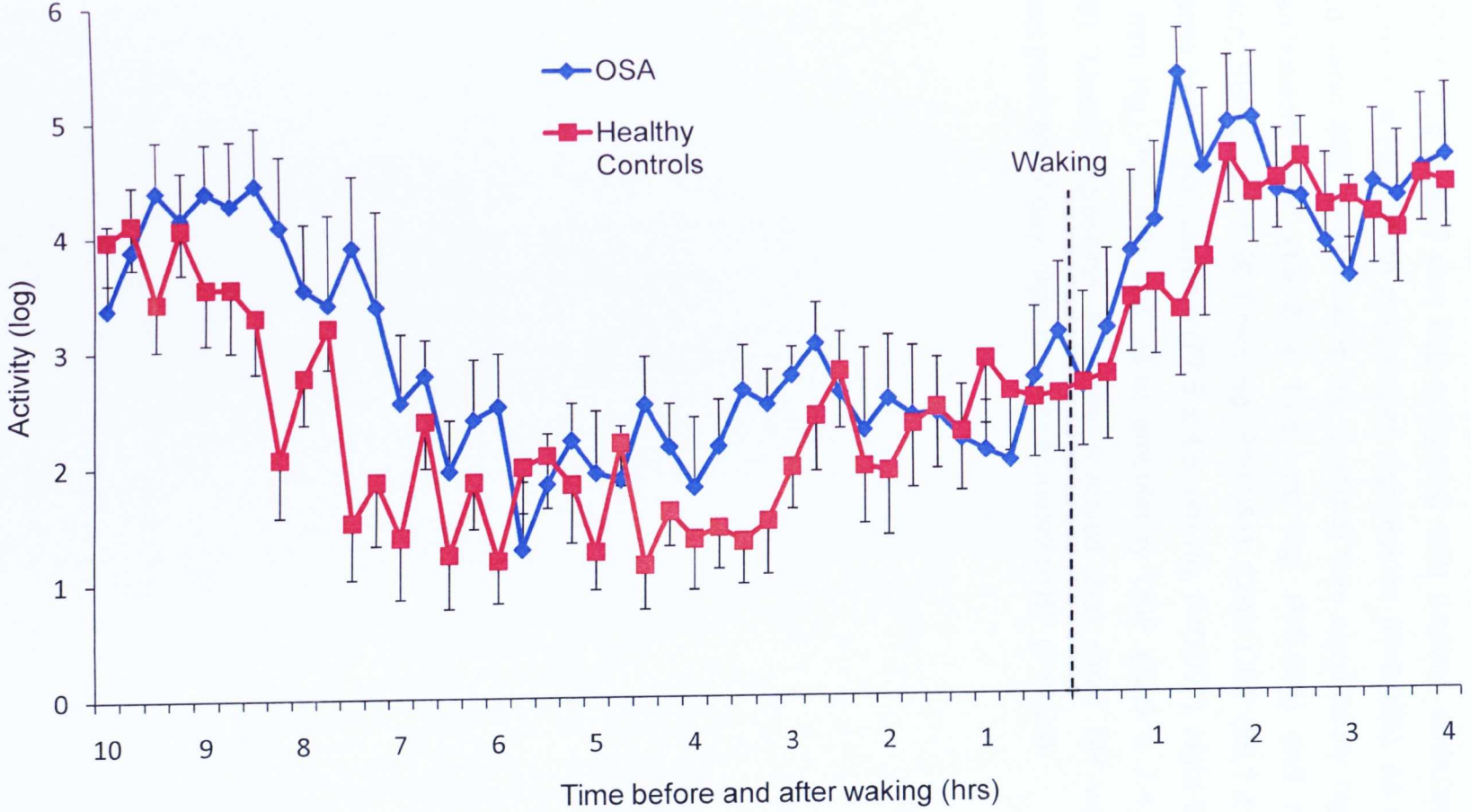

Figure 5.4: Activity (log) before and after waking in OSA patients and healthy controls.

5.3.5 Clinic blood pressure and 24-hr ambulatory monitoring in OSA

Clinic SBP and DBP measurements were compared to ambulatory BP measurements in 7 male OSA patients (Table 5.2). There was a trend for higher clinic SBP (152.6 ± 19.0 mm Hg) compared with daytime ambulatory SBP (134.8 ± 9.5 mm Hg), which approached significance (P=0.058). As would be expected, clinic SBP (measured in the daytime) was significantly higher than 24-hr ambulatory SBP (130.3 ± 13.5 mm Hg, P=0.038) and night-time ambulatory SBP (123.0 ± 20.1 mm Hg, P=0.041). Clinic DBP (88.1 ± 3.4) was significantly higher than daytime (77.5 ± 4.8 mm Hg, P=0.007), night-time (65.1 ± 10.6 mm Hg, P=0.002) and 24-hr ambulatory DBP (73.0 ± 7.4 mm Hg, P=0.003). Linear regression analyses revealed that clinic BP was not a significant predictor of day, night or 24-hr ambulatory BP (P>0.05).

Table 5.2: Clinic and ambulatory blood pressure measurements for 7 male OSA patients.

Patient diagnosis	Age (yrs)	Clinic SBP/DBP (mm Hg)	Daytime ambulatory SBP/DBP (mm Hg)*	Night-time ambulatory SBP/DBP (mm Hg)†	24-hr ambulatory SBP/DBP (mm Hg)
Mild OSA	48	154/ 91	146/ 86	137/ 72	143/ 83
Mild OSA	60	151/ 93	131/ 81	95/ 53	116/ 70
Moderate OSA	31	128/ 89	127/ 76	130/ 78	125/ 74
Severe OSA	24	148/ 82	150/ 73	155/ 77	154/ 77
Severe OSA	34	146/ 89	126/ 70	124/ 62	124/ 65
Severe OSA	58	150/ 86	131/ 69	103/ 55	120/ 63
Severe OSA	58	191/ 87	133/ 88	118/ 59	130/ 79

*10:00 – 20:00h †00:00 – 06:00h

5.3.6 Blood pressure and OSA severity

Age, BMI, collar size, AHI and ODI were entered into multiple regression analyses to determine significant predictors of clinic SBP and DBP in 11 male OSA patients. Significant predictors of clinic SBP were AHI (P=0.007) and age (P=0.038), explaining 69% of the variation in SBP. Increases in AHI of 10 events/hr and in age of 10 yrs were associated with increases in clinic SBP of 3.9 and 6.2 mm Hg, respectively. None of the variables entered into the multiple regression model were predictors of clinic DBP (P<0.05).

5.4 Discussion

The main finding of this study is that systolic blood pressure reactivity is greatest during the night in OSA, compared with healthy controls for which it peaks following waking. Absolute blood pressure was also higher in OSA over the 14-hr recording period. Secondary analyses revealed that clinic blood pressure was higher than ambulatory measurements in a subgroup of OSA patients and did not predict higher day, night or 24-hr ambulatory blood pressure. Clinic systolic blood pressure was, however, associated with age and OSA severity (apnoea-hypopnoea index).

Although there was no significant difference in the mean SBP reactivity between healthy controls and OSA patients, the timing of peak reactivity differed between groups. As hypothesised, peak SBP reactivity in OSA patients occurred during sleep, around 4-6 hrs prior to waking. Interestingly, the lowest SBP reactivity in OSA patients was during the first 2 hours following waking, the time when BP reactivity was highest in the healthy controls, and in non-OSA hypertensives (Jones et al., 2006). Greater increases in SBP for a given level of activity during the night may increase the risk of cardiovascular events at this time. Therefore, these findings may help to explain the increased risk of myocardial infarction between 00:00 and 06:00 h in OSA (Kuniyoshi et al., 2008). There were no significant differences in MAP reactivity, despite a slight trend for greater MAP reactivity after waking in healthy controls compared to OSA patients. Both groups displayed similar patterns of DBP reactivity, with peak reactivity occurring 0-2 hrs after waking, suggesting that increased risk of cardiovascular events is more likely to be associated with increases in SBP rather than DBP. The unique profile of SBP reactivity may be a useful tool for

providing information about the risk of sudden cardiac events in individuals with OSA.

Mean SBP, DBP and MAP were significantly higher in OSA patients compared to healthy controls over the 14-hr period. Healthy controls exhibited falls in BP during sleep, and although there were no significant interactions between time and SBP or MAP in this relatively small sample of OSA patients, there was a trend for reduced dipping in the OSA patients. It has previously been found that the nocturnal decline in BP is inversely related with cardiovascular mortality, independent of overall 24-hr BP and other risk factors (Ohkubo et al., 1999). Non-dipping status in individual OSA patients may heighten their cardiovascular risk above that already associated with this population. This highlights the importance of a thorough assessment for diagnosis of hypertension and may have implications for clinical practice. Suzuki et al. (1996) found that of a sample of 40 OSA patients 19 were systolic non-dippers, and that the respiratory disturbance index was a significant predictor of non-dipping. However, AHI and ODI were not significant predictors, and therefore it is important to assess 24-hr ambulatory BP in all OSA patients and not just those with high OSA severity. In the current study, a subgroup of OSA patients (n=7) completed 24-hr ambulatory BP monitoring. Mean daytime (10:00-20:00 h), night-time (00:00-06:00 h) and 24-hr ambulatory BP measurements were compared with clinic BP. These specific times were chosen in order to remain consistent with previous studies (Staessen et al., 1991, Owens et al., 1999). Transition times (06:00-10:00 h and 20:00-00:00 h) are typically not included in the day and night-time mean blood pressures because bed rest between individuals is not consistent for these periods, and cannot be used reliably (Staessen et al., 1991). In the current study clinic DBP was significantly higher than daytime ambulatory DBP, and there was a strong trend for higher clinic SBP compared to daytime ambulatory SBP. There was no significant relationship between clinic BP and day, night or 24-hr ambulatory BP, suggesting that BP readings performed in the clinic do not reflect ambulatory BP and may be subject to effects of white-coat hypertension. These findings strengthen the case for ambulatory monitoring for diagnosis of hypertension and assessment of dipping status in OSA. Measures of absolute BP and BP

reactivity may provide further information regarding cardiovascular risk in individual patients.

Despite the differences in clinic and ambulatory BP, there were significant relationships between clinic SBP, age and OSA severity. Increases in AHI of 10 events/hr and in age of 10 yrs were associated with increases in clinic SBP of 3.9 and 6.2 mm Hg, respectively. Other variables, including BMI, collar size, and ODI were not significant predictors of BP, although a larger sample size, including both genders and a greater variance amongst independent variables may allow important predictors of BP to be identified.

Rate-pressure product (RPP) is another measure that can provide useful information regarding cardiovascular risk (Forjaz et al., 1998a). It is the product of SBP and HR, and there is significant evidence to suggest that it is associated with myocardial oxygen consumption (Kitamura et al., 1972, Gobel et al., 1978). Rate-pressure product was significantly greater in OSA patients (8910 ± 1266) compared with healthy controls (6915 + 1266, $P<0.0005$). Both groups exhibited reduced RPP during sleep but the pattern was shifted upwards in OSA patients. Cardiovascular risk increases with elevated RPP (Robinson, 1967, Dentry et al., 1970), thus providing further evidence of increased cardiovascular risk in the OSA population.

It may be speculated that there are increases in activity at night in OSA due to frequent sleep disturbances. However, there was no significant difference in activity between OSA and controls in the current study, which may suggest that reduced nocturnal dipping is due to the greater BP reactivity and/or an endogenous component. However, the 15-min means used in the current study may have masked short bursts of activity so that no differences were identified between the groups. Activity during sleep, measured using actigraphy, is commonly used to assist the diagnosis of sleep and circadian rhythm disorders and is recognised by the American Academy of Sleep Medicine as a useful adjunct for clinical assessment (Morgenthaler et al., 2007). However, activity tends to be assessed over short epochs (e.g. 30 sec) and a validated algorithm is used to determine whether the patient is asleep or awake for each epoch so that total sleep time can be estimated (Oakley, 1997). The measurement of BP

within in this time frame for assessment of reactivity would require more invasive techniques, and it is unclear whether actigraphy would detect the majority of apneic events which lead to the increases in BP.

A key limitation of the current study is the use of a young healthy control group, rather than a group of hypertensives matched for characteristics such as age, gender and BMI. However, the BP reactivity profile found in the healthy control group is comparable to that previously identified in hypertensives (Jones et al., 2006). The study was also limited by the number of BP measurements taken during sleep, which were reduced to minimise sleep disturbances, meaning that BP reactivity slopes calculated for the sleep period contained fewer data points that those before 23:00 h and after 06:00 h. It is therefore possible that the BP reactivity values for sleep may have been more susceptible to the influence of outliers. However, the consistency of the control group reactivity profile to previous studies, where measurements were taken every 20-mins during sleep (Jones et al., 2006), indicates that the method was robust.

5.5 Conclusion

Unlike healthy controls and hypertensive patients, OSA patients show a unique profile of BP reactivity, with a peak occurring during sleep. This may explain, in part, the higher nocturnal blood pressures and incidence of myocardial infarction during the night in this population. Given the discrepancies between clinic and ambulatory measurements of BP, the present study strengthens the argument for ambulatory monitoring for all OSA patients. The methods used for determining BP reactivity profiles may provide useful information regarding risk of sudden cardiac events in individual OSA patients.

Chapter 6

Study 4

Chronic effects of leisure-time physical activity on blood pressure and symptoms of obstructive sleep apnoea

6.1 Introduction

The prevalence of obstructive sleep apnoea (OSA) is rapidly increasing, and yet it has been estimated that as many as 80-90% of OSA cases are undiagnosed (Young et al., 1997). Although knowledge of the condition has grown over the years, many OSA patients still go untreated (Silverberg et al., 2002). The condition, which is characterised by frequent episodes of upper airway collapse during sleep, is an independent risk factor for hypertension (Lavie et al., 2000, Nieto et al., 2000). Obstructive sleep apnoea has also been associated with increased risk of stroke, cardiac arrhythmias, and heart failure (Parish and Somers, 2004). Sleep disturbance due to frequent apnoeic and hypopneic episodes causes other symptoms, such as severe sleepiness and fatigue during the day, thus reducing quality of life (Silverberg et al., 2002). These symptoms also increase the probability of long-term sick leave (Sivertsen et al., 2008) and the risk of motor vehicle accidents (Barbe et al., 1998, Teran-Santos et al., 1999). Results of previous studies suggest that the problems of BP control and daytime sleepiness may be connected and that severe sleepiness in OSA patients increases the risk of developing hypertension (Kapur et al., 2008).

Successful interventions for reducing OSA severity and improving sleep quality have typically included soft tissue surgery (such as uvulopalatopharyngoplasty) (Riley et al., 2000), weight-loss surgery (Charuzi et al., 1992, Pillar et al., 1994) or dietary interventions (Harman et al., 1982, Sampol et al., 1998, Kajaste et al., 2004). However, exercise training has also been proven to be beneficial, both as a separate treatment (Norman et al., 2000) and as an adjunct therapy to CPAP (Giebelhaus et al., 2000). Given the wealth of evidence relating OSA with hypertension, it is important to examine the effects of treatments on blood pressure to establish the most beneficial therapies for reducing hypertension and therefore the risk of cardiovascular events. However, the efficacy of exercise as a treatment for hypertension and cardiovascular risk in the OSA population warrants further investigation.

Successful treatment of daytime sleepiness in OSA patients is vital, given the risks associated with road accidents and also the severe effect it can have on quality of life. The relationships between OSA severity and symptoms of daytime sleepiness and fatigue have been investigated previously (Aguillard et

al., 1998, Hong and Dimsdale, 2003). However, findings of these studies have been inconsistent, with some researchers suggesting that daytime sleepiness is correlated with the AHI, and others suggesting that it is related to fitness and physical activity rather than OSA severity. These discrepancies may be due to the definitions used to describe daytime sleepiness and fatigue and the techniques employed to measure them. It is likely that results of multiple sleep latency tests, sleepiness questionnaires and measures of physical and subjective fatigue during exercise describe distinctively different problems, all of which may be associated with OSA. Although study results suggest that chronic physical activity may have an important part to play in lessening the symptoms of OSA, there are certain limitations to previous investigations, including sample size and the lack of control for confounding factors, which may have led to important relationships going undetected. A recent study by Basta et al. (2008) addressed this problem and found significant effects of regular exercise on daytime sleepiness in OSA whilst controlling for a range of patient characteristics. However the study lacked comparisons of energy expenditure between OSA and the general population and did not report the effects of physical activity on OSA severity (AHI) or BP. The aim of the current study is to investigate the chronic effects of leisure-time physical activity on BP, OSA severity and daytime sleepiness. It is hypothesised that greater leisure-time activity is associated with lower BP and less daytime sleepiness in OSA, independent of potentially confounding factors such as BMI, age and gender.

6.2 Methods

6.2.1 Participants

Ninety-six patients (78 males) attending the Liverpool Sleep Clinic and 118 healthy controls (77 males) took part in the study. Participant characteristics of the two groups are given in Table 1. The OSA group consisted of patients with mild (n=25), moderate (n=26) and severe (n=45) OSA, with AHI ranging from 5.1 to 113. The study was given NHS ethical approval and all participants gave their written informed consent before taking part.

6.2.2 Measurements

A leisure-time physical activity questionnaire, adapted from a standard questionnaire developed by Lamb and Brodie (1990), was used to estimate values of energy expenditure over a 2-week period in OSA patients and healthy controls. Using an extensive list of leisure-time activities patients were asked to report the time spent performing physical activity during the previous two weeks. These data were then converted into metabolic equivalents (METs) where 1 MET is the energy expended by sitting quietly and equates to 4.2KJ/kg/hour/m^2 (McKeag and Moeller, 2007) from which the number of total KJ over the fortnight were calculated for each participant. Participant characteristics of age, gender, BMI, SBP, DBP and MAP were recorded. For OSA patients, the apnoea-hypopnoea index (AHI) and oxygen desaturation index (ODI) were recorded in order to determine the severity of the sleep apnoea. The Epworth Sleepiness Scale (ESS) questionnaire was also completed by each OSA patient. This is a measure of the probability of falling asleep in certain situations and is quantified on a scale of 0-24, with higher values indicating a greater chance of sleep during the daytime (Johns, 1993).

6.2.3 Grouping of participants – energy expenditure

Participants were divided into groups according to estimated leisure-time energy expenditure. In order to promote and maintain health, it has been recommended that adults undertake a minimum of 30 min moderate exercise per day for 5 days a week (Haskell et al., 2007). This amounts to approximately 840 KJ per day (Pate et al., 1995) and therefore a minimum leisure-time energy expenditure of 4200 KJ per week is recommended. Participants were divided into 3 groups. Due to the large number of patients reporting physical activity levels of above the 8400 KJ per fortnight recommendation these participants were divided into 2 groups using a median split. Therefore the patients were assigned to the following physical activity groups: low (<8400 KJ, n=37); moderate (8400 to 23,100 KJ, n=30); and high physical activity (≥23,100 KJ, n=29). The control group was divided according to the same energy expenditures and therefore sample sizes for the three groups above were 35, 41 and 42, respectively.

6.2.4 Statistical Analyses

Multivariate general linear models were performed with physical activity group as a fixed factor and age, BMI and gender as covariates. The first analysis included OSA patients and controls. The dependent variables entered were SBP, DBP and MAP, with physical activity and health status (i.e. OSA or healthy control) as fixed factors, in order to assess the relationships between BP, physical activity and health status. For the second analysis, involving OSA patients only, AHI, ODI and ESS were entered as dependent variables to investigate the relationships between physical activity, OSA severity and daytime sleepiness. Linear regression analysis was used to determine the slopes between continuous covariates and dependent variables.

6.3 Results

6.3.1 Participants

Table 6.1 provides patient characteristics for OSA patients and healthy controls. Measures of OSA severity (AHI and ODI) and daytime sleepiness are available for OSA patients only. Blood pressure (SBP, DBP and MAP) was significantly higher in the OSA group ($P<0.0005$). The mean age and BMI of the OSA patients were significantly greater than healthy controls ($P<0.0005$), and the proportion of females within the group was lower. This highlights the need to control for these characteristics when investigating the effects of health status on blood pressure, and therefore the following analyses include covariates of age, BMI and gender where appropriate. There was no significant difference in mean fortnightly energy expenditure between OSA and controls ($P<0.05$).

Table 6.1: Participant characteristics for OSA patients and healthy controls.

Variable	OSA patients (n=96)	Healthy controls (n=118)
Age (years)	51 ± 10	37.9 ± 11.7†
Gender (male/female)	78/18	77/41
Body Mass Index (kg/m2)	35.9 ± 7.64	23.5 ± 2.3†
Systolic blood pressure (mm Hg)	141.2 ± 17.8*	123.1 ± 14.3†
Diastolic blood pressure (mm Hg)	88.3 ± 11.7*	78.9 ± 9.0†
Mean arterial pressure (mm Hg)	105.9 ± 12.0*	93.7 ± 10.0†
Estimated energy expenditure (KJ/fortnight)	18,126 ± 19,761	17,118 ± 14,972
Apnoea-hypopnoea index (events/hr)	36.2 ± 26.9	-
Oxygen Desaturation Index (events/hr)	30.4 ± 30.1	-
Epworth Sleepiness Scale (0-24)	10.9 ± 5.6	-

* n=74; † significantly lower than OSA (P<0.0005)

6.3.2 Blood pressure and OSA

There was a significant effect of health status on SBP, DBP and MAP ($P<0.0005$), with significantly higher blood pressures in the OSA patients compared with healthy controls while controlling for age and gender (Figure 6.1). There was no significant effect of physical activity group on SBP, DBP and MAP ($P>0.05$). There was, however, a significant effect of age on blood pressure ($P<0.005$) and linear regression analysis revealed that for every 1-yr increase in age, SBP, DBP and MAP increased by 0.7, 0.3 and 0.4 mmHg, respectively. There was a significant effect of gender with lower SBP and MAP in females ($P<0.05$), although no effect of gender was found for DBP ($P=0.183$).

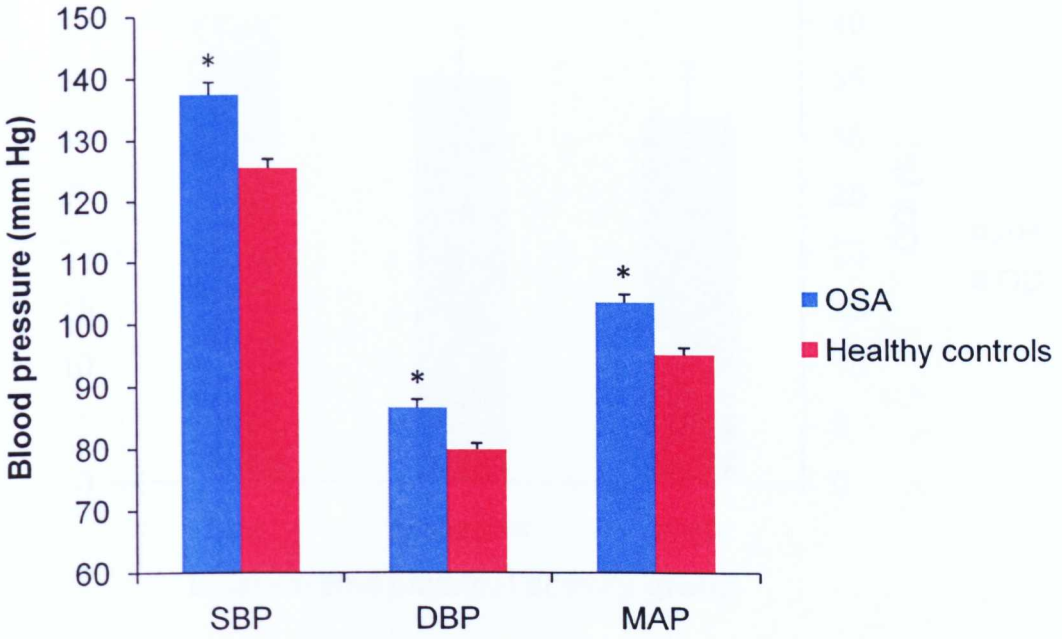

Figure 6.1: Systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) in obstructive sleep apnoea (OSA) patients versus healthy controls

6.3.3 Physical activity and OSA severity

The AHI and ODI provide quantitative measures of OSA severity. There was no significant effect of physical activity group on AHI (P=0.366) or ODI (P=0.140), despite trends for reduced OSA severity with increasing energy expenditure. There was, however, a significant difference of 13.8 (±7.0%) in ODI between the low physical activity and high physical activity groups (P=0.05, Figure 6.2). There was a significant effect of BMI on AHI and ODI (P<0.0005) and linear regression analysis revealed that for every 1 kg/m^2 increase in BMI, AHI and ODI increased by 1.5 events/hr and 1.7 events/hr respectively. However, there was no significant difference in BMI between the three physical activity groups (p<0.05).

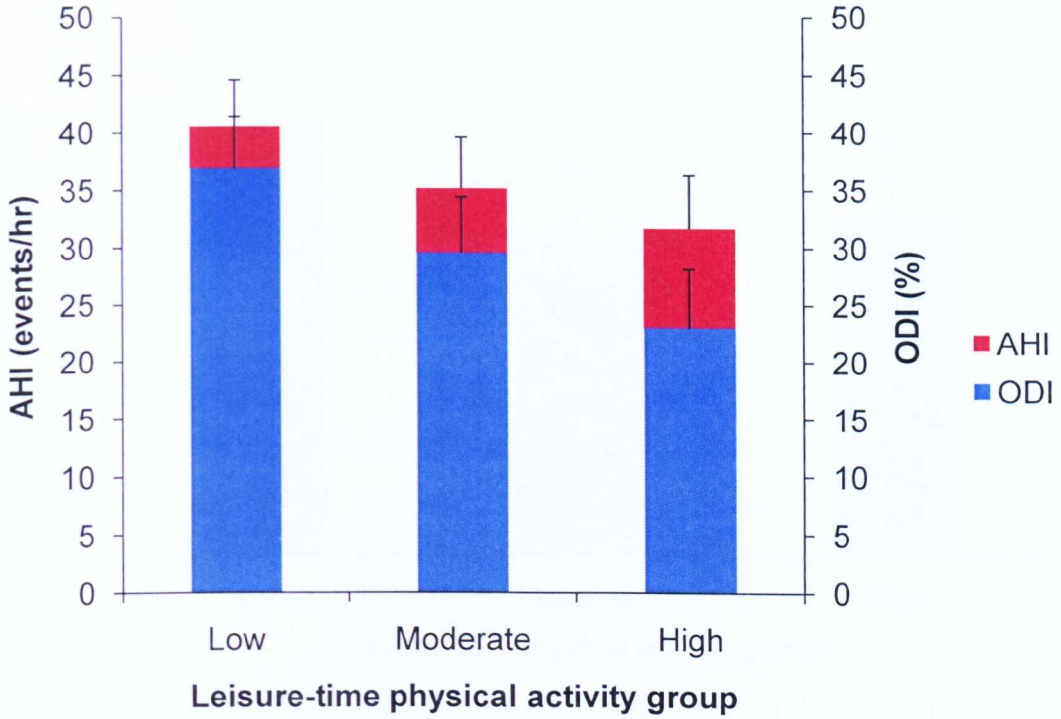

Figure 6.2: Apnoea-hypopnoea index (AHI) and oxygen desaturation index (ODI) across the 3 physical activity groups, *Significantly different from the low activity group (P<0.05)

6.3.4 Physical activity and daytime sleepiness

The ESS was used as a measure of subjective daytime sleepiness. There was a significant effect of physical activity on daytime sleepiness (P=0.019), with significantly lower ESS scores (±SD) in the high activity group (10.9 ±5.8) and moderate activity group (9.2 ±4.4) compared with the low activity group (12.9 ±5.8, P<0.05) (Figure 6.3). Daytime sleepiness was not significantly different between the moderate and high physical activity groups (P=0.512). However, linear regression analysis indicated that AHI was a significant predictor of daytime sleepiness (r =0.30, P=0.003), although an increase of 0.06 in ESS score for every unit increase in AHI would not be considered clinically significant.

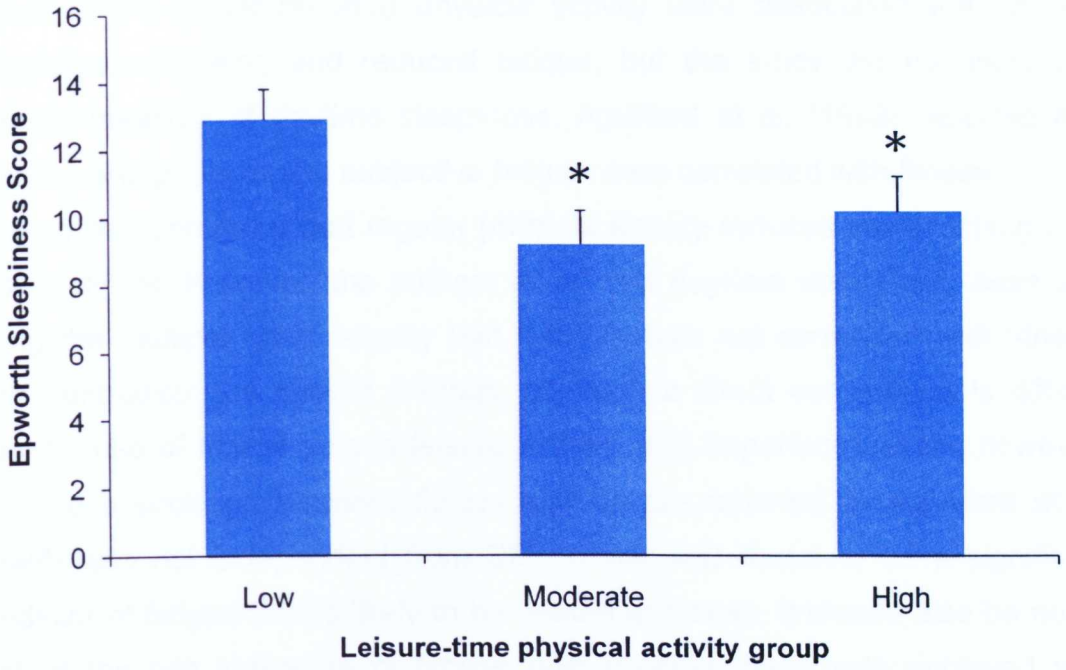

Figure 6.3: Daytime sleepiness (Epworth Sleepiness Score) across the 3 physical activity groups, *Significantly different from low activity group ($P<0.05$).

6.4 Discussion

Obstructive sleep apnoea was associated with higher systolic, diastolic and mean arterial pressure compared with healthy controls, when adjusted for age and gender. However, levels of leisure-time physical activity had no effect on blood pressure in either OSA patients or healthy controls. Physical activity also had no effect on AHI, although significant differences were found in ODI between the low activity and high activity groups. Physical activity had a significant effect on Epworth Sleepiness Score, with reduced daytime sleepiness in the two more active groups compared with the low activity group. Therefore, the main finding of this study is that increases in leisure-time physical activity may reduce key symptoms of OSA, but are less successful in reducing blood pressure or OSA severity independent of changes in BMI, age and gender.

Obstructive sleep apnoea patients whose energy expenditure equated to less than the recommended amount of physical activity (8600 KJ/fortnight) suffered from significantly more daytime sleepiness than those performing the recommended amount or above. Hong and Dimsdale (2003) reported that

higher levels of self-reported physical activity were associated with greater subjective well-being and reduced fatigue, but the study did not include a specific measure of daytime sleepiness. Aguillard et al. (1998) reported that measures of physical and subjective fatigue were correlated with fitness, and so it could be speculated that regular physical activity reduces daytime fatigue in OSA patients. However, the authors found that daytime sleepiness, assessed using the multiple sleep latency test (MSLT), was not correlated with fitness. This contradicts the current findings, although a direct comparison is difficult due the use of fitness versus leisure activity. It is important to note, however, that the association between fitness and fatigue reported by Aguillard et al. (1998) was not independent from BMI, which was found to be a significant predictor of fatigue and is likely to be related to fitness. It should also be noted that of the two indicators of fitness only maximal heart rate achieved was correlated with fatigue. Maximal oxygen uptake, a standard measure of fitness, was not correlated with fatigue. In the current study covariates were included in the analyses and therefore the significant effect of physical activity on daytime sleepiness was independent of BMI and also gender. Basta et al. (2008) investigated the effects of physical activity on daytime sleepiness in OSA. Similar to the present study, a physical activity questionnaire was used to estimate energy expenditure. However, in contrast with the current study participants were asked to estimate the average time spent per week on particular activities. It may be speculated that this method leads to an over-estimation of energy expenditure reported by the individual patients. In the current study patients were questioned on their physical activity retrospectively, giving details of activities performed in the previous two weeks. It is likely that this approach may provide a more accurate representation of actual physical activity levels. Despite these differences in data collection, the current findings are consistent with those of Basta et al. (2008) who found that weekly energy expenditure was associated with daytime sleepiness when adjusted for patient characteristics. However, caution must be taken when drawing conclusions, because the relationship between physical activity and sleepiness could be in either direction. It is possible that individuals with naturally higher daytime sleepiness find it more difficult to be physically active.

There have been inconsistencies in the literature as to whether OSA severity is correlated with physical activity. In the present study we found no significant effects of self-reported physical activity on AHI, a finding that supports those of Hong and Dimsdale (2003) who reported no correlation between respiratory disturbance index and self-reported habitual activity. However, Peppard and Young (2004) reported that performing 3-6 hrs of exercise per week reduced the odds ratio (95%CI) for having an AHI of over 15 (moderate OSA) to 0.39 (0.19-0.80) when compared to 0 hrs of exercise. Despite no effects of physical activity on AHI in the current study, there was a significant reduction in ODI in the most physically active group when compared with the least active group. This would suggest that leisure-time physical activity may have some benefits in reducing OSA severity, even if it is limited to reducing the level of oxygen desaturation during sleep rather than the number of apneoas and hypopnoeas.

Body mass index was correlated with AHI and ODI. A reduction of 1 kg/m^2 in BMI was associated with a reduction in AHI and ODI of 1.5 events/hr and 1.7%, respectively. Although leisure-time physical activity was not directly associated with AHI it may reduce the number of apnoeas indirectly via weight loss. In the current study there was no significant difference in BMI between physical activity groups, although there are other factors not measured such as energy intake and work-related physical activity that may account for this. Previous studies have found that weight loss can cause substantial reductions in OSA severity, sometimes reducing AHI to normal levels of <5 events/hr (Sampol et al., 1998). Many weight-loss interventions previously studied have involved surgical procedures (Charuzi et al., 1992, Pillar et al., 1994, Valencia-Flores et al., 2004). Not only are these forms of treatment costly, but they often provide only short-term solutions. Despite large initial reductions in AHI in 14 OSA patients following surgery, Pillar et al. (1994) found significant increases in AHI after only 5 to 10-yr follow-ups. Therefore lifestyle interventions such as physical activity may be crucial in providing more long-term reductions in OSA severity and symptoms. Exercise training interventions have been shown to reduce OSA severity, both in exercise-only interventions (Norman et al., 2000) and as adjunct therapy to continuous positive airway pressure (CPAP) (Giebelhaus et al., 2000). The promotion of mild-to-moderate intensity leisure-time activities may be a suitable treatment option for OSA patients. In the

current study mean changes (95%CI) in ESS scores between the more physically active groups compared with the least active group were -3.7 (-6.3 to -1.0) and -2.7 (-5.5 to 0.1). These differences in daytime sleepiness between activity groups compare with mean changes (95%CI) found for parallel-group studies, -3.8 (-4.6 to -3.1), and crossover designs, -1.8 (-2.6 to -1.1), investigating the effects of continuous positive airway pressure (CPAP) (Giles et al., 2006). This is the current treatment for OSA, which has been reported to be successful in the reduction of AHI (Giles et al., 2006). There is also strong evidence for improvement of BP control and diurnal BP profiles with CPAP (Wilcox et al., 1993, Akashiba et al., 1999, Logan et al., 2003, Haentjens et al., 2007), particularly in OSA patients suffering from excessive daytime sleepiness (Robinson et al., 2006). However, compliance with this treatment is often poor (Engleman et al., 1994), and therefore physical activity may provide a useful alternative or adjunct therapy for CPAP. Lifestyle interventions involving weight loss via low calorie diets have been found to reduce AHI and improve sleep quality in patients with mild OSA (Tuomilehto et al., 2009), and therefore weight loss via physical activity may provide a suitable treatment for OSA patients for whom surgery is deemed unnecessary.

In the current study greater leisure-time physical activity levels had no significant effect on SBP, DBP or MAP. In an experimental study of weight loss in OSA patients Tuomilehto et al. (2009) reported minor reductions in BP. Although the mean changes were not significantly different from baseline, there were BP reductions in a number of OSA patients substantial enough to allow them to discontinue anti-hypertensive treatment following the trial. The lack of direct relationships between BP and physical activity may be due to limitations in the BP measurements, which in the current study were taken in the sleep clinic. In some individuals clinic BP is exaggerated from normal ambulatory values, a phenomenon known as white-coat hypertension (Pickering et al., 1988). More reliable BP values for the diagnosis of hypertension may be obtained from 24-hr ambulatory recordings of BP (Mancia, 1990). Further research is needed to determine the effects of chronic physical activity on ambulatory BP in OSA patients. Other methodological considerations for the current study include the use of self-reported activity surveys, the accuracy of which may be questioned. More direct measures of physical activity, such as

accelerometry, may have provided a more reliable measure. The validity of the Epworth Sleepiness Scale has also been investigated, in terms of its ability to reflect objective measures of sleepiness. According to Chervin et al. (2000) the ESS was significantly associated with self-rated problem sleepiness but not with objective sleepiness measured via the Multiple Sleep Latency Test (MSLT) or OSA severity (AHI). However, previous validation studies have found significant correlations between ESS, AHI and MSLT scores (Johns, 1993). Although linear regression analysis in the present study indicated that AHI was a significant predictor of ESS, the gradient of the slope was very small and therefore was unlikely to be of clinical relevance. Despite discrepancies in the literature, the ESS provides a simpler and less time-consuming alternative to the MSLT, and continues to be used regularly in both research and clinical settings. Finally, caution must be taken when describing relationships between variables, because cause and effect cannot be assumed with the current study design. For example, in present study patients with low activity levels on average suffer from greater daytime sleepiness. It may be concluded that physical activity has a role to play in reducing daytime sleepiness. However, it also possible that daytime sleepiness causes individuals to be less active and therefore the causal relationships require clarification with experimental designs.

6.5 Conclusion

The current study provides further evidence that OSA is associated with hypertension, independent of age and gender. Leisure-time physical activity was associated with reduced daytime sleepiness and ODI following adjustments for patient characteristics. Although physical activity was not associated with reductions in AHI or BP, exercise interventions may reduce these indirectly via changes in BMI. Therefore, interventions focusing on leisure-time activities may provide useful alternative or adjunct therapy to traditional CPAP treatment. The effects leisure-time physical activity on OSA severity, symptoms and BP in combination with CPAP requires further investigation via experimental methods.

Chapter 7

Study 5

Diurnal variation in the mechanical and neural components of the cardiac baroreflex

This work has been accepted for publication in the July issue of Hypertension, 2011.

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Chapter 8

Study 6

Postural influences on diurnal variation in baroreflex gain

8.1 Introduction

The baroreflex is a key mechanism for blood pressure (BP) homeostasis (Benarroch, 2008). It has previously been shown that cardiac baroreflex function varies with time of day (Hossmann et al., 1980, Conway et al., 1983, Parati et al., 1988) and that diminished integrated gain in the morning can be attributed to changes in the mechanical or neural component depending on whether BP is rising or falling, respectively (chapter 7). These diurnal effects have been described when participants are in the supine position. However, there are many activities in day-to-day living that produce physiological challenges and that have been associated with changes in baroreflex function, such as exercise (Willie et al., 2011) and the assumption of an upright posture (O'Leary et al., 2003). The risk of vasovagal syncope is greatly increased in the morning (Mineda et al., 2000, Zoghi et al., 2008), which may be associated with insufficient baroreflex function to maintain adequate BP during orthostatic stress (Cooper and Hainsworth, 2002). Despite evidence for diurnal variation in orthostatic tolerance (Lewis et al., 2010), there is little information on the alterations in baroreflex function with postural changes in relation to time of day.

A number of studies have been performed to investigate the effects of orthostatic stress on baroreflex function. The current consensus is that orthostatic stress augments vascular sympathetic baroreflex sensitivity (O'Leary et al., 2003) and reduces cardiac baroreflex sensitivity (O'Leary et al., 2003, Hughson et al., 1994, Taylor and Eckberg, 1996, Jasson et al., 1997, Iellamo et al., 1996, Pickering et al., 1971, Saeed et al., 2009). However, other studies report either an increase (Pawelczyk and Raven, 1989), or no change in the cardio-vagal arm of the baroreflex (Cooper and Hainsworth, 2002). These discrepant findings may be attributed to methodological differences such as the use of spontaneous techniques versus more invasive neck suction and pharmacological methods (O'Leary et al., 2003). Following a controlled incremental orthostatic challenge to presyncope (60° HUT with 5-min incremental stages of lower body negative pressure) Lewis et al. (2010) found that reductions in baroreflex function from baseline, assessed via spontaneous methods (alpha index), were not significant between morning and afternoon trials. However, the rate of decline was significantly greater in the morning (-0.87 ± 1.07) than in the afternoon (-0.16 ± 0.35 ms/mm $Hg^{-1}min^{-1}$), as was the

time to presyncope consistent with the observation that baroreflex gain is diminished in the morning. However, time of day differences in baroreflex function with changes in posture from supine to standing have not been investigated using the gold standard Oxford method, which in contrast to spontaneous baroreflex indices, enable the robust assessment of cardiac baroreflex function under near open-loop conditions.

Therefore, the first aim of this study is to use the modified Oxford method to determine the combined influences of posture and time of day on cardiac baroreflex function. Previous findings (chapter 8) indicate diminished integrated gain in the morning compared to afternoon whilst supine. It is hypothesized that integrated gain is reduced with standing compared to supine, and that these posture-induced reductions are greater in the morning. Therefore, the diurnal variation in gain previously observed when supine is accentuated for standing posture. The second aim is to investigate the contribution of mechanical and neural components to changes in baroreflex gain in order to unravel the mechanism responsible. Although this research question has been addressed previously (Saeed et al., 2009), the validity of the closed-loop-spontaneous transfer function method used has recently been under question (Kamiya et al., 2011). Given that diminished baroreflex gain with orthostatic stress is thought to involve parasympathetic nervous system withdrawal resulting in a reduction of available vagal nerve activity with which to regulate heart rate (Hughson et al., 1994), it is hypothesized that changes in integrated gain with standing are due to alterations in the neural component.

8.2 Methods

8.2.1 Participants

Six healthy subjects (4 males, 2 females) with a mean ± SD age of 24.5 ± 4.3 (range: 21-33 yr) and body mass index of 21.5 ± 2.6 kg/m^2 were recruited for the study. The participants were a sub-sample of those who participated in the previous study of diurnal variation in baroreflex gain (chapter 7).

8.2.2 Measurements

Beat-to-beat BP via photoplethysmography (Finometer, TNO-TPD Biomedical Instrumentation) and electrocardiogram (ECG lead CM5, Corometrics Neo-Trak

502) were recorded non-invasively. To account for potential drift, finger blood pressure measures were verified at the brachial artery in the contralateral arm by sphygmomanometry. These measures were acquired continuously via an analog-to-digital converter (Powerlab/16SP ML795; ADInstruments, Colorado Springs, CO, USA) at 200 Hz per channel. Analysis was performed offline using the arterial BP and ECG waveforms to determine the timing of the R waves and beat-to-beat values for systolic (SBP), diastolic (DBP) and mean arterial pressure (MAP). Ultrasound imaging (Terason t3000, Burlington, MA, USA) was used to measure beat-to-beat carotid artery diameter, which was analysed using custom edge tracking software as has been previously described. All off-line data processing was performed using custom written software in LabView 8.2 (National Instruments, Texas, USA) on a Macintosh 2.26 GHz MacBook Pro computer.

8.2.3 Experimental Protocol

All trials were completed in a temperature-controlled laboratory (22-23°C). A venous cannula was inserted into the right anticubita vein and, following a 15-minute stabilization period in the supine position, baseline data were recorded for 5 minutes. Participants then underwent baroreflex testing using the modified Oxford method technique, completing a supine and a standing trial separated by a minimum of 15 minutes. Participants completed this protocol at two times of day: morning (0700h) and afternoon (1600h). Testing sessions were separated by a minimum of 48 hours and completed in a counterbalanced fashion. For the morning trial a standardized carbohydrate meal was consumed at 0500h and for the afternoon trial two identical meals were consumed at 0500h and 1400h in order to keep dietary intake as consistent as possible between testing sessions.

Oxford trials were repeated until a valid trial was completed, i.e. the drop and rise in blood pressure were >15 mmHg relative to baseline levels. Doses given for SNP and PE were typically 150 and 250μg, respectively, although SNP was reduced for standing trials to approximately 100μg depending on the individual's mass and previous responses to the drugs. Integrated, mechanical and neural baroreflex gains were determined using custom-written software (LabVIEW 8.2, National Instruments, Texas, USA) as described in chapter 2.3.

8.2.4 Statistical Analysis

Linear mixed models, as described in chapter 2.4, were used to compare baroreflex gains between supine and standing trials. Values are means ± SE unless otherwise stated. All data were analyzed using SPSS 17 (SPSS, Chicago, IL).

8.3 Results

8.3.1 Participants

All participants completed at least one supine and standing Oxford trial in both the morning and afternoon. Following an assessment of the quality of the recordings the data for one female was excluded, and therefore analyses of integrated baroreflex gain were based on n=5 (4 males, 1 female). Due to difficulties in obtaining carotid diameter measurements during the standing trials, the mechanical and neural gains for some participants were excluded, limiting the sample sizes for the secondary analyses to n=4 (G_{down} morning trials); n=3 (G_{down} afternoon trials); and n=4 (G_{up} afternoon trials).

8.3.2 Baseline cardiovascular variables

Table 8.1 shows average resting values over a 5-minute baseline period for supine and standing trials in both the morning and afternoon. There was no significant diurnal variation in baseline HR, SBP, DBP or MAP. The diurnal variation in carotid diameter found in Chapter 7 during supine rest remained significant in the current study (P=0.037). Baseline measures of HR and DBP were significantly greater during standing compared with supine posture (P<0.05), with trends for increased SBP (P=0.13) and MAP (P=0.064). Conversely, carotid diameter was significantly reduced with standing compared to supine; trends that were consistent at both times of day.

Table 8.1: Summary of baseline cardiovascular variables for supine and standing postures in the morning and afternoon (n=5).

Variable	Morning		Afternoon	
	Supine	Standing	Supine	Standing
Heart rate (beats/min)	62.4 ± 13.6	80.5 ± 17.1*	59.6 ± 14.3	78.5 ± 17.8*
Systolic blood pressure (mm Hg)	118.8 ± 14.7	137.0 ± 21.9	127.9 ± 23.3	137.2 ± 23.6
Diastolic blood pressure (mm Hg)	57.9 ± 5.3	76.3 ± 21.5*	61.9 ± 8.6	77.1 ± 10.9*
Mean arterial pressure (mm Hg)	76.3 ± 6.3	93.3 ± 21.9	79.8 ± 10.6	94.9 ± 14.5
Carotid Diameter (mm)	5.68 ± 0.50	5.33 ± 0.57*	5.85 ± 0.63†	5.39 ± 0.80*

* $P < 0.05$ vs. Supine; † $P<0.05$ vs. Morning

8.3.3 Postural influences on diurnal variation in integrated baroreflex gain

Table 8.2 shows the integrated G_{up} and G_{down} for supine and standing postures at both times of day. There was significant diurnal variation in integrated gain for G_{down} and G_{up} when participants were in a supine position ($P<0.05$). The assumption of an upright posture caused significant reductions in G_{down} and G_{up} ($P<0.05$). For G_{down} the greatest reductions were observed in the afternoon, falling from 14.3 ± 0.7 ms/mm Hg (supine) to 5.4 ± 0.4 ms/mm Hg (standing). In the morning G_{down} was reduced from 6.2 ± 0.5 ms/mm Hg (supine) to 4.9 ± 0.4 ms/mm Hg (standing), which although significantly different from each other ($P=0.04$) eliminated the diurnal variation in G_{down} whilst standing ($P= 0.42$, Figure 8.1). Although the diurnal variation was still highly significant for G_{up} in the standing position ($P<0.0005$, Figure 8.2), the difference between morning

and afternoon was reduced from 5.6 ± 1.3 (supine) to 2.7 ± 0.5 ms/mm Hg (standing).

Table 8.2: Integrated baroreflex gains and correlation coefficients for supine and standing trials in the morning and afternoon.

	Morning		Afternoon	
	Supine	Standing	Supine	Standing
G_{down} Integrated gain (ms/mm Hg)	6.2 ± 0.5	4.9 ± 0.4*	14.3 ± 0.7†	5.4 ± 0.4*
R value ± SE	0.96 ± 0.015	0.95 ± 0.009	0.98 ± 0.007	0.93 ± 0.014
G_{up} Integrated gain (ms/mm Hg)	11.7 ± 0.7	3.6 ± 0.4*	17.3 ± 1.1†	6.3 ± 0.3*†
R value ± SE	0.89 ± 0.042	0.82 ± 0.037	0.96 ± 0.015	0.89 ± 0.037

* $P < 0.05$ vs. Supine; † $P<0.05$ vs. Morning

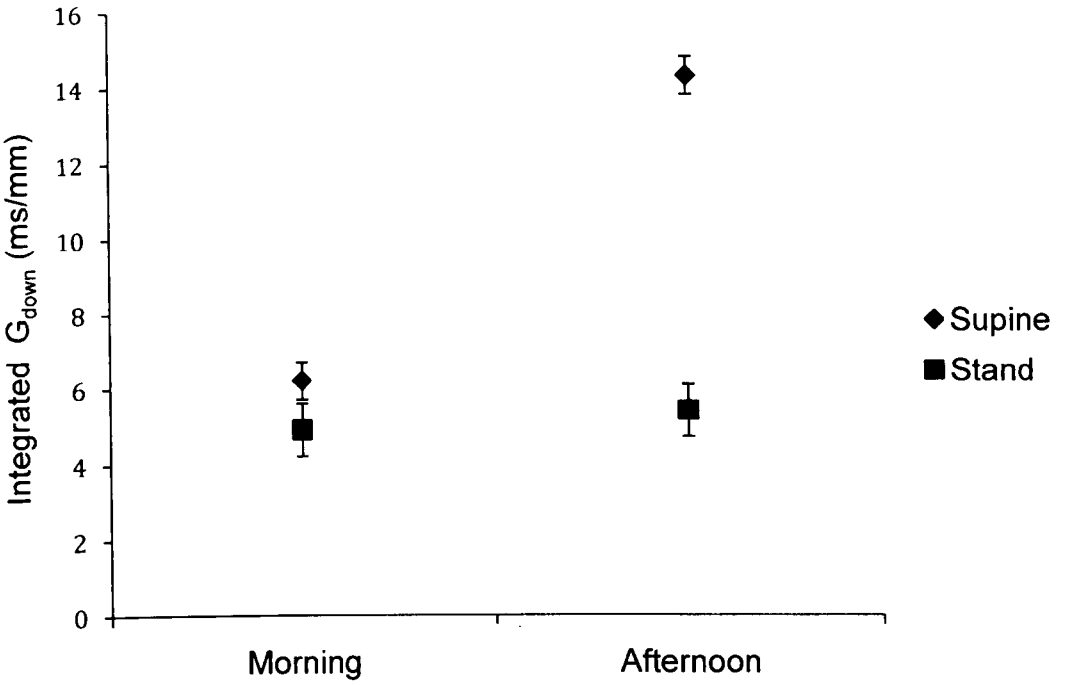

Figure 8.1: Integrated G_{down} for supine and standing postures in the morning and afternoon.

8.3.4 Mechanical and neural components

Table 8.3 shows mechanical and neural gains for supine and standing trials at both times of day. Due to difficulties in obtaining carotid diameter during some of the standing trials, the results do not include a number of trials that were eliminated from the analyses on the basis of quality (assessed subjectively). Significant reductions in neural gain from supine to standing were found for falling pressures in the afternoon ($P<0.0005$, $n=3$) and rising pressures in both the morning ($P<0.0005$, $n=5$, Figure 8.3) and afternoon ($P<0.0005$, $n=4$). Although significant changes were found for neural G_{down} in the morning ($P=0.029$, based on $n=4$), the results indicated an increase with standing with no changes in the mechanical component despite reductions in integrated gain. Significant mechanical changes with posture were found in the afternoon, with reductions in G_{up} ($P=0.002$, $n=4$) and, increases in G_{down} ($P=0.001$, $n=3$).

Table 8.3: Mechanical and neural baroreflex gains and correlation coefficients for supine and standing trials in the morning and afternoon.

	Morning		Afternoon	
	Supine	Standing	Supine	Standing
G_{down} Mechanical gain (ms/mm Hg)	0.011 ± 0.001	0.011 ± 0.001	0.009 ± 0.001	0.026 ± 0.005*†
R value ± SE	0.70 ± 0.084	0.83 ± 0.086	0.79 ± 0.027	0.67 ± 0.135
G_{up} Mechanical gain (ms/mm Hg)	0.011 ± 0.001	0.010 ± 0.001	0.019 ± 0.001†	0.012 ± 0.002*†
R value ± SE	0.84 ± 0.030	0.71 ± 0.058	0.88 ± 0.063	0.81 ± 0.077
G_{down} Neural gain (ms/mm Hg)	239.4 ± 50.2	396.1 ± 33.0*	1018.8 ± 129.7†	99.0 ± 24.5*†
R value ± SE	0.62 ± 0.055	0.88 ± 0.040	0.80 ± 0.018	0.64 ± 0.082
G_{up} Neural gain (ms/mm Hg)	670.2 ± 69.5	197.1 ± 38.3*	567.7 ± 69.5	229.6 ± 40.1*
R value ± SE	0.79 ± 0.026	0.55 ± 0.069	0.93 ± 0.027	0.74 ± 0.097

* $P < 0.05$ vs. Supine; † $P<0.05$ vs. Morning

Figure 8.2: Integrated G_{up} for supine and standing postures in the morning and afternoon.

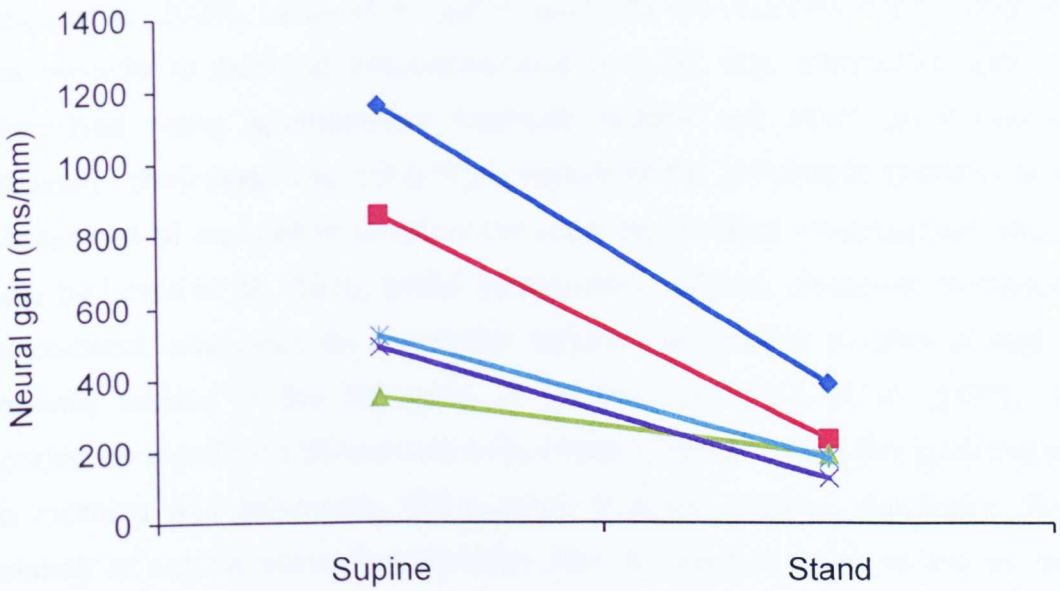

Figure 8.3: Neural G_{down} for supine and standing postures in individual participants (n=5) in the afternoon.

8.4 Discussion

The main findings of the study are that (1) standing posture was associated with significant reductions in integrated gain at both times of day, (2) postural-related reductions in G_{down} were greater in the afternoon, thus eliminating the diurnal variation in baroreflex gain for falling pressures, and (3) postural-related reductions in G_{up} were similar for morning and afternoon, maintaining the diurnal variation in gain for rising pressures. Our secondary aim was to investigate the contribution of mechanical and neural components to changes in baroreflex gain. Our results indicate that the falls in integrated gain with upright posture were predominantly associated with reductions in the neural component as hypothesized, although the limitations associated with these findings are discussed later.

In the present study a change in posture from supine to standing was associated with reduced integrated baroreflex gain, supporting the findings of many previous studies (O'Leary et al., 2003, Hughson et al., 1994, Taylor and Eckberg, 1996, Jasson et al., 1997, Iellamo et al., 1996, Pickering et al., 1971, Saeed et al., 2009). Lewis et al. (2010) explored the changes in baroreflex gain with regards to postural influences and time of day. Baroreflex gain was determined using spontaneous methods before and after an incremental orthostatic challenge. The contrasting nature of the orthostatic challenges and assessment of baroreflex function between the current investigation and the study by Lewis et al. (2010) make comparisons difficult. However, evidence of the postural influences on baroreflex function in relation to time of day are presently lacking in the literature. In contrast to Lewis et al. (2010), who reported no significant differences in baseline (supine) baroreflex gain between the morning and afternoon, the present findings suggest significant diurnal variation in supine baroreflex function with diminished gains in the morning. These results are consistent with previous evidence of diurnal variation in supine subjects (Hossmann et al., 1980, Conway et al., 1983). Lewis et al. (2010) reported that although the rate of decline in baroreflex gain was greater in the morning compared with the afternoon, there was no significant diurnal variation in the gain values identified at presyncope. In the current study there was no significant difference in G_{down} between morning and afternoon when

participants were in the standing position, but significant diurnal variation was still apparent for G_{up}. Although Lewis et al. (2010) reported that the decline in BP was greater in the morning, the lack of diurnal variation in G_{down} during standing suggests that the increased risk of syncope in the morning is not due to poor BP control via the cardiac baroreflex. The diminished cardiac baroreflex gains observed with falling pressures when standing would appear to increase the risk of syncope irrespective of time of day. Other mechanisms must be involved that lead to the elevated risk of syncope in the morning. It is possible that the increased risk in the morning may be due to inadequate maintenance of BP through the sympathetic baroreflex at this time. Orthostatic hypotension as a result of venous pooling stimulates an increase in sympathetic outflow and parasympathetic inhibition, leading to peripheral vasoconstriction and increased heart rate and contractility (Naschitz et al., 2006). Studies have shown that the sympathetic baroreflex is enhanced when an upright posture is assumed (O'Leary et al., 2003), however diurnal variation in these responses to posture has not been examined to date. Lewis et al. (2010) reported lower initial cerebral blood flow velocity in the morning compared to afternoon, which may explain the more rapid time to presyncope at this time of day, as opposed to the physiological state when presyncope is eventually reached. Alternatively, it is possible that cerebral hypoperfusion is related to impaired cerebral autoregulation in the morning. However, pilot studies (unpublished) have detected no significant diurnal variation in cerebral autoregulation.

The findings of the current study indicate that the ability of the cardiac baroreflex to cope with rising pressures is reduced when an upright posture is assumed, particularly in the morning. The risk of cardiovascular events is greatest between 0600 and 1200h (Muller et al., 1987), as is the risk of stroke (Elliott, 1998). The response to increases in BP that occur while individuals are in the standing position may be reduced due to diminished baroreflex gain, thus increasing the risk of cardio- and cerebro-vascular events in vulnerable populations.

The results of the current study indicate that the falls in integrated gain with upright posture were predominantly associated with reductions in the neural component. Significant reductions in neural gain from supine to standing were

found for falling pressures in the afternoon and rising pressures at both times of day. Nevertheless, the reductions in baroreflex gain for rising pressures in the afternoon were also associated with significant changes in the mechanical component. There was also evidence of reductions in carotid diameter with standing compared to supine, supporting previous findings of reduced carotid diameter with upright sitting versus supine postures (Saeed et al., 2009). The examination of the contributions of mechanical and neural components to changes in baroreflex gain was limited by the elimination of some of the trials due to the quality of the carotid imaging. It should be reiterated that the results ought to be interpreted with caution, since some of the analyses do not include data from all 5 participants. Some unexpected results were observed, including increases or lack of changes in component gains despite overall reductions in integrated gain. Therefore, where significant results were found the individual participant responses have been examined to investigate the consistency of the response. The finding of reduced neural gain for falling pressures in the afternoon and rising pressures at both times of day was supported by consistent responses between participants, i.e. every participant included in the analysis displayed a reduction in neural gain from supine to standing. Significant reductions in mechanical G_{up} with the standing posture were found in the afternoon, and although decreases were seen in all 4 participants included in the analysis, the reductions in 2 participants were marginal. From these findings it may be speculated that the neural component is the most influential component associated with reductions in integrated baroreflex gain when an upright posture is assumed. These results support the hypothesis that diminished baroreflex gain is associated with parasympathetic withdrawal and therefore reduced vagal nerve activity with which to regulate heart rate (Hughson et al., 1994). However, further studies are warranted to investigate these initial findings. Saeed et al. (2009) examined the contributions of the components of the cardiac baroreflex to differences in gain between supine and sitting positions. They found that diminished integrated gain associated with an upright-seated position was due to reductions in the vascular (mechanical) arm of the baroreflex. However, the validity of closed-loop-spontaneous transfer function analysis, as used by Saeed et al. (2009), has been questioned (Kamiya et al., 2011). The modified Oxford method used in the present study allows a near open-loop analysis of the cardiac baroreflex system.

8.5 Conclusion

The findings of this investigation support those of previous studies in suggesting that baroreflex gain is reduced when an upright posture is assumed. Analyses of the baroreflex components indicate that reductions in neural gain when in the standing position may be the cause of the diminished integrated gain, although further studies are warranted. This is the first study to use standard pharmacological methods to examine the effects of time of day on postural differences in gain. Diurnal variation in baroreflex gain is eliminated when BP falls during the standing position, yet is maintained during stand when BP is rising. These results suggest that the increased risk of syncope in the morning is not associated with diurnal variation in cardiac baroreflex function, and that other mechanisms, such as the sympathetic baroreflex or cerebral autoregulation, are behind the increased morning risk. The existence of diurnal variation in G_{up} even after postural changes suggests that the risk of cardiovascular events in the morning remains, irrespective of whether an individual is supine or standing. Further investigations are needed to unravel the mechanisms behind the interactions between time of day and postural changes.

Chapter 9

Synthesis of findings

9.1 Conclusions

The work undertaken in this thesis was designed to explore the control of BP in humans, providing new data in key areas that warranted further research. The aims of this thesis were: 1) to investigate sources of variation in human blood pressure control, in particular the effects of blood pressure status, physical activity and time of day; 2) to explore and apply the investigations above to a clinical population of patients with obstructive sleep apnoea, who suffer from both circadian-related issues and generally high blood pressure status; and 3) to explore mechanisms responsible for diminished blood pressure control in the morning in the general population. These aims have been achieved through completion of a series of studies reported within this thesis. These separate studies were designed according to the specific objectives laid down in the introduction. Thus the following conclusions can be made:

1. Blood pressure status was a significant predictor of acute BP changes following exercise. However, when statistical artefacts of regression to the mean and mathematical coupling are not controlled for, it can lead to spurious results and the masking of other important moderators of blood pressure responses.

2. Obstructive sleep apnoea patients exhibited a unique systolic BP reactivity profile, with peak reactivity at night, which may help to explain the increased risk of myocardial infarction at this time in this population.

3. Leisure-time physical activity was associated with reduced daytime sleepiness in patients with OSA, and may be a useful adjunct therapy to CPAP.

4. Diminished cardiac baroreflex sensitivity in the morning was due to reduced mechanical gain when BP is rising, and therefore therapies targeting the mechanical component may reduce the cardiovascular risk in the morning. Diminished baroreflex sensitivity in the morning in response to falling BP was due to reduced neural gain.

5. Cardiac baroreflex sensitivity was reduced in the morning and afternoon when an upright posture was assumed, and was primarily attributed the

decreases in neural gain. Although observed at both times of day, reductions in baroreflex sensitivity due to the change in posture occurred to a greater extent in the afternoon. This caused the diurnal variation that was reported in the supine position to be attenuated for rising BP, and eliminated entirely for falling BP when participants changed to a standing position.

9.2 Implications

The conclusions drawn from the studies in this thesis have implications for the future work of researchers, clinical populations, in particular OSA and hypertensive patients, and also the general population. The specific implications of each study are outlined below in relation to these groups.

9.2.1 Implications for researchers

The studies in this thesis provide further knowledge of BP control and the influence of physical activity, time of day, and BP status, as well as related mechanisms and methodological issues. The main conclusion from study 1 was that the importance of BP status in predicting PEH can be exaggerated when statistical artefacts of regression to the mean and mathematical coupling are not controlled for. Researchers should be aware of the problems of spurious correlations when initial and change values are entered into correlation or regression analyses, particularly when interpreting the results of previously published studies. In future studies researchers should control for these artefacts, not only to minimise spurious correlations, but to ensure that other important predictor variables are not overlooked. This is the case for any study involving initial and change values, but is particularly vital for studies investigating the effectiveness of interventions to enhance aspects of health, especially when the results will provide evidence for position statements and guidelines. Study 2 provided evidence to show that using an appropriate study design can minimise the effects of regression to the mean and mathematical coupling. In studies of BP responses to exercise or other interventions, researchers are advised to use ambulatory BP measurements on a control day and post-intervention day. The use of multiple measurements rather than a small number of baseline values reduces these statistical artefacts, and therefore minimises the chance of spurious correlations and allows other important moderators of BP to be identified.

Study 3 involved the use of BP reactivity profiles, which may be a useful tool for determining cardiovascular risks in patients. However, further research is needed to evaluate its predictive value and to examine its potential use in other populations. The findings of study 3 also highlight the importance of taking into account physical activity when making BP measurements, particularly given the effects of time of day on BP responses to activity. Time of day should be controlled for when investigating BP responses to exercise or other physiological challenges. In study 4 it was found that physical activity was associated with reduced daytime sleepiness, although the cause-effect relationship should be interpreted with caution. In the future researchers should explore the use of physical activity interventions with longitudinal studies, potentially in conjunction with CPAP. Given the results in this thesis, it is recommended that ambulatory BP monitoring is used as it is more relevant and reliable than clinic measurements. Researchers should also control for patient characteristics such as age, gender and BMI.

Studies 5 and 6 provided further information about the components of the integrated baroreflex response involved in diurnal variation and changes in posture. This has implications for future studies in which researchers are investigating potential treatments or interventions to reduce the risk of cardiovascular events and syncope. From the current study results it would be recommended that the mechanical component is targeted for improving control of rising BP, and that the neural is targeted for falling BP. Further research is needed to confirm the findings of study 6, in which the neural component was found to be reduced when changing from supine to standing. Given the significant differences seen between morning versus afternoon, and supine versus standing, these two studies also highlight the need to control for time of day and posture when assessing baroreflex function.

9.2.2 Implications for clinical populations

The results of study 1 indicated that $\dot{V}O_{2\,max}$ was a significant predictor of PEH, with greater reductions in BP in the less fit individuals. This has implications for clinical populations of hypertensive patients in whom fitness tends to be lower.

The results suggest that these individuals will benefit most from the BP-lowering effects of post exercise. Time of day for exercise was also a significant predictor, and the findings indicate that hypertensive patients should exercise in the afternoon rather than the morning to gain the greatest reductions in BP following exercise. Although study 1 results suggest that the importance of BP status in predicting PEH has been exaggerated in previous studies, the results from study 2 confirm that it does still play a role alongside other moderators. This is a positive outcome for the promotion of exercise as an anti-hypertensive treatment. Hypertensive populations should be encouraged to exercise regularly, particularly since the results show that individuals with high BP will benefit most from the BP-lowering effects of exercise.

The results of study 3 indicate that BP reactivity is high at night is OSA patients. This may explain, in part, why the risk of MI is elevated at this time in this clinical population. Better BP control, particularly a night, is needed in these patients. This may involve the use of CPAP and combined with other treatments. In study 4 it was found that *leisure-time* physical activity in OSA patients was not related to BP, suggesting that other possibilities for interventions will need to be pursued. On the other hand, physical activity was associated with lower daytime sleepiness, which is a key symptom that can lead to increased risk of road traffic accidents, sick leave and reduced quality of life. Further research in the form of longitudinal studies is needed to determine the cause and effect in this relationship. Finally, an increase in physical activity should help to reduce BMI, which was associated with OSA severity (AHI, ODI). Therefore, it is recommended that OSA patients combine CPAP therapy with a healthy diet and regular exercise in order to lose weight, control their BP and reduce their daytime sleepiness.

Although studies 5 and 6 involved young healthy participants, the results may help to unravel the mechanisms involved in diurnal variation in BP control associated with elevated cardiovascular risk in the morning. The results highlight that BP control is worse in the morning, suggesting that clinical populations, such as those with atherosclerosis and/or hypertension, should avoid intense exercise in the morning as well as other stress-inducing activities. The mechanical component of the baroreflex response was the site responsible

for most of the reduction in control of rising BP is the morning. It is recommended that individuals aim to improve their vascular health, for example though regular aerobic exercise, in order to enhance this component and improve their BP control when most at risk. Study 6 revealed that even when changing to a standing posture baroreflex function is reduced further and control of rising BP is still worse in the morning than the afternoon.

9.2.3 Implications for the general population

The results of study 1 apply to the general population as well as clinical populations. It is recommended that exercise is performed in the afternoon for the greatest BP-lowering effects post exercise. In study 2 it was found that age, BMI and $\dot{V}O_{2\,max}$ were significant predictors of PEH. This has implications for the general population because those individuals who are older, overweight and unfit and therefore most at risk of developing hypertension, should benefit most from the BP reductions following exercise.

The results of study 3 confirmed previous research findings that BP reactivity is greatest in the morning in the general population. Therefore in older populations, where the risk of cardiovascular events begins to increase, it is recommended that morning exercise is avoided because BP responses to activity are higher at this time. Although no significant relationships between leisure-time physical activity and BP were identified in study 4 in the control group, this study may have been limited by the use of one-off clinic BP measurements. It has been suggested that ambulatory BP measurements are more accurate and reliable and therefore may have been more successful in indentifying the benefits of exercise, which is recommended for maintaining healthy BP levels in the general population.

Studies 5 and 6 highlight the need for maintaining a healthy lifestyle within the general population. Although the studies are in the young healthy volunteers, the diurnal variation is still present in BP control with a reduced ability to cope with rises and falls in BP in the morning. The mechanical component was identified as the site primarily involved in this reduced morning baroreflex response. Carotid distensibility has been shown to reduce with age (Monohan

et al., 2001) and therefore it is important for the general population to maintain vascular health through exercise and diet in order to avoid large reductions in baroreflex function.

9.3 Recommendations for future research

There are several potential areas for future research that have emerged from the studies reported in this thesis. These are predominantly concerned with: physiological mechanisms involved in diurnal variation in BP control in both healthy and clinical populations; and the effectiveness of chronic physical activity in the improvement of BP control in hypertensives and other clinical populations. Some recommendations for future research include:

9.3.1 The chronic effects of exercise training on ambulatory BP: A meta-analysis.

The meta-analytical approach applied in study 2 revealed reductions in BP following acute exercise deemed to be of clinical significance. It seems a logical progression to investigate the effectiveness of chronic exercise on blood pressure and its cardio-protective effects. Although meta-analyses have been used to investigate the effects of aerobic training (Fagard, 2001, Kelley et al., 2001), the main outcome measures were changes in resting SBP and DBP, as opposed to ambulatory measurements, which are more relevant to the everyday control of BP and related cardiovascular events. Future meta-analyses should aim to investigate the combined effects of exercise *duration, intensity* and mode to determine the most effective protocols for BP control. It is important to note that mathematical coupling and regression-to-the-mean may influence any proposed anti-hypertensive intervention involving initial BP status and BP changes, and therefore should be controlled for.

9.3.2 Blood pressure reactivity profiles in clinical populations

Following the identification of BP reactivity profiles in OSA patients that differ substantially to those found in health controls (study 3) and hypertensives (Jones et al., 2006), similar techniques may be applied to populations with sleep disorders other than OSA or those suffering from daytime sleepiness only. This will determine whether the reactivity profile is unique to OSA or whether other populations with sleep- and circadian-related disturbances may be at risk of

nocturnal cardiovascular events. Shift workers may provide an interesting non-clinical population to consider for future studies of BP reactivity. Further investigations with large sample sizes are required to determine the effectiveness of BP reactivity profiles as a tool for identifying individuals at risk.

9.3.3 Physical activity as an adjunct therapy to CPAP for reducing symptoms and severity of OSA

In study 4 leisure-time physical activity was associated with reduced ODI and daytime sleepiness in OSA patients. Although cause and effect cannot be assumed with cross-sectional study designs, it is possible that physical activity would provide a useful treatment for reducing OSA severity and daytime sleepiness in OSA patients. Future studies should use experimental designs to examine the potential of physical activity interventions as an adjunct to traditional CPAP therapy. Given the discrepancies between clinic and ambulatory measurements of BP in OSA patients reported in study 3, potential anti-hypertensive benefits of combined exercise and CPAP therapy should be assessed using ambulatory BP measurements.

9.3.4 Diurnal variation in cardiac and sympathetic baroreflex function in healthy and clinical populations

The findings of studies 5 and 6 contribute to the understanding of mechanisms involved in BP control, including how diurnal variation in the separate components of the cardiac baroreflex may potentially contribute to greater risk of cardio- and cerebro-vascular events. Although it is important to initially delineate these mechanisms in healthy individuals in order to document the 'normal' physiological responses, the study of young healthy individuals is limited in as far as generalising the results to clinical populations. Future studies should follow up the findings of studies 5 and 6 in clinical populations associated with hypertension and cardiovascular disease. Patients with OSA would be a particular interesting population to study, especially given the altered circadian profiles of BP control reported in this thesis. The studies in this thesis have focused on the baroreflex control of heart rate. However, the control of peripheral resistance via sympathetic nerve activity is also important. Future studies should investigate both cardiac and sympathetic baroreflex function, given the influence of both these mechanisms on the maintainenance of

cardiovascular homeostasis (Rudas et al., 1999). In addition to this, exploration of diurnal variation in baroreflex function following exercise may help to explain circadian variation previously found in BP responses to activity.

Chapter 10

References

10.1 References

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