# EFFECTS OF ACUTE SLEEP RESTRICTION ON LABORATORY AND AMBULATORY PHYSIOLOGICAL REACTIVITY IN YOUNG ADULTS

Thesis submitted for the Degree of Doctor of Philosophy

**Éanna D. O' Leary, B. A. (International), H. Dip. Psych.**


School of Psychology

National University of Ireland, Galway

Galway

May, 2013

**Primary Supervisor:**

Prof. Jack James

**Co-Supervisor:**

Dr Brian Hughes

## TABLE OF CONTENTS

## ABSTRACT

**Introduction.** Reduced sleep duration has been associated with adverse health outcomes, in particular negative cardiovascular health. The mechanisms by which sleep loss may influence cardiovascular health are unclear but may be related to alterations in physiological stress responding. The current project sought to assess the association between sleep duration and cardiovascular response to laboratory social stress in addition to examining ambulant cardiovascular functioning using experimental partial restriction of sleep duration in a sample of healthy young adults. To objectively monitor adherence to the sleep manipulation, participants were provided with a wrist activity monitor. Associated physiological processes, including salivary markers of neuroendocrine stress functioning and inflammation were additionally monitored to further explore the effects of acute sleep restriction on stress system functioning across both laboratory and ambulatory contexts.

**Methods.** Resulting from pooled data collected from 128 college students, five empirical studies are reported, incorporating successive methodological refinements to help advance understanding of the effects of sleep loss on physiological responding. In a sample of 93 college students, Study 1 examined laboratory cardiovascular reactivity (i.e., systolic and diastolic blood pressure [SBP and DBP, respectively], and heart rate [HR]) during evaluative social stress, including assessments of hemodynamic determinants of blood pressure, to determine if cardiovascular reactivity to laboratory stress (CVR) was altered under conditions of experimental sleep restriction relative to a rested group. The primary aim of Study 1 was to examine laboratory CVR as a possible mechanism through which sleep restriction may be related to increased cardiovascular risk. In Study 2, in order to assess possible additional physiological effects of laboratory stress that may exist across stress systems, salivary α-amylase response (sAA) to social stress, as a measure of sympatho-adrenal-medullary system

(SAM) activity, was examined in 113 college students following acute sleep restriction, compared to a rested group. The SAM axis releases the hormones epinephrine and norepinephrine and denotes the instantaneous sympathetic response to stress. However, as only two previous examinations of sAA response following sleep loss exposure obtained objective assessment of sleep duration (using actigraphy), further examinations of sAA response following verified short sleep durations were required. A secondary aim sought to shed light on the sensitivity of the sAA response to a laboratory stress protocol (as used in Study 1), exposing participants to negative social evaluation, presented by video relay. Study 3 extended the traditional CVR laboratory protocol, testing the potential for the findings of the previous laboratory based CVR observations in Study 1, to generalise to conditions outside of the laboratory setting. Further, few studies have measured how sleep loss affects cardiovascular response to naturalistic stressors, while periods of interpersonal contact have been suggested as a useful task for investigating CVR in the field. One hundred and six participants underwent ambulatory blood pressure and HR monitoring while engaging in everyday activities, comparing cardiovascular arousal during periods of high and low stress and interpersonal contact, whilst rested and sleep restricted. Examination of nocturnal blood pressure dipping was also conducted to explore if the habitual nocturnal reduction in blood pressure is associated with reduced sleep duration. In Study 4, in order to establish whether reduced sleep duration influences ambulant measures of hypothalamic-pituitary-adrenal (HPA; i.e., cortisol) activity, advancing the previous limited and incomplete research of such effects, the awakening response of cortisol was examined. Relative to SAM axis activation (Study 2), the HPA axis controls the longer-term neuroendocrine stress response. One hundred and twenty college students were requested to provide four waking saliva samples, in their own homes, over the first 45 mins post-awakening, for the assessment of the awakening response in cortisol. The previous studies (1 and 3) examined the influence of

sleep restriction, social stress, and interpersonal contact, on measures of laboratory and ambulatory CVR. Study 2 examined levels of sAA, while Study 4 assessed measures of HPA activity, investigating sleep loss related change in cortisol awakening response and SAM related activation. However, failure to obtain adequate amounts of sleep has also been suggested to promote low-level systemic inflammation, itself associated with cardiovascular risk. In Study 5, a brief exploratory investigation into the effects of sleep restriction on levels of a novel marker of low level systemic inflammation, C-reactive protein (CRP) detectable in saliva, was examined in 120 college students, offering a preliminary examination of both the validity of the marker and its ability to detect change associated with reduced sleep duration.

**Results.** Study 1 confirmed that relative to a rested group, acute sleep restriction was associated with alterations in the hemodynamic determinants of blood pressure in response to social stress (vascular response evident), while overall blood pressure response (SBP, DBP, and HR) remained unchanged between rested and sleep restricted groups. In Study 2, marked increases in basal sAA activity following one night of partial sleep restriction were identified. The data additionally corroborated previous findings regarding the sensitivity of α-amylase to laboratory social stress exposure, while further demonstrating a significant increase in sAA in response to an acute social stress protocol where the primary social evaluative element was presented by virtual (i.e., video-relayed) observers. Study 3 suggested the potential role that sleep restriction may have in negatively affecting both nocturnal ambulatory cardiovascular indices and reactivity to naturally occurring interpersonal contact (i.e., increased SBP and DBP), in ways that have been implicated in the etiology of heightened risk for cardiovascular disease. In Study 4, acute sleep loss was related to morning stimulation of the HPA-axis and, in particular, dampening of morning cortisol levels. Finally, in Study 5, while CRP, assessed in saliva, remained statistically unchanged following sleep restriction, increased CRP level in

conjunction with greater body mass index, a known correlate of systemic inflammation, suggested an association between sleep restriction and augmented salivary CRP response.

**Conclusions.** The findings provide evidence of alterations to both overall measures of cardiovascular function and underlying hemodynamic determinants, in addition to associative (neuroendocrine and inflammatory) physiological changes related to cardiovascular functioning, following acute sleep restriction, in both laboratory and ambulant conditions. Such data highlight lines of research leading to pathway clarification, though which reduced sleep duration may, over time, influence cardiovascular health.

## ACKNOWLEDGEMENTS

This work was supported by a Project Grant (No. RP/2007/225) from the Irish Health Research Board awarded to Prof. Jack James and a doctoral scholarship awarded to the present author. The latter was issued as part of a programme grant awarded to Dr Brian Hughes, who served as Director of the Centre for Research on Occupational and Life Stress (CROLS), as part of the Irish Social Sciences Platform funded by the Programme for Research in Third Level Institutions, administered by the Irish Higher Education Authority and co-funded by the European Regional Development Fund.

I would like to thank all the administrative and technical staff of the School of Psychology, National University of Ireland, Galway (NUIG), especially Mr Declan Coogan for his cardiovascular/bioassay laboratory problem solving ability and Mr Joseph Mee/Mr John Dring for programming the social stressor task. Thanks are also owed to the technical staff of the Department of Pharmacology & Therapeutics, in particular Mr Danny Kerr and Mr Brendan Beatty, in addition to Mr Maurice Martyn and Mr Michael Coughlan from the Department of Microbiology, both at NUIG, for their assistance and laboratory expertise. Thanks, also, to Dr Matt Bristow, Anglia Ruskin University, Cambridge, U. K., Ms Elizabeth Schrader, CROLS laboratory Research Assistant, and the staff of Salimetrics Europe Ltd., for their technical assistance with salivary assaying.

In particular, I express my thanks to Dr Hughes, NUIG, for his mentorship, advice, and continued support. I also owe special thanks to Dr Siobhán Howard, Mary Immaculate College, University of Limerick, for being an outstanding colleague, mentor, and friend throughout this research and for providing guidance for which I am most thankful.

I am very grateful to my postgraduate colleagues in the School of Psychology, both past and present, for their camaraderie, in particular my fellow graduate researchers and

friends at CROLS, Ann Marie, Eimear, and Niamh. I extend this gratitude to my siblings, Aoife and Cian, thank you also for your encouragement.

To prospective students contemplating a PhD and to those writing up their theses; I wish you the best of luck. Finally, I thank my primary supervisor, Prof. James, for always giving freely of his time and whose direction, skill, and support I am fortunate to retain.

This thesis is dedicated to Teresa O'Leary, for her unending fortitude, and the late Dónal O'Leary; equally responsible for bestowing early life lessons in the importance of resilience.

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ACRONYMS

ABP: Ambulatory blood pressure

ACTH: Adrenocorticotropin hormone

ANCOVA: Analysis of covariance

ANOVA: Analysis of variance

APPs: Acute phase proteins

$AUC_G$: Area under the total response curve with respect to the ground

AVP: Arginine vasopressin

BMI: Body mass index

bpm: Beats per minute

CAC: Coronary artery calcification

CAR: Cortisol awakening response

CD: Compensation deficit

CHD: Coronary heart disease

CO: Cardiac output

CRH: Corticotropin-releasing hormone

CRP: C-reactive protein

CV: Coefficient of variation

CVD: Cardiovascular disease

CVR: Cardiovascular reactivity

DBP: Diastolic blood pressure

EC: Event code

ELISA: Enzyme-linked immuno sorbent assay

ELSA: English longitudinal study of ageing

EP: Epinephrine

ESS: Endothelial shear stress

HP: Hemodynamic profile

HPA: Hypothalamic-pituitary-adrenal

HR: Heart rate

hr: Hour

hs-CRP: High-sensitivity C-reactive protein assay

IL-6: Interleukin-6

LVM: Left ventricular mass

MFI: Multidimensional fatigue inventory

MI: Myocardial infarction

min: Minute

mmHg: Millimetres of mercury

MMPI: Minnesota multiphasic personality inventory

MnInc: Mean increase in cortisol

NE: Norepinephrine

NREM: Non-rapid-eye-movement

nm: Nanometer

OR: Odds ratio

pg/ml: Picogram/millilitre

PSG: Polysomnography:

rpm: Revolutions per minute

REM: Rapid-eye-movement

RR: Relative risk

SAM: Sympatho-adrenal-medullary

SBP: Systolic blood pressure

SNS: Sympathetic nervous system

SV: Stroke volume

TPR: Total peripheral resistance

TSST: Trier social stress test

TSST-C: Trier social stress test for children

u/min: Units per minute

u/ml: Units per millilitre

ug/dl: Milligrams/decilitre

µL*:* Microlitre

# Chapter 1.

# INTRODUCTION[1]

*"Sleep that knits up the ravell'd sleave of care,*

*The death of each day's life, sore labour's bath,*

*Balm of hurt minds, great nature's second course,*

*Chief nourisher in life's feast."*

***Macbeth*: Act 2, Scene 2**

Sleep is a universal human characteristic. A central constituent of our behaviour, just as eating or socialising, it is an activity that we regularly engage in. In terms of health, while we may all have heard about the need for maintaining healthy lifestyle behaviours, such as regular physical activity or balanced diet, public health information regarding the importance of maintaining adequate sleeping habits has been comparatively absent. Indeed, sleep, and its maladaptation, barely features in the teaching of the medical professions. When researchers from Oxford university investigated British medical education in the late 1990's, they discovered that the average amount of time devoted to sleep and sleep disorders in undergraduate teaching was just 5 mins, rising to 15 mins with additional preclinical training (Martin, 2003; Stores & Crawford, 1998). One's general practitioner is therefore unlikely to be an expert on the subject. However, our collective imperative to engage in this behaviour to a degree thought to be beneficial to health, and in so doing achieve sleep of adequate duration, has been noted to be under threat.

[1] Elements of this chapter have been published as a book chapter: O'Leary, É. D., Howard, S., & James, J. E. (2012). Sleep duration and health: Examining psychosomatic stress-related pathways. In K.E. Moore, K. Kaniasty & P. Buchwald (Eds.), Stress and Anxiety. Application to Economic Hardship, Occupational Demands and Developmental Challenges (pp. 119 - 130). Berlin: Logos.

*1.2. A Sleep-Sick Society?*

Reviewing contemporary culture, and societies' appetite for time-saving devices and strategies, James Gleick (2000) noted "The mere presence of an alarm clock implies sleep deprivation, and what bedroom lacks an alarm clock?" (p. 122). Indeed, increasingly, sections of the population experience regular sleep loss due to modern lifestyles, increased work pressure, and psychosocial stress. Evolution equipped humans, in common with all other animals, with biological mechanisms to make us sleep at approximately the same time every day. However, for significant proportions of our population, daily cycles of sleep and activity are no longer governed by dawn and dusk, replaced instead by accumulated demands imposed by a largely "24-hr" society (Rajaratnam & Arendt, 2001). This trend is a result of less dependency of daylight hours (i.e., increased shift-work) and long working hours (Knutson, Van Cauter, Rathouz, DeLeire, & Lauderdale, 2010), increased time spent commuting to and from the workplace (Chatzitheochari & Arber, 2009), or alterations in lifestyle (e.g., referring to the double burden of taking care of family's needs and work-related responsibilities; Sigurdson & Ayas, 2007).

As a result, in recent decades, it has been found that sleep duration in adults and adolescents has decreased by 1.5 − 2 hrs and it has been suggested that the pressure on sleep time will continue to grow due to lifestyle choice (i.e., increased television and internet usage) or pressures imposed by work and family demands (Bixler, 2009; Van Cauter, Spiegel, Tasali, & Leproult, 2008). Clinically, it is well established that people experiencing psychological ill health often achieve suboptimal sleep, such that disrupted sleep is included in the diagnostic criteria for some of the most common mental health problems, including Major Depression and Generalized Anxiety Disorder (American Psychiatric Association). Further, frequent sleep loss is not only common among younger and working adults, but is a

growing problem among young children and older adults (Am-Meijer & Enober, 2000; Feinsilver, 2003).

### *1.2.1.Characteristics of sleep*

During sleep, the brain's activity changes in characteristic ways over the course of the night. As assessed using polysomnography (PSG), through the application of electrodes to the head, face, and chest, normal sleep has been classified into two main types of sleep pattern: rapid-eye-movement (REM) sleep and non-rapid-eye-movement (NREM) sleep. NREM sleep is broken down into three distinct stages: N1, N2, and N3. These stages are characterised by increasingly larger and slower brain waves. N1 sleep is very light sleep; N2 is slightly deeper sleep; and N3, also called slow-wave sleep, is the deepest NREM sleep stage. Sleep can also be estimated using wrist actigraphy, which involves wearing a wristwatch-like device that counts wrist movements and thereby identifies sleep versus wake stages. The advantage of actigraphy is that can be easily used for multiple days, which provides a better measure of habitual behaviour (Knutson, 2013). Finally, subjective estimates of sleep duration and quality may also be collected.

Sleep characteristics also change across the lifespan. A typical pattern of declining slow-wave sleep (NREM stage 3) and increased time awake in bed is evident with increasing age (Dement et al., 1985). Frequency of awakenings is known to increase in the elderly, but perhaps more importantly, the elderly find it more difficult to get back to sleep after an awakening, and as a result of this there is a significant amount of intra-night wakefulness (Feinsilver, 2003). Such awakenings in the elderly have been reported to be an expression of the disorganisation of sleep resulting from changes in circadian and homeostatic regulation (Hofman, 2000) as evidenced by their reduced slow-wave sleep. Age-related reductions in mental and physical activity may also influence homeostatic drive (Stanley, 2005).

As sleep is in part governed by behaviour (in addition to circadian and homeostatic sleep rhythms), sleep can be viewed as occurring within a framework of physical and social settings, and may be shaped by socio-cultural values, beliefs, and practices. Social pressures and poor environmental conditions may cause decrements in sleep quality and quantity amongst certain higher risk populations (i.e, lower socieconomic position; Stranges et al., 2008), and sleep may therefore play a role in the relationship between individuals access to, and control over, health-protective resources and health outcomes (Van Cauter & Spiegel, 1999). Further, in some societies and cultural settings, sleep is a communal phenomenon, while solitary sleep is considered to be the cultural ideal in many Anglo-European societies. In Japan, the social acceptability of the unique form of napping called *inemuri* (literally, 'to be present and sleep'), in which the sleeper is in a situation not ordinarily meant for sleep (i.e., at work or at a social event), is another example of the influence of cultural values on sleep patterns. How we sleep, where we sleep, with whom, and for how long we sleep are therefore, in part, moulded by our culture and attributed customs and practices (Owens, 2004).

### *1.2.2. Prevalence of Sleep Loss*

The evidence that sleep loss is a common feature of contemporary life comes from several interlinked sources. First is the observation that many people report high levels of sleepiness during waking hours. For example, a 2011 study of Americans sleep habits found that 43 per cent of Americans between the ages of 13 and 64 said they rarely or never got a good night's sleep on weeknights. More than half (60 per cent) said that they experienced a sleep problem every night or almost every night (i.e., waking in the night, waking up too early, or feeling un-refreshed when they get up in the morning). Notably, the participants reported very active technology use in the hour before trying to sleep. Almost all of those surveyed (95 per cent) used some type of electronics, such as a television, computer, video

game or smart phone at least a few nights a week within the hour before bed. A similar picture has emerged in Europe. A 2002 study interviewing 18,980 randomly selected participants, representative of the general population of the U.K., Germany, Italy, Portugal, and Spain, found that excessive daytime sleepiness (i.e., having the tendency to fall asleep easily during the day or having periods of sudden and irresistible sleep during the day, at least 3 days weekly) was reported in 15 per cent of the sample, being highest in the U.K. and Germany, with the lowest sleep duration reported by U.K. participants (Ohayon, Priest, Zulley, Smirne, & Paiva, 2002). Further reports indicate, for example, that 10 per cent of middle-aged Finns were found to be excessively tired during the day; 9 per cent of Swedish adults were found to be suffering from excessive daytime sleepiness while in Poland, 21 per cent of adults reported to be moderately sleepy during the day (Martin, 2003). Indeed, the problem may have increased over time. While objective evidence regarding the historical changes in daytime sleep related function is difficult to find, there is some. A standard psychological test of personality, the Minnesota Multiphasic Personality Inventory (MMPI), was regularly administered to large numbers of Americans since the 1930s, and it has been shown that the proportion of men who felt tired during the day was significantly higher in later decades (i.e., 1980s) compared to earlier populations tested. However, some researchers believe the situation to be considerably worse. In a review undertaken by the U.S. National Commission on Sleep Disorders Research it was estimated that as many as 70 million Americans (more than a quarter of the population) were suffering from sleep deprivation or some type of sleep problem, resulting in a direct cost to the U.S. national health care bill of approximately 16 billion dollars a year. The Commission's report concluded that a convincing body of scientific evidence indicates that many Americans are severely sleep deprived and, therefore, excessively sleepy during the day, such that the deaths, illness, and damage due to sleep deprivation and sleep disorders represents a substantial problem for

American Society (Leger, 1994). More recent reports indicate significantly greater economic costs of sleep loss, including costs associated with absenteeism, disability, reduction or loss of productivity, industrial and motor vehicle accidents, hospitalisation, and health care utilisation, resulting in sleep loss becoming classified as an increasingly unmet public health problem (Colten & Altevogt, 2006; Hossain & Shapiro, 2002).

A further reason for considering sleep loss to be a common problem is that many of us experience less total sleep time than we want, or need. Conventional wisdom holds that we need approximately 8 hrs of sleep per night. The experimental evidence suggests that the underlying sleep tendency for a healthy adult (i.e., the amount of sleep that a person would take if completely liberated from work schedules and other constraints) is between 7.5 and 8.5 hrs each night (Bonnet & Arand, 1995; Kamdar, Kaplan, Kezirian, & Dement, 2004; Martin, 2003). For most, however, the reality falls short of this. Current figures suggest that six in ten Americans (63 per cent of those sampled) are getting less sleep that they feel allows them to function at their best on weeknights, sleeping an average of 6 hrs and 40 mins (National Sleep Foundation, 2011). Considering European averages, a recent survey reported that while over 5,500 British adults were found to sleep approximately 7 hrs a night, they still found it difficult to get out of bed in the morning (Foster & Roenneberg, 2012). The authors cite the possible source of the problem as “social jet lag” or the discrepancy between what our body clock wants us to do (optimum sleep duration) and what our social clock wants us to do (decrements to this optimum amount due to lifestyle demands).

While it has been argued that such decreases in sleep duration do not reflect a secular trend across different populations, it has been acknowledged that it is as yet unclear whether the proportions of short or long sleepers have increased over time, which may be of greater relevance (Bin, Marshall, & Glozier, 2012). Looking at sleep duration across the lifespan, some reports suggest the proportion of adult short sleepers (< 6 hrs per night) may be rising

(Grandner, 2012). In older populations, Banks et al. (2010) report data from wave 4 (2008–09) of the English Longitudinal Study of Ageing (ELSA), a prospective cohort study representative of older men and women living in England beginning in 2002, with participants being measured biannually thereafter. Sleep duration reported in ELSA was found to be 6 hrs 51 mins per night, while 10 per cent of participants reported to be short sleepers (5 hrs or less) and 10 per cent reported to be long sleepers (8 hrs or more). Similar trends are identifiable in younger cohorts. A recent study conducted in university students aged 17 to 30, including almost 17,500 participants, from 24 countries, including Ireland, found that while the majority of those sampled (63 per cent) reported average sleep time of 7 to 8 hrs, a sizable proportion (21 per cent) were short sleepers. Further, compared to a reference category of typical sleepers (7 - 8 hrs) per night, short sleepers defined as < 6 hrs (6 per cent of those sampled) or 6 – 7 hrs (15 per cent of those sampled) were more likely to report poor self-rated health than those who slept for 7 to 8 hrs a night (Steptoe, Peacey, & Wardle, 2006). The Asian countries in the survey, particularly Japan, Korea, and Thailand, were notable for having a high proportion of respondents with poor self-rated health as well as short average sleep durations.

In sum, the increased demands associated with a "24-hr society" may be marginalising sleep, leading to trends of chronic short sleep, yet sleep is not an optional behaviour. Nature imposes sleep upon us, such that it is a psychological and physiological imperative. It has been shown that lack of sleep makes us inefficient at work, dangerous behind the wheel of a car and emotionally unattractive to be with, damaging social relationships (Dinges, 2009; Kamphuis, Meerlo, Koolhaas, & Lancel, 2012). In short, it lowers the quality of our lives, increases accidents, while further evidence suggests sleep loss may also make us more vulnerable to certain illnesses.

*1.3. Epidemiological Research*

Due to the adverse changes in sleeping habits the question arises, which long-term health consequences might result from sleep deficiency? Obtaining sufficient sleep is increasingly becoming recognised as an important domain of healthy behaviour and epidemiological surveys, relating self-reported sleep duration to health, implicate poor sleep as a predictor of cardiovascular risk.

Over four decades of epidemiological evidence indicate that sleep duration is associated with mortality in a U-shaped manner, such that the lowest risk is most often found amongst those reporting sleep durations of 7 - 8 hrs (Grandner, Hale, Moore, & Patel, 2010). In general, it appears mortality risk increases with greater deviations (i.e., "shorter" and "longer" sleepers) from this 7 - 8 hrs, associated with greater mortality risk (Grandner & Drummond, 2007). In relation to "short sleepers", which were usually defined as < 6 or 7 hrs, a meta-analysis of epidemiological data revealed that the pooled relative risk (RR) of short sleep for cardiovascular related mortality was 1.06 and RR for long sleepers of 1.38 (Gallicchio & Kalesan, 2009). While longer sleep suggests an even greater RR compared to short, it is suggested that the risk associated with short and long sleep represent separate phenomena and should be considered separately (Bliwise & Young, 2007).

For example, sleep duration and quality have commonly been associated with blood pressure level. Cross-sectional epidemiologic evidence has generally found that self-reported short sleep durations are associated with higher blood pressure or higher prevalence of hypertension (Cappuccio et al., 2007; Choi et al., 2008; Gottlieb et al., 2006; Kawabe & Saito, 2008; Kotani, Saiga, Sakane, Mu, & Kurozawa, 2008; Stang, Moebus, Möhlenkamp, Erbel, & Jöckel, 2008). Gottlieb et al. (2006) hypothesised that as hypertension carries a high risk for cardiovascular disease, effects of short sleep duration on incidents of hypertension may increase the risk of cardiovascular disease and mortality. In a sample of 5,909

individuals, they found that those who reported < 6 hrs of sleep were indeed at an increased risk for hypertension. Two of these studies observed a significant association in women but not men (Cappuccio et al., 2007; Stang et al., 2008), while further studies found no association between sleep and blood pressure, including among elderly adults (van den Berg et al., 2007) and one among children aged 3 – 10 years (Bayer, Neuhauser, & von Kries, 2009); perhaps suggesting that associations between sleep and blood pressure may be modified by age. Cross-sectional data have also been collected using wrist actigraphy, as previously mentioned, an objective measure to estimate sleep duration. Wrist actigraphy was designed as an alternative assessment method (compared to participant self-report) to gather objective data on sleep-wake parameters (see Sadeh, Hauri, Kripke, & Lavie, 1995). The actigraph is a small watch-like device that records movements that can be worn throughout the sleep period. The presence of movement, as recorded by the device at wrist level, is interpreted as time awake, and the absence of movement as sleep time. Using this methodology, sleep duration was estimated from 3 to 6 days of wrist actigraphy in participants 35 – 50 years old (Knutson et al., 2009). It was found that verified shorter sleep durations and lower sleep maintenance (the percentage of time between sleep onset and sleep end that was spent sleeping) were both associated cross-sectionally with increases in blood pressure. Cumulatively, cross-sectional studies generally support a relationship between insufficient sleep and higher blood pressure level.

Several prospective epidemiologic studies have additionally examined cardiovascular outcomes in relation to sleep duration. Gangwisch et al. (2006) monitored 4,810 individuals who reported initial baseline measures of self-reported sleep duration and then screened for hypertension incidence (e.g., general practitioner diagnosis) over an 8- to 10- year period. They found that subjects between the ages of 32 and 59 years at baseline, who reported averaging ≤ 5 hrs of sleep per night, were at an increased risk for developing hypertension

over the follow-up period compared to those reporting sleeping 7 – 8 hrs per night, after adjusting for numerous potential confounds. In the aforementioned cross sectional study of adults aged 35-50 years using wrist actigraphy (Knutson et al., 2009), further prospective analyses of these data indicated that sleep duration was significantly associated with incident hypertension over a 5-year period. The odds ratio (OR) for shorter average sleep duration predicting hypertension was 1.37, suggesting that for every hour less sleep, there was a 37 per cent higher odds of incident hypertension. These researchers also examined incident coronary artery calcification (CAC), which is a predictor of the development of coronary heart disease (CHD). Results indicated a significant negative association between sleep duration and incidence of CAC. Longer measured sleep duration was associated with a decreased adjusted odds of incident calcification over 5 years (OR = 0.67 per hr), suggesting that every extra hour of sleep was associated with a 33 per cent lower odds of CAC (King et al., 2008). Similarly, research into incidence of non-fatal myocardial infarction (MI) or fatal CHD corroborates findings on incident hypertension and CAC. In a sample of 71,617 females, a baseline self-report assessment of sleep duration followed by a 10-year follow up period monitoring occurrence of CHD-related events, found that, after adjustment for age, short sleep duration (< 5 hrs) was associated with a significantly increased risk of both incident CHD-related events (Ayas, White, Al-Delaimy, et al., 2003).

Finally, recent meta-analyses have underscored the associations between short sleep durations and cardiovascular risk. In a systematic review and meta-analysis of the effects of sleep duration on cardiovascular diseases, Cappuccio et al. (2011), examining 15 separate prospective studies which included duration of sleep at baseline, with a follow-up period of > 3 years, found that short sleepers had a greater risk of CHD and stroke than those sleeping 7 – 8 hrs per night. Further, the most up-to-date evidence reported by Guo et al. (2013), investigating the associations between sleep duration and hypertension across 24 separate

examinations, suggested that while both shorter and longer sleep time was associated with increased risk of hypertension in cross sectional studies, only short sleep emerged as a significant predictor of greater risk for hypertension when examining pooled longitudinal data.

Overall, while much cross sectional and epidemiological evidence suggests that accumulation of short sleep over years may be associated with cardiovascular related risk, such as hypertension, the underlying mechanisms linking sleep loss to the progression of cardiovascular related disease are poorly understood (Faraut, Boudjeltia, Vanhamme, & Kerkhofs, 2012; Knutson, 2010). More rigorous studies are therefore required to fully understand this association. The majority of the observational studies described above had consistent findings, but we must still consider the methodological limitations of these investigations. Firstly, the vast majority of these studies relied on a self-reported measure of sleep duration, and as such, the accuracy is uncertain. Recent analysis comparing sleep durations estimated from wrist actigraphy to self-reported sleep in a sample of over 600 middle-aged adults indicated only moderate agreement between these measures (Lauderdale, Knutson, Yan, Liu, & Rathouz, 2008). In addition, there may be important mediating mechanisms that are not taken into account using epidemiological evidence. The experimental examination of the stress response, and the resulting activation of the classical stress systems, offers good research potential and clinically significant endpoints which align with the cardiovascular risks associated with short sleep; it is to this possible mediator of effects that we now turn.

### *1.4. Stress: A Psychological and Physiological Experience*

The stress response is commonly conceptualised as the psychological and physical response to perceived threat or challenge, which unfolds as a series of interacting psychological and physiological elements.

### *1.4.1. Physiological Responding*

The various physiological responses an individual experiences in response to threatening environmental influences was first described by Walter Bradford Cannon (Cannon, 1929b) in his description of the 'fight or flight' response to theat. Cannon's theory arose from his observation that when an animal was faced with a stressor in their environment, increases in heart rate (HR), blood pressure, glucose and respiration were evident. Such physiological changes were necessary for an adaptive response to the threat (resulting in the animal's preparation to either fight the source of the stress or flee from it), and rapid homeostasis (or stabilisation) of important physiological systems occurred once the threat was no long present. Hans Selye expanded on Cannon's earlier work, coining the term "stress", which he conceptualised as a non-specific physiological response to any kind of demand that an organism faces (Selye, 1956). The resulting physiological response(s) refer to Selye's characterization of the stress response as a "general adaptation syndrome", organised into three stages. The first stage is the general alarm reaction, during which numerous biological systems (e.g., cardiovascular and neuroendocrine systems) are activated in response to the stressor. The second stage would lead to resistance, with the activated biological systems returning to normal. However, if the stressful stimulus is maintained, the organism loses its resistance and enters a phase of exhaustion, regarded as the third stage of the syndrome.

### *1.4.2. Psychological Responding*

More recent conceptualisations of stress include Lazarus and Folkman's (1984) transactional model of stress. In essence, the theory postulated that it is not the stressor in isolation that determines its potential to cause harm or ill health; rather, it is a person's appraisal of the stressful situation, in addition to the coping mechanisms and resources

available to deal with the stressor, which determines the eventual final effect of the stressor challenge.

### *1.4.3. Allostasis and Allostatic Load*

While the Lazarus and Folkman model implicates the importance of the individual as influencing the nature and magnitude of the stress response, subjective experience does not always correlate with the output of physiological mediators of stress (e.g., no correlation observed between subjective and cortisol responses to stressful daily activities; Pottier et al., 2011). Therefore, while the subjective experience of stress is an important determinant, the measurement of the physiological responses of the body to environmental challenges constitute the primary means of connecting experience with resilience or the risk of disease.

The allostatic load model expands the stress-disease literature by proposing multi-systemic physiological dysregulation that contribute to disease risk (Juster, McEwen, & Lupien, 2010). The term 'allostasis', originally conceived by Sterling and Eyer (1988), literally means 'creating stability through change', with stability referring to homeostasis of the major physiological processes altered as a consequence of stressful challenge. Allostasis is adaptive in the short term (when balance is restored rapidly). However, as defined by McEwan and Stellar (1993), a maladaptive state, referred to as allostatic load, may result from the "cumulative strain on the body produced by repeated ups and downs of physiologic response, as well as the elevated activity of physiological systems under challenge…the impact of wear and tear on a number of organs and tissues, can predispose the organism to disease. We define this state of the organism as allostatic load" (p. 2094). Therefore, persistent and prolonged activation of physiological systems in response to stressors may result in 'allostatic load' or a wearing down of bodily systems due to constant activity. As summarised by McEwen and Seeman (1999), allostatic load may emerge from one of four sources: frequent exposure to stress; multiple exposure to novel stress in which habituation

does not occur, indicating lack of adaptation; or delays in recovery from stress (when the initial response is activated but doesn't subside in a timely manner. However, rather than operating independently, the systems may interact leading to synergistic effects on the body. The concepts of allostasis and allostatic load were therefore additionally introduced in recognition of the need to integrate the effects of stress on the entire body and have moved researchers away from a univariate approach (i.e., the examination of one marker of stress system activity) of understanding the effects of stress on the body, toward a more multivariate approach (i.e., the examination of multiple markers). Thus, the fourth potential source of allostatic load, results from an abnormal stress response from one system (e.g., neuroendocrine system) which leads another system (e.g., inflammatory) to overcompensate.

### *1.4.5. The Stressful Nature of Sleep Loss*

While a distinction is often made between stress and sleep loss as separate entities, for example, reports of psychological stress have been shown to contribute to sleep loss and insomnia (Âkerstedt, 2006), sleep loss and stress have similar effects on stress system functioning, with experimental evidence supporting the stressful nature of sleep loss. Sleep patterns have relationships with perceived stress and mood, observed in both older (Reynolds, Kupfer, Hoch, & Stack, 1986) and younger adults (Kamphuis et al., 2012). One recent study found that sleep-deprived participants reported greater subjective stress, in addition to affective changes (increased anxiety and anger) to laboratory stress following one night of total sleep deprivation (Minkel et al., 2012).

Further, various studies have shown activation of the classical physiological stress systems subsequent to reduced sleep time, such as the neuroendocrine release of cortisol (Meerlo, Sgoifo, & Suchecki, 2008). For example, a robust argument in favour of the stressful nature of sleep loss arises when we consider its effects on cortisol on the day following sleep reduction. Under regular sleep – wake conditions, the 24-hr profile of cortisol

in healthy adults displays an early morning maximum and declining levels throughout the day, with a trough centred around midnight and a rapid elevation in late sleep. In contrast, a state of sleep loss in healthy young men (observed across both partial and total sleep loss, in addition to semi-chronic curtailment of the sleep period) results in an elevation of evening cortisol levels the following day and in a delayed onset of the circadian trough of cortisol secretion (Leproult, Copinschi, Buxton, & VanCauter, 1997; Spiegel, Leproult, & Van Cauter, 1999). It has been suggested that such effects may be the result of an alteration (following sleep loss) in the rate of cortisol recovery from the early morning maximum (Van Reeth et al., 2000). Although the magnitude of the physiologic changes found in such short-term studies was modest, the changes provide a potential mechanism whereby long-term sleep loss may affect health through stress system dysregulation.

Additionally, McEwen (2006) specifically highlight sleep loss as potent physiological stressor, and as a primary candidate involved in the disruption of allostasis contributing to allostatic load, especially if sleep loss if allowed to continue over the long term. He suggests that relatively brief loss of sleep promotes an exacerbation of allostatic processes, with progressively more severe physiologic and behavioural consequences as the sleep loss is prolonged. Therefore, if an individual experiences repeated challenges to allostatic processes, as evidenced by the laboratory evidence under conditions of acute sleep loss, relative to periods of being fully rested, with continued challenge (i.e., chronic sleep loss) it is hypothesised that the resulting disruption to physiological allostasis would contribute to increased risk of stress related illness.

On the premise that human behaviour, including sleeping behaviour, is governed by that behaviours antecedents (i.e., increasing sleep drive balanced against lifestyle demands) and consequences (i.e., with shorter sleep durations, increased time awake but with indicated increased health risks), sleep, and an individual's choice (forced or otherwise) to engage in

this behaviour to a greater or lesser extent, is amenable to modification. Thus, stress responses, indicative of the physiological consequences of sleep loss, are of interest from a psychophysiological perspective, as if found to result in aversive outcomes (particularly in relation to cardiovascular health, as suggested by epidemiological evidence), strengthen the argument for the potential health benefits of limiting behaviours leading to short sleep durations.

As such, experimental evidence is in favour of the stressful nature of sleep loss, while examination of the classical stress systems, such as the cardiovascular system in response to such stress, provides an outcome variable that can be measured in both laboratory and field settings, allowing assessment of the disruption to stress processes. The following section will introduce physiological reactivity to stress, beginning with the cardiovascular system, and associated physiological stress systems will be addressed subsequently.

### *1.4.6. Stress-Induced Physiological Responses: The Reactivity Hypothesis*

Reactivity refers to change in physiological activity from rest (i.e., baseline) to stressful experience, and it is proposed that individuals who experience persistent and/or pronounced physiological responses to psychosocial stress are at a higher risk of developing cardiovascular disease (e.g., Obrist, 1981). As such, there is a marked distinction made between "healthy" and "pathologic" reactivity to stress. The shorter-term response to acute stress (as alluded to previously in terms of adaptive allostasis) is protective, and indeed necessary, for example, enhancing immune function, promoting memory of dangerous events, increasing blood pressure and HR to meet the physical and behavioural demands of fight or flight, and making fuel more readily available to sustain intensified activity (VanItallie, 2002). At the same time, over activity of the stress system contributes to the chronic wear and tear (McEwen, 2000) that render the organism more vulnerable to risk of illness. Indeed, when considering stress in the etiology of heart disease, in comparison with

more traditional risk factors (e.g., cholesterol), studies of both maladaptive acute and chronic stress effects on cardiovascular health, indicate risk levels comparable to those associated with elevated cholesterol (Dimsdale, 2008).

There is an additional "social dimension" to an individual's physiological response to stress (Van Cauter & Spiegel, 1999). Interpersonal laboratory stressors have often been shown to produce greater physiological responses than more cognitive tasks (Linden, Rutledge, & Con, 1998), while data supports the superior predictive power of social interaction tasks (versus non-social tasks) in predicting ambulatory cardiovascular functioning (Ewart & Kolodner, 1993; Linden & Con, 1994). Positive social interactions have also been shown to exert beneficial effects on physiological reactivity. For example, the availability of positive social interactions alongside exposure to acute social stress in the laboratory is associated with attenuated cortisol and cardiovascular responses (e.g., Ditzen et al., 2007). Such beneficial social interaction in the presence of stressful experience suggests advantageous pathways involving behavioural processes, including facilitation of positive health behaviours and psychological processes, linked to appraisals, emotions or moods (e.g., depression), and feelings of control (see Uchino, 2006). There is evidence linking social support to these psychological processes (Felsten & Wilcox, 1992; Golden et al., 2009), although direct evidence for their mediational role on health outcomes is lacking (House, 2001).

As a general proposition, the reactivity hypothesis is not specific with regard to the particular physiological processes that may be implicated in health risk (James, Gregg, Matyas, Hughes, & Howard, 2012), with research indicating the involvement of multiple (and multiplicative) hemodynamic and non-hemodynamic variables (e.g., Mullington, Haack, Toth, Serrador, & Meier-Ewert, 2009; Phillips & Hughes, 2011). Accordingly, researchers

routinely monitor several parameters simultaneously, with significant empirical effort being undertaken to examine cardiovascular reactivity (CVR) to stress.

### *1.5. The Cardiovascular Response to Stress*

The reactivity hypothesis is most commonly used in reference to blood pressure and HR reactivity. Such attention is in keeping with the empirical support for the notion that blood pressure level is a key predictor of cardiovascular disease, and that blood pressure-lowering regimens are important to cardiovascular disease protection (Czernichow et al., 2011).

Blood pressure represents the force (pressure) exerted by blood against the arterial walls during a cardiac cycle. The cardiac cycle (when blood is pumped out of the heart into the aorta and the pulmonary artery, before re-entering the heart from the venae cavae and the pulmonary veins), is commonly referred to as the heartbeat, while HR is the number of cycles occurring within a particular time frame. This cycle is divided into two phases – contraction (known as systole, when the heart contracts and forcefully ejects blood into the circulatory system, corresponding to the maximum period of pressure, termed systolic blood pressure [SBP]) and relaxation (known as diastole, characterised by filling of the ventricles, corresponding to the lowest period of pressure, termed diastolic blood pressure [DBP]). A normal resting HR is considered to be approximately 60 - 80 beats per min (bpm).

CVR can be defined as the arithmetic difference between baseline measures of cardiovascular function and the change in these measures brought about by an eliciting stimulus (i.e., a stressor; Allan & Scheidt, 1996). Designed for short-term use to deal with physical threats, the purpose of such "flight-or-flight" responses, as Cannon (1929a) described them, is to prepare the organism for immediate physical action. Increased cardiovascular activation (in addition to associated physiological processes such as neuroendocrine activity and cortisol release), facilitates the supplementary supply of

nutriments needed for such physical activity to support the requirements of fight or flight. However, as Turner (1994) notes, modern stressors, though still evoking physiological adjustments that are similar to those seen in response to physical threat, often are in excess of situational demands, with the result that the reactions shown in response to stressors may substantially exceed metabolic requirements. Therefore, such excess (or prolonged) responsivity is central to the CVR hypothesis, linking exaggerated cardiovascular reactions with elevated risk for hypertension and CHD (Manuck, 1994). In order to be a useable risk factor for disease, the reliability of CVR as a stable characteristic suggests there is moderate consistency in CVR over time and across tasks, and it is highly reliable within tasks (Kelsey, Ornduff, & Alpert, 2007). Moreover, the extensive epidemiological evidence of reduced sleep duration and increased risk for cardiovascular pathologies indicates a potential role of CVR in determining health risk.

### *1.5.1. Mechanisms of Action*

Several underlying mechanisms of action have been proposed. It has been suggested that repeated exposures to stressors (due to their metabolic disproportionality to physical demands) over time results in elevations in blood pressure and HR, leading to structural changes to the heart and blood vessels (Obrist, 1981). The repeated eliciting of such responses may contribute to cardiac and vascular hypertrophy (Lovallo & Gerin, 2003). For example, an exaggerated increase in SBP prolongs myocardial relaxation (corresponding to the aforementioned systole phase of the heart beat), resulting in an increase in left ventricular filling pressure. These changes occur before the development of left ventricular hypertrophy and likely represent early hypertensive heart disease (Kucukler, Yalçin, Abraham, & Garcia, 2011; Mottram, Haluska, Yuda, Leano, & Marwick, 2004). Consistently, psychological stress-induced increase in blood pressure has been demonstrated to be an independent contributor to left ventricular hypertrophy in those with increased resting blood pressure

(Munakata et al., 2002). In addition to structural adaptation, elevated reactivity has also been suggested to precipitate autoregulation (i.e., adjustments to the circulation in order to compensate for the over perfusion in cardiac activity, and associated blood flow beyond that necessary for the particular stressful challenge). These auto-regulatory adjustments can take the form of increased arterial resistance and the resetting of resting blood pressure levels (Carroll, 1992; Obrist, 1981).

While exaggerated cardiovascular response to stress has traditionally been the focus of the reactivity hypothesis, evidence has also emerged regarding the possibility that low cardiovascular responses may also be associated with poor health outcomes. This purported low cardiovascular response is represented by a cardiovascular stress response pattern that is lower than that seen during typical states of homeostatic function (Phillips, Ginty, & Hughes, 2013), and has been associated with a range of negative health states. For example, low CVR has been associated with smoking (e.g., Girdler, Jamner, Jarvik, Soles, & Shapiro, 1997), obesity (Carroll, Phillips, & Der, 2008), depression (e.g., York et al., 2007), poor self-rated health (Phillips, Der, & Carroll, 2009), and addiction (to exercise, alcohol, and other substances; e.g., Heaney, Ginty, Carroll, & Phillips, 2011; Lovallo, Dickensheets, Myers, Thomas, & Nixon, 2000). As one might expect, given the evidence supporting the reactivity hypothesis, the observed low stress responsivity has not been associated with increased levels of negative cardiovascular health outcomes. However recent data suggests low cardiovascular responses may underlie the Type D personality, shown to be a predictor of poor health outcomes in cardiac populations (Howard, Hughes, & James, 2011; O'Leary, Howard, Hughes, & James, 2013). As a result, both large and small cardiovascular stress reactions may be considered potentially maladaptive, though much remains to be clarified regarding the correlates of low cardiovascular responses relating to future cardiovascular health risk.

### *1.5.2. Cardiovascular Reactivity and Health*

A number of empirical studies have corroborated such interpretations by linking increased CVR to a number of indicators of subsequent longer term health and coronary disease. One relevant disease state is hypertension, clinically defined as a resting blood pressure of greater than 140/90 mmHg (Chobanian et al., 2003). However, given the continuous relations of blood pressure to cardiovascular risk, any definition of high blood pressure is at least somewhat arbitrary (Freitag & Vasan, 2003). Thus, increased resting blood pressure is a major independent risk factor for the future development of essential hypertension and CHD (i.e., Burt et al., 1995; Miura et al., 2001), even when increases are observed within the normotensive range (Treiber et al., 2003). In one meta-analysis of studies (results drawn from 31 separate cohorts composed of 169 associations between a reactivity score and future outcomes), Chida and Steptoe (2010) reported that those exhibiting higher reactivity scores had a 23 per cent increase in risk of hypertension; an association corroborated by others. Several studies have reported positive associations between reactivity and subsequent blood pressure levels in children, adolescents, and adults (Matthews et al., 2004; Matthews, Woodall, & Allen, 1993; Murphy, Alpert, Walker, & Willey, 2007). Although, others have failed to find strong support for the unique predictive ability of CVR. Carroll et al. (2001) reported only modest support for the relationship between laboratory reactivity and development of hypertension in a 10-year follow up. Nonetheless, further corroboration of the potential negative health effects of increased reactivity are provided by findings linking heightened CVR with cardiovascular risks such as atherosclerosis and increased left ventricular mass (LVM).

In addition to associations of increased CVR and elevated blood pressure, Chida and Steptoe (2010) found that greater reactivity to stress was associated longitudinally with poor cardiovascular status, increased LVM, subclinical atherosclerosis, and clinical cardiac events. While prediction of the development of actual cardiovascular disease is unclear, such

findings indicate that heightened cardiovascular responsivity appears to be a marker for, if not a causal factor in, the development and progression of cardiovascular disease (CVD). Furthermore, the specific physiological elements that underlie the overall blood pressure response may be of interest and may contribute to our understanding of increased cardiovascular response as a marker of disease risk.

### *1.6. Sleep Loss and Laboratory CVR*

As reviewed, recent epidemiological studies identify sleep loss as an important risk factor for cardiovascular health, including increased hypertension risk, yet the relations between sleep loss and cardiovascular activity remain equivocal. Some studies have reported that sleep reduction produces modest increases in blood pressure and/or HR (i.e., Lusardi et al., 1999; Muenter et al., 2000), while others have reported little or no effects (Meney, Waterhouse, Atkinson, Reilly, & Davenne, 1998; Miró, Cano-Lozano, & Buela-Casal, 2002; Smith & Maben, 1993).

Evidence for the effects of sleep loss on CVR to laboratory stress is equally inconsistent. Kato et al. (2000) examined the effects of one night of normal rest compared to one night of total sleep deprivation in a sample of eight healthy adults, finding that sleep loss resulted in a modest increase in resting blood pressure level on the morning after sleep deprivation. However, HR and blood pressure to novel acute stressful stimuli (i.e., timed mental subtraction and cold pressor task) were unchanged following sleep loss. The authors concluded that sleep loss does not potentiate cardiovascular responses to stressful stimuli. On the other hand, the mental arithmetic task used as part of the stress protocol failed to elicit significant increases in HR or blood pressure in the control (i.e., normal sleep) condition, thereby limiting interpretation. Nonetheless, a more recent study, despite reporting robust increases in both HR and blood pressure during laboratory stress at rest, also reported minimal cardiovascular change as a result of sleep loss. Using a comparable methodology to

the Kato et al. investigation, Yang et al. (2012), in a sample of 28 healthy individuals, reported no difference in mean arterial pressure, and only marginally significant change to HR, in response to laboratory stress (mental arithmetic) comparing a rested and sleep deprived condition.

There are, however, some recent data to suggest that sleep-related increases in blood pressure reactivity may be evident in cardiovascular response to alternate laboratory stressors. Franzen et al. (2011) exposed 20 healthy young adults to evaluative social stress in the laboratory following rest and again following total sleep deprivation, finding significantly increased SBP response to stress in the sleep loss condition. While such data suggest sleep-related increases in blood pressure may operate through additive effects of stress exposures which are specifically evaluative in nature, in comparison to mental stress alone, taken together, the influence of sleep loss on CVR to laboratory stress remains unresolved. As the underlying mechanisms upon which sleep may act to influence health are, at present, poorly understood (Faraut et al., 2012), this warrants further investigation, examining if sleep loss affects health through psychophysiological pathways, such as CVR to laboratory challenge.

### *1.7. Sleep Loss and Ambulatory CVR*

Examining the possible mechanisms linking sleep disturbances and cardiovascular disease, ambulatory blood pressure (ABP) assessment may offer significant exploratory advantage over clinical/laboratory measurements. Notably, ambulatory assessment provides a profile of cardiovascular activity away from clinical/laboratory settings, thereby providing a representation of blood pressure response in non-artificial settings. Further, ambulant monitoring additionally typically involves repeated assessment, over 24-hr periods, thereby increasing reliability of observed effects. Moreover, a recent review documented the superiority of ambulatory over clinic blood pressure measurements in the prediction of major cardiovascular events (Verdecchia, Angeli, Gattobigio, & Porcellati, 2003). Such superiority

in the prediction not only of end-organ damage but also of clinical events has also been emphasised by the results of the Systolic Hypertension in Europe (Syst-Eur) trial. In this study, the incidence of cardiovascular events and mortality rate were more closely predicted over a follow-up period by ABP values compared to office blood pressure measurements (Staessen et al., 1999).

ABP monitoring devices, using either a microphone to measure Korotkoff sounds (arterial sounds heard through a stethoscope applied to the brachial artery distal to a blood pressure cuff) or a cuff that senses arterial waves using oscillometric techniques, allows multiple readings during all of a patient's ambulant activities. The monitor is worn continuously throughout the daytime and nighttime period and returns measurements at pre-defined intervals. In the limited number of studies that have examined the effects of sleep loss using ABP monitoring, findings have been inconsistent. While some data support cardiovascular increases (SBP and DBP) following sleep loss (Tochikubo, Ikeda, Miyajima, & Ishii, 1996), others show a mixture of both increases and decreases. Lusardi et al. (1996) found that following a period of reduced sleep (43 per cent of usual sleep time) in young normotensive participants, ambulatory blood pressure monitoring revealed decreased SBP and DBP during the nocturnal period, in addition to increased SBP and HR post-awakening, compared to a night of full rest.

However, a shortcoming of the present state of knowledge is that few controlled studies have examined whether sleep loss affects not only the basal activity of stress systems (i.e., mean daytime, nighttime, or 24-hr activity) but, also, their *reactivity* to a "real" stressor (Meerlo et al., 2008). Thus, an important issue yet to be sufficiently examined regarding previous inconsistent results relates to measurement strategy. Ambulatory monitoring allows examination of blood pressure and HR responses during daily activities, thereby allowing assessment of reactivity to ambulant stimuli (Pickering, 1996). Ambulatory assessment has

also become a frequently used method of assessing the generalisability of laboratory-based CVR to real-life settings (i.e., Ottaviani, Shapiro, Goldstein, James, & Weiss, 2006). In this way, blood pressure change (if any) observed under laboratory conditions as a result of sleep loss can be further examined in relation to more ecologically valid ambulant contexts.

### *1.7.1. Generalising Beyond the Laboratory*

A key assumption of the reactivity hypothesis is that response to acute stress tasks in the laboratory reflects how an individual would typically respond to stress in real life (Turner, Girdler, Sherwood, & Light, 1990), such that, over time, repeated reactivity leads to increased risk of disease. For example, Kamarck, Schwartz, Janicki, Shiffman, and Raynor (2003) examined CVR to a battery of laboratory stressors (e.g., mental arithmetic or performance of a speech) in a sample of over 300 healthy participants, finding SBP and DBP CVR, as measured in the laboratory, predicted ambulatory responses to demanding or uncontrollable activities in daily life. In a further review, Zanstra and Johnston (2011) concluded that cardiovascular response to a battery of laboratory stressors was related to stressor responses in real life, when the latter was assessed by examining the response to an objective stressor or stress measured using self-report. Identification of events or behaviours that are associated with increases in cardiovascular responses in the field, which are, objectively, sufficiently frequent or intense to be likely to produce a long-term effect on the cardiovascular system, is therefore essential to the examination of cardiovascular correlates of common and naturally occurring activities.

One such activity, interpersonal contact, has been suggested as a useful task for investigating CVR in the field (Bastard et al., 1999; Brondolo et al., 2009; Holt-Lunstad, Jones, & Birmingham, 2009). For example, using a population of undergraduate students, Lehman and Conley (2010) found that momentary reports of evaluative stress during naturally occurring interactions were associated with concurrent increases in both SBP and

DBP. As such, examining the effect sleep loss has on cardiovascular responses to such activities would increase our currently limited understanding of the effects shorter sleep durations have in the "real-world", in domains such as daily functioning (Grandner, Patel, Gehrman, Perlis, & Pack, 2010). Although these two types of cardiovascular responses (laboratory and naturalistic) are correlated, it is often the case that the responses obtained in real life are larger than those obtained in the laboratory, further underscoring the ecological validity of these real life stressors (Phillips & Hughes, 2011; Zanstra & Johnston, 2011).

It is therefore indicated that laboratory-based stressor responses at least partially reflect responses in everyday life and that ambulatory stress reactions, such as reactivity to naturally-occurring interpersonal interactions, may be additionally studied as part of a real life stress paradigm. As sleep loss has been shown to increase negative emotionality surrounding interpersonal interaction (Kahn-Greene, Lipizzi, Conrad, Kamimori, & Killgore, 2006), and interpersonal contact has been suggested as a useful task for examining CVR in ambulant settings, an examination of periods of stress and interpersonal contact while rested and sleep restricted, and resulting effects on ambulatory cardiovascular response, would elucidate the generalisability of stress reactivity observed under laboratory conditions. Further, such naturalistic observations, yet to be tested satisfactorily, may be key to demonstrating whether shorter sleep affects not only mean levels of ambulatory cardiovascular activity, but also reactivity to ambulatory stimuli (Meerlo et al., 2008).

### *1.7.3. Nocturnal Blood Pressure*

Ambulatory cardiovascular monitoring can also be used to identify abnormal patterns of nocturnal blood pressure, and in so doing, demonstrate a number of patterns of blood pressure response that may be relevant to health outcomes. Ambulatory measurement is the only non-invasive technique that permits blood pressure to be monitored during the night, permitting examination of changes associated with the sleep-wake cycle. Daytime and

nightime, for the purposes ambulant classification, have been defined as the waking and sleeping periods as measured using participant self-report (or through defined fixed time intervals ; Rosansky, Menachery, Wagner, & Jackson, 1995).

In normotensive individuals, sleep is associated with reduced blood pressure, referred to as the "dipping" phenomenon, whereby SBP and DBP may decline by 10 per cent to 20 per cent during sleep (Rafey, 2009). The "non-dippers" (those who fail to show sufficient decline in nocturnal blood pressure) vs." dippers" (those demonstrating more typical blood pressure declines) classification was first introduced by O'Brien et al. (1988) who reported a more frequent history of stroke in non-dippers compared to dippers. Such classification was based on the hypothesis that target organ damage and prognosis are worse when the blood pressure load is persistent throughout the entire 24-hr period, compared to being limited to the daytime hours only (Verdecchia, 2000). While much attention has been paid to this binary classification, separating individuals into those who demonstrate/fail to demonstrate a blood pressure decline over the nighttime period, individuals demonstrating a marked nocturnal fall in blood pressure, known as "extreme" dippers, have also been defined (O'Brien et al., 2000). Such responses are characterised by marked reduction in nocturnal blood pressure (i.e., greater than 20 per cent decline in blood pressure relative to daytime values; Kario & Shimada, 2004).

While the categorisation of continuous variables is common in the social sciences (Taylor, West, & Aiken, 2006), the dipper/non-dipper/extreme dipper classification has been criticised because it implies an arbitrary di- or tri-chotomisation of a continuous variable (i.e., the day−night difference in blood pressure), potentially leading to loss of valuable information (MacCallum, Zhang, Preacher, & Rucker, 2002) and because the definitions of daytime and nighttime (i.e., diary vs. fixed time intervals) have differed across studies. However, such classification appears to be useful from a clinical perspective. Alterations in

nocturnal dipping pattern may be seen in healthy normotensive individuals (i.e., Aranda et al., 2010), but is relatively more prevalent among those with various disease states (Lorendo, Nelesen, Ancoli-lsrael, & Dimsdale, 2004). Supportive evidence from prospective data indicate hypertensive patients who are non-dippers are at greater risk of cardiovascular morbidity than dippers (Verdecchia et al., 1994). There is also evidence that hypertensive patients who are extreme dippers are at increased risk of ischemic stroke, possibly as a result of cerebral hypoperfusion due to excessive nocturnal blood pressure fall (Kario et al., 2001). Data also suggest (again among hypertensive patients), extreme dippers have more marked cerebrovascular damage than dippers (Rifai & Ridker, 2001) and increased arterial stiffness in normotensive individuals with risk components of the Metabolic Syndrome (Epstein & Ross, 1999).

Dipping pattern could be influenced by inadequate sleep, and associated sleep disorders (Matthews et al., 2008). Amongst studies of normotensive and hypertensive men and women, non-dippers had more night time movement as measured by actigraphy than did dippers (Herity, 2000; Leary, Donnan, MacDonald, & Murphy, 2000). Schillaci at al. (2007) additionally reported that individuals who reported longer duration of sleep also had greater blood pressure dipping from day to night.

To summarise, evidence suggests that ABP is a strong predictor of cardiovascular risk, while nocturnal blood pressure measured by ABP is also a sensitive predictor of cardiovascular outcome amongst at risk populations (Dolan et al., 2005), suggesting that the measurement of nighttime blood pressure could be an important part of cardiovascular assessment (O'Brien, 2010). As such, assessing the effects of sleep loss across both laboratory and ambulatory settings allows determination of effects to a greater degree of certainty, as opposed to laboratory assessment alone.

*1.7.4. Hemodynamic Profile*

It is known that changes in overall blood pressure response (i.e., laboratory or ambulatory) are underpinned by changes in underlying hemodynamic variables. Cardiac output (CO; the volume of blood pumped from the heart within a certain timeframe, usually one minute, which is the product of HR and stroke volume [volume of blood pumped out of the heart with each beat]) and total peripheral resistance (TPR; overall resistance to blood flow relating to all peripheral vasculature in the systemic circulation), and changes therein, bring about overall (SBP/DBP) blood pressure change (Turner, Sherwood, & Light, 1994). A number of researchers have scrutinised these underlying determinants in an effort to classify individuals based on their pattern of reactivity to stress. For example, Girdler et al. (1990) classified participants' response to laboratory stressors as either "myocardial" or "vascular" responses on the basis of increases in CO and TPR, respectively. Reactivity patterns of CO and TPR have also been found to be stable over time for certain tasks, leading to the conclusion that individual differences in hemodynamic response patterns may be implicated in cardiovascular pathology (Gregg, James, Matyas, & Thorsteinsson, 1999; Kasprowicz, Manuck, Malkoff, & Krantz, 1990; Lawler et al., 2001).

However, previous methods used in the classifying of individuals as myocardial or vascular reactors have varied between studies. Additionally, previous methods have not accounted for the reciprocal relationship between CO and TPR. It is known, for example, that an increase in one parameter is usually accompanied by a relative decrease in the other (Guyton & Hall, 1997). In response to these shortcomings, Gregg, Matyas, and James (2002) proposed a model that explains variations in blood pressure in terms of a dynamic compensatory relationship between CO and TPR (see James et al., 2012; James & Gregg, 2004b; Ottaviani et al., 2006). Thusly defined, overall blood pressure changes can be interpreted as myocardial (reflecting change in CO accompanied by insufficient

compensatory change in TPR) or vascular (reflecting change in TPR accompanied by insufficient compensatory change in CO). Mixed responses have also been classified, identified as those who show equivalent change in both CO and TPR.

Furthermore, although change in blood pressure requires change to either CO or TPR or both, the underlying relationship between CO and TPR can undergo marked change without observable significant change in overall blood pressure level. A difficulty with previous approaches was that they confounded two separate concepts, compensation deficit (CD) and hemodynamic profile (HP), which can be independently expressed. CD measures the extent to which CO and TPR compensate, thereby indicating degree of change in blood pressure (increase, decrease, or no change). On the other hand, HP measures the nature the compensation, whether it is myocardial (CO predominates) or vascular (TPR predominates) or both (i.e., a "mixed response" involving equal amounts of change in CO and TPR). The validity of the model originally proposed (Gregg et al., 2002), has been subsequently confirmed (James & Gregg, 2004b; Ottaviani et al., 2006). As such, even small (or non-significant) shifts in overall blood pressure can mask more vigorous change at the hemodynamic level, with the result that non-significant between-group differences in overall blood pressure (i.e., between rested and sleep restricted participants) may be characterised by significant between-group differences in hemodynamic patterning.

The importance of examining such underlying processes is that HP appears to be implicated in the development of cardiovascular pathology. Specifically, it is believed that blood pressure changes characterised as vascular confer increased risk of disease (i.e., Obrist, 1982). Elevated TPR accompanied by normal CO represents a defining characteristic of hypertension (Julius, 1988). In a prospective study of young (17 - 29 year old) hypertensive males, Lund-Johansen (1991) reported that subjects with high cardiac index (relating CO to the size of the individual)/normal TPR pattern, changed direction, to a low cardiac index/high

resistance pattern, at 10- and 20-year follow up. Such data implicate restructuring of hemodynamic patterns over time, accounting for the appearance of the high TPR/ low CO (i.e., vascular) pattern noted in established hypertension. As such, examination of these parameters may reveal critical pathways by which sleep loss influences cardiovascular stress responses, with data indicating these variables to be sensitive to experimental sleep reduction in the laboratory (James & Gregg, 2004b).

### *1.8. Cardiovascular System and Stress Response: Summary*

In sum, excess (or prolonged) cardiovascular responsivity (often in excess of situational demands) is central to the CVR hypothesis, linking exaggerated cardiovascular reactions with elevated risk for hypertension and CHD (Manuck, 1994). Recent epidemiological studies identify sleep loss as an important risk factor for cardiovascular health, including increased hypertension risk, yet the relations between sleep loss and cardiovascular activity remain equivocal. While some studies have reported that sleep loss produces modest increases in laboratory measures of blood pressure and/or HR, with some recent data to suggest that sleep-related increases in blood pressure reactivity may be evident in cardiovascular response to evaluative social stressors, the findings remain as yet inconclusive.

ABP may offer advantage over laboratory measurements, providing a profile of repeated assessments of cardiovascular activity away from laboratory settings. Examination of events or behaviours which are associated with increases in cardiovascular responses in the field, such as interpersonal contact, would elucidate the generalisability of stress reactivity observed under laboratory conditions. Further, a shortage of literature exists to help us understand the effects of shorter sleep durations in the "real-world", in domains such as daily functioning (Grandner, Patel, et al., 2010). Such naturalistic observations may be important to demonstrating whether sleep loss influences reactivity to commonly encountered ambulant

stimuli. Ambulatory cardiovascular monitoring can also be used to identify patterns of nocturnal blood pressure, screening for patterns of blood pressure response that may be relevant to health outcomes.

It is known that changes in overall blood pressure response are underpinned by changes in underlying hemodynamic variables. Examination of CO and TPR, which together mediate changes in blood pressure, allows researchers to examine in greater detail the cardiovascular response to stress, with previous data revealing these variables to be sensitive to experimental sleep reduction in the laboratory (James & Gregg, 2004b).

Examining the effect sleep loss has on both laboratory and ambulant blood pressure offers a useful direction in the examination of the influence of sleep loss on cardiovascular health outcomes. In particular, consideration of the cardiovascular response after presentation of a stressor (both in the laboratory or naturally occurring in the field) combined with examination of the underlying hemodynamic changes mediating the blood pressures response, may extend our understanding of the potential mechanisms by which long term sleep loss may, as indicated by epidemiological data, confer increased risk of damage to the cardiovascular system.

### *1.9. Taking a Multivariate View: Stress and Health*

As previously noted in relation to cardiovascular stress responses, the reactivity hypothesis is not specific with regard to the particular physiological processes which may be implicated in health risk. Consistently, research indicates the involvement of multiple physiological biomarkers. A biomarker is defined as a biological indicator, such as blood or saliva, that reflects underlying physiological processes, including both normative processes and pathogenic states (Baum & Grunberg, 1995). For example, as previously outlined, elevated blood pressure responses (when persistent) are considered a biomarker of cardiovascular disease. Similarly, elevated pro-inflammatory cytokines are biomarkers of

inflammation and elevated body temperature is a biomarker of infection. Multi-biomarker assessments have become increasingly popular among psychophysiological researchers, as a way of linking behavioural, environmental, and social factors to an individual's health and well-being, with a greater degree of certainty (Wang et al., 2006; Zethelius et al., 2008). Moreover, such integrative research was underlined by the Committee on Future Directions for Behavioural and Social Sciences Research at the National Institutes of Health (Singer & Ryff, 2001). Highlighting areas of scientific opportunity where significant investment is most likely to improve both U.S. and global health outcomes, the meeting particularly encouraged research that assesses multiple behavioural and psychosocial pathways to health and well-being, a task that requires an integration of biological and social science.

### *1.10. The Neuroendocrine Response to Stress*

The term 'neuroendocrine' classically refers to hormone signalling involving the hypothalamus, pituitary gland, and peripheral body systems. In stress responding, the autonomic sympatho-adrenal-medullary (SAM) system and the hypothalamic-pituitary-adrenal (HPA) axis are traditionally considered to be the main neuroendocrine systems involved in the integrated stress response (Axelrod & Reisine, 1984). The effects of sleep loss on the activation of these systems and the release of stress hormones (as illustrated in Figure 1, and overviewed below) may have direct functional consequences for the way we deal with everyday challenges (Meerlo et al., 2008), while chronic activation of the neuroendocrine systems has been associated with the development of various disease states, including cardiovascular disease.

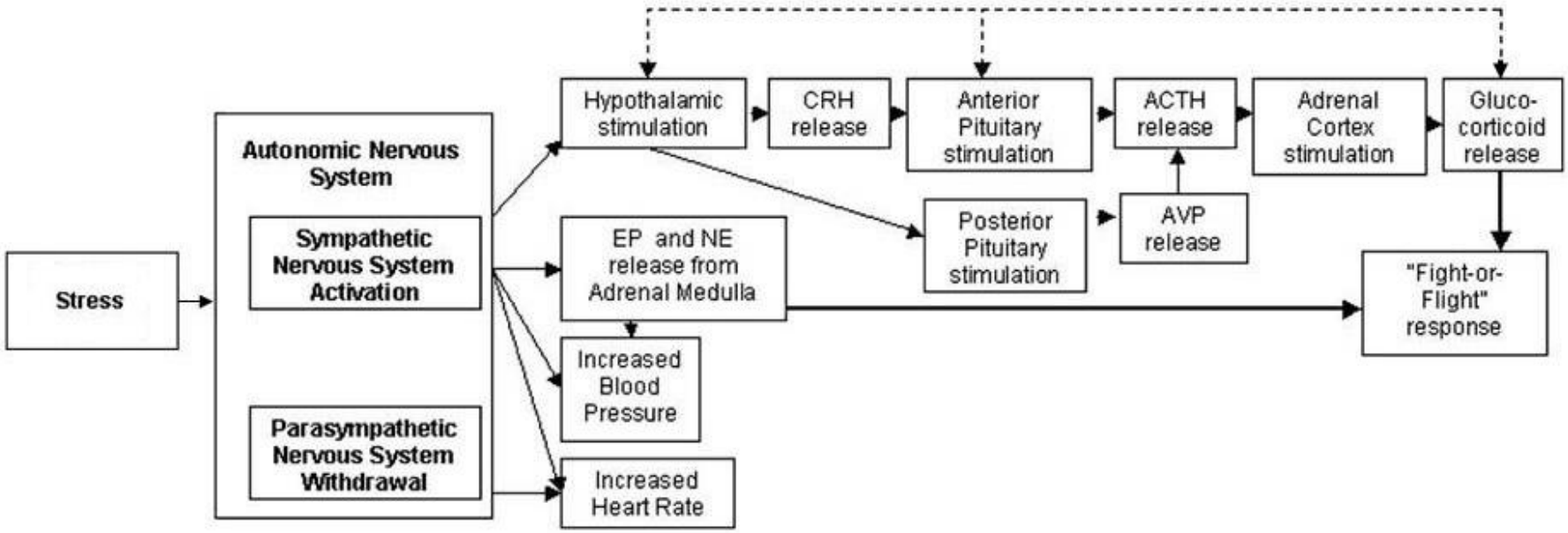


*Figure 1.* A simplified representation of components of the autonomic sympatho-adrenal-medullary axis and the hypothalamic-pituitary-adrenal axis stress systems. CRH = corticotrophin-releasing hormone, ACTH = adrenocorticotropin hormone, EP = epinephrine, NE = norepinephrine, AVP = arginine vasopressin. Solid lines represent direct or indirect stimulatory pathways. Dashed lines represent direct or indirect inhibitory pathways. (Adapted from Klein and Corwin, 2002).

*1.10.1. Sympatho-Adrenal-Medullary (SAM) Axis*

Described by Cannon (1929a), in specifying the "fight-or-flight" response, hormones first became thought of as physiological biomarkers of stress responding. As outlined by Piazza et al. (2010), the fight-or-flight response begins with the immediate activation of the sympathetic branch of the autonomic nervous system (SNS), which stimulates the release of the catecholamines, epinephrine (EP) and norepinephrine (NE), from sympathetic neurons and the adrenal medulla (for review see Klein & Corwin, 2002); this SNS-stimulated release of EP and NE from the adrenal medulla is also known as the SAM axis. As nervous impulses directly stimulate the adrenal medulla, the SAM axis has much faster and more immediate effects than the slower acting HPA axis. Known effects of EP and NE include decreased blood flow to the organs of the gastrointestinal tract, the skin, and the kidneys. This selective decrease in blood flow to some organs, ensures maximum blood flow to other areas, such as the brain, heart, and skeletal muscles when a stressor is encountered, allowing effective behavioural "fight-or-flight" responding.

Circulating or excreted levels of EP and NE, which are metabolised quickly and indicate immediate changes in SAM axis activation, are the primary biomarkers of SAM axis activity and can be measured reliably in urine, plasma, and cerebrospinal fluid samples (Christensen, Vestergaard, Sørensen, & Rafaelsen, 2007; Westermann, Hubl, Kaiser, & Salewski, 2002). However, venipuncture is a comparatively invasive procedure, placing considerable burden on research participants (and research designs), requiring specialised phlebotomy personnel. Additionally, as catecholamines are secreted slowly while the bladder fills, urinary catecholamine assessments provide a relatively static index of SAM axis activity, which makes it difficult to examine the impact of stressor onset and cessation (Baum & Grunberg, 1995).

As a substance that can be relatively easily and cost-effectively assessed, a growing literature suggests that the salivary enzyme α-amylase (sAA) may serve as a minimally invasive surrogate marker of SAM activity (for reviews see Granger et al., 2006; Nater & Rohleder, 2009a). Based on early observations by Gilman et al. (1979) that sAA increased in response to stressful challenge, Chatterton et al. (1996) reported a significant positive correlation between sAA and plasma NE and EP in response to physical and some psychological stressors, suggesting the possible use of sAA as a marker of SNS and SAM system activation. Since then, numerous studies have confirmed the responsivity of sAA to psychosocial stress, in addition to physical exercise, while additional evidence for the association between amylase responses and sympathetic activation have been reported (for a review see; Nater & Rohleder, 2009a). The rationale behind these associations is that catecholamine release in response to SNS activation stimulates salivary gland receptors that, in turn, alter activity of these glands, and in so doing, alter the release of certain markers (Granger et al., 2006). Although the main function of sAA is the enzymatic digestion of carbohydrates (Rohleder & Nater, 2009), sAA appears to be a viable surrogate marker of SAM axis activation, in its parallel of stress-related increases in NE and EP (Chatterton et al., 1996; Nater, Ditzen, & Ehlert, 2012; Rohleder, Nater, Wolf, Ehlert, & Kirschbaum, 2004). In a recent study, 66 healthy participants undertook a laboratory social stress protocol (Trier Social Stress Test [TSST]), while saliva and blood samples were taken for analysis of sAA activity and NE/EP concentration, respectively. sAA, NE, and EP showed significant increases in response to the acute stress induction. Further, controlling for a number of confounds, stress responses in sAA significantly predicted stress responses in plasma NE (Thoma, Kirschbaum, Wolf, & Rohleder, 2012). With regard to diurnal patterns, sAA exhibits moderately low levels upon awakening, dropping briefly at 30 mins post-awakening

and increases gradually throughout the day (Nater, Rohleder, Schlotz, Ehlert, & Kirschbaum, 2007).

### *1.10.2. Stress and the SAM Axis*

On encountering a stressor, NE slows digestion and gastrointestinal motility, increases plasma glucose levels, and dilates pupils, whereas EP increases HR and cardiac contractility, relaxes smooth muscle, and increases blood pressure and glucose release. It has been noted that the rise in catecholamine levels depends on stressor severity, doubling in their resting levels during daily activities, and rising to three to five times their resting levels during moderately severe stressors (Kvetnansky, Sabban, & Palkovits, 2009). These catecholamines, and their concomitant effects on other physiological functions such as blood pressure and HR, serve as objective sympathetic indicators of the stress an individual is exposed to. Although SAM axis arousal is necessary in the context of acutely stressful situations, continual activation of this system may ultimately arise in maladaptation (McEwen, 2000).

### *1.10.3. Hypothalamic-Pituitary-Adrenal (HPA) Axis*

Whereas SAM axis activation is designed for an immediate response to threat, HPA axis activation is a relatively more prolonged hormonal response, that is observed approximately 15 – 20 mins following stressor onset (see review by Dickerson & Kemeny, 2004). One of the primary biomarkers of the HPA axis is cortisol. The secretion of cortisol is triggered once a stressor is perceived by the cerebral cortex, which alerts the neurons of the paraventricular nucleus of the hypothalamus to release corticotrophin-releasing hormone (CRH). CRH travels to the pituitary gland, where it stimulates the release of the adrenocorticotropin hormone (ACTH), as well as arginine vasopressin (AVP). Although AVP acts centrally to support the fight-or-flight response, ACTH circulates to the cortex of the adrenal glands to stimulate glucocorticoid release, including corticosteroids (e.g., cortisol; for

review see Kudielka & Kirschbaum, 2005). This cycle creates a negative feedback loop, in which increasing cortisol levels then go on to hinder further production of CRH and ACTH, in a circuit known as the hypothalamic-pituitary-adrenal (HPA) axis. Eventually, cortisol mobilizes energy stores, serves as an anti-inflammatory hormone, and communicates with the immune system (Dickerson & Kemeny, 2004).

Secretion of cortisol follows a circadian pattern, with an initial peak in the morning (peaking 20 - 45 mins post-awakening) gradually decreasing throughout the remainder of the day. There are two different forms of cortisol in the body; "free" and "bound". Bound (to proteins) cortisol is found in serum and accounts for the largest proportion of cortisol in the body. As changes in the binding proteins can alter measured serum total cortisol concentrations, free cortisol, found in other bodily fluids such as saliva and urine, is a purer measure of cortisol, as it is not bound to proteins (Hamrahian, Oseni, & Arafah, 2004).

### *1.10.4. Stress and the HPA Axis*

HPA axis activation is adaptive in the short term, but repeated or prolonged activation can have detrimental effects on health, and lead to a dysregulation of the negative feedback loop. Specifically, prolonged exposure to stressors appears to uncouple cortisol from its ability to inhibit further CRH and ACTH secretion, which leads to an overproduction of cortisol (Dickerson & Kemeny, 2004). Elevations in cortisol, in turn, are associated with chronic health problems, psychological disorders, and problems with memory, learning, and attention (Sapolsky, 2000). The possible role of cortisol in the development of central adiposity and the development of cardiovascular disease has specifically been highlighted. Prompted by the relationship between abdominal obesity and excess cortisol, as a characteristic of Cushing's syndrome, fuelled speculation that chronic stress or dysregulation of the HPA axis with excessive glucocorticoid production, may also lead to central adiposity with its related adverse health consequences see (see VanItallie, 2002). Because CRH itself

has anorexogenic properties, an exaggerated release of cortisol in response to CRH, followed by negative feedback inhibition of synthesis and/or release of CRH from the hypothalamus with increased cortisol levels, may play a role in the mishandling of fat associated with excess cortisol (Raber, 1998).

Conversely, reductions in cortisol have also been associated with negative health. The HPA axis is vital for supporting normal physiological functions and, notably, is an important regulator of other physiological systems. For example, cortisol can inhibit many aspects of immune system functioning. It can be considered the body's own natural anti-inflammatory agent, because it can preferentially inhibit proteins that play a central role in regulating inflammation (Dickerson & Kemeny, 2004), itself associated in the etiology of negative cardiovascular health (Libby, 2002). McEwen et al. (1999) also give the example of reduced cortisol secretion in response to stress, as one of the potential contributory factors to allostatic load; resulting in the secretion of pro-inflammatory cytokines (which are normally counter regulated by cortisol). It is therefore important to consider increases or decreases in stress system (e.g., HPA axis) activity in the context of simultaneous activity of associated systems, to enable greater comprehension of potential effects on adaptive functioning.

### *1.11. Sleep Loss and Stress Axes Activity*

*1.11.1. Sleep Loss and the Sympatho-Adrenal-Medullary (SAM) Axis*: Previous strategies, testing the relationship between sleep and sympathetic activity, have typically employed protocols that were prolonged in duration, relied on assessment of urinary catecholamines (e.g., Müller, Riemann, Berger, & Müller, 1993) or involved a single plasma catecholamine determination after a night of total sleep loss (Chen, 1991). However, effects on sympathetic activity may be observed following a single night of partial sleep loss. In a study by Irwin et al. (1999) 17 male volunteers were exposed to baseline rested sleep on one night and again to partial sleep loss (awake from 3am – 6am) on a second night. Sleep was

monitored somnopolygraphically and NE and EP were assessed by blood draw. The results indicated that sleep onset was associated with a decline in plasma levels of NE and EP, with a nadir occurring 1 hr after sleep onset. In contrast, partial sleep loss resulted in increases in both markers. Such results suggest that even brief disruption of sleep can alter biomarkers of SAM stress activity. However, as blood draw is associated with significant stress reactions (Meeran, Hattersley, Mould, & Bloom, 1993), the observed effects may include extraneous effects of sampling method used. Saliva sampling has the advantage that it is non-invasive, making multiple sampling easy and, relatively, stress free. As non-invasiveness has been outlined as an important premise in stress research (Gröschl, 2008), to rule out the confounding effects of additional stress induced by blood draw, salivary amylase may be assayed as an relatively non-invasive index of the SAM system (as has been done previously; Thoma et al., 2012).

Recent findings have begun to emerge which suggest sAA may also be a useful marker in the context of sleep research (Nater & Rohleder, 2009a), especially in relation to the possible sensitivity of sAA to sleep loss (Seugnet, Boero, Gottschalk, Duntley, & Shaw, 2006). However, amongst the small number of studies that have reported on sleep-related variables and sAA response, findings have been inconsistent. For example, resting levels of sAA (measured after awakening) in healthy young adults, were found to be unrelated to self-reported awakening time, sleep duration or sleep quality (Nater et al., 2007). Alternatively, others have shown that when sleep duration is experimentally manipulated, the greater the sleep duration prior to a morning assessment of sAA (no sleep, 3 hrs sleep, 7 hrs sleep), the lower the observed α-amylase concentration (Figueiro & Rea, 2011). Recently, poor sleep efficiency (but not duration) has also been associated with higher social stress related sAA activity (Trier Social Stress Test for Children; TSST-C) in 8-year old children As only two of these previous examinations (Räikkönen et al., 2010; Seugnet et al., 2006) obtained objective

assessment of sleep duration (using actigraphy and polysomnography, respectively), further examinations of sAA response following verified short sleep durations is required. Based on such conflicting results, potentially as a result of uncertain accuracy using self-reported sleep time (Lauderdale et al., 2008), and as a potentially valuable non-invasive marker of SAM activity reducing the confounding effects of additional measurement stress, the association (if any) between objectively measured acute sleep loss and resulting stress related sAA activity requires further scrutiny before being confirmed as a viable pathway denoting reduced sleep duration and SAM axis function.

*1.11.2. Sleep Loss and the Hypothalamic-Pituitary-Adrenal (HPA) Axis:* In relation to HPA axis function, there is a temporal association between sleep structure and HPA axis activity. The early phase of nocturnal sleep, characterised by extended epochs of slow-wave sleep, often referred to as deep sleep, is the only time of day during which secretory activity of the HPA axis is subject to a pronounced inhibition, resulting in minimum concentrations of cortisol (Born & Fehm, 1998). While sleep has been shown to have a moderate but stable inhibitory effect on the secretion of cortisol, sleep loss or reduced sleep quality has been previously demonstrated to result in increased cortisol levels (Balbo, Leproult, & Van Cauter, 2010). This negative relationship has been replicated in correlational studies (Rodenbeck, Huether, Rüther, & Hajak, 2002) and in experimental studies using total (Vgontzas et al., 2003) and partial (Omisade, Buxton, & Rusak, 2010) sleep loss. However, other studies did not detect such effects (Voderholzer et al., 2012) finding cortisol profile was substantially maintained following sleep loss, or, reported decreased cortisol levels after sleep loss (Wu et al., 2008).

Such inconsistencies are further evidenced in an additional feature of the daily distribution of cortisol; the rapid increase in cortisol levels 30 – 45 mins after awakening (Pruessner et al., 1997; Wust et al., 2000). This increase, a phenomenon termed the cortisol

awakening response (CAR), appears to be a distinct feature of the HPA axis, superimposed upon the typical circadian rhythmicity of cortisol secretion. Comprehensive assessment of the CAR entails measurement of two different dimensions, overall cortisol secretory activity and the robustness or magnitude of the response (in relation to awaking from sleep; Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003). Thus, to fully capture such effects, multiple assessments over the awakening period are required. Furthermore, the CAR does not require artificial laboratory conditions or administration of exogenous agents/stressors used to examine reactivity; rather awakening itself is a consistent, recurring, and strong stimulus for HPA reactivity (Wilhelm, Born, Kudielka, Schlotz, & Wüst, 2007).

Published reports examining associations between sleep-related parameters (e.g., sleep duration and wake timing) and the CAR are accumulating; however, further study is needed. Findings from several studies to date indicate that the CAR is neither related to nocturnal sleep duration (Federenko et al., 2004; Pruessner et al., 1997; Wust et al., 2000), nor to wake timing (Pruessner et al., 1997; Wust et al., 2000), while a handful of other results suggest associations between wake time and CAR parameters (Edwards, Clow, Evans, & Hucklebridge, 2001; Kudielka & Kirschbaum, 2003). However, using methodological improvements (i.e., objective measurement of sleep time), more recent data are now hinting at a pattern of decreased awakening cortisol response flowing sleep loss (Gribbin, Watamura, Cairns, Harsh, & LeBourgeois, 2011; Wu et al., 2008). Notably, these findings may be considered preliminary due to small sample sizes ($N$ = 10 and seven, respectively) and incomplete (see Clow, Hucklebridge, Stalder, Evans, & Thorn, 2010) CAR profiling (i.e., single morning sample in serum; Wu et al., 2008). Consequently, inconsistencies in findings concerning CAR response and sleep-related parameters (e.g., sleep duration), and the limited extent of that research, indicate the need for further investigation.

*1.12. Neuroendocrine System and Stress Response: Summary*

In order to advance understanding of how biological and behavioural processes interact to determine risk or resilience, it has been suggested that multiple measurements of stress-related biological processes be attained (Kario & Shimada, 2004; Ohkubo et al., 2002). A significant limitation of research in this area is that HPA and SAM axes reactivity are rarely assessed simultaneously (Rifai & Ridker, 2001). The SAM axis releases the hormones EP and NE and promotes the immediate 'fight-flight' response to stress, whilst the HPA axis controls the longer-term stress response, principally through the glucocorticoid cortisol. Whereas cortisol typically peaks approximately 15 mins after exposure to stress before gradually returning to baseline, and increases gradually over the first hour post-awakening, sAA displays a near immediate stress-response and returns to baseline approximately 10 mins after stress exposure. This asymmetry in response pattern is consistent with the faster reactivity of the SNS compared to the HPA axis (Gordis, Granger, Susman, & Trickett, 2006; Takai et al., 2004). Therefore, examination of concurrent actions (i.e., reduced sleep duration) across systems permits a more thorough understanding of the physiological correlates of sleep loss on stress system functioning, when compared to examination of activity exclusively in a single system.

As a diagnostic fluid, saliva offers distinctive advantages over serum, as it can be collected relatively non-invasively, a particularly important consideration in stress research. As a result, it has the advantage of not introducing additional participant stress that may otherwise distort results. The salivary enzyme α-amylase has been proposed as a marker of stress-induced activity of SAM activity and recent studies have underscored the usefulness of sAA in this regard (Nater & Rohleder, 2009a; Thoma et al., 2012). In relation to HPA axis function, while cortisol has been widely used to assess HPA axis activity, assessment of the

rapid increase in cortisol levels after awakening provides an examination of cortisol responding relating to the sleep-wake transition.

While both SAM and HPA function are indicators of the body's response to stress, and chronic dysfunction of these systems have been associated with health risk, associations between biomarkers of these systems (sAA and CAR) and sleep loss, to date, have been inconsistent. Investigations are therefore needed to clarify the effect of verified sleep loss on sAA as a purported non-invasive biomarker of SAM related stress reactivity, in addition to more rigorous CAR profiling in a larger sample. It may be the case that some of the reported health consequences, including cardiovascular, of chronically restricted sleep are mediated by augmented activation in these stress systems. As such, examining the effect sleep loss has on such markers of neuroendocrine function offers a useful direction in the examination of the effects of sleep loss on negative health outcomes.

### *1.13. Inflammatory Processes*

Research has also suggested that failure to obtain adequate amounts of sleep promotes low-level systemic inflammation, itself associated with cardiovascular risk (Faraut et al., 2012). Inflammatory mediators are thought to elevate during cardiovascular disease progression, for example, atherosclerosis is characterised by a non-specific local inflammatory process which is accompanied by a systemic response (Epstein & Ross, 1999; Libby, 2002). However, inflammatory activity has been suggested to not only play a role in atherogenesis and coronary artery disease (Esch, Stefano, Fricchione, & Benson, 2002), but also in other diseases of the circulatory system. For example, elevated blood pressure (i.e., arterial hypertension) may induce an inflammatory state in the arterial wall through both immune response and mechanical signalling pathways (Taylor, 1999). It has been hypothesised that vascular shear stress, exacerbated by increased cardiovascular activation, can lead to inflammation in the vascular wall. Mechanistically, increases in an individual's

blood pressure cause increases in endothelial shear stresses (ESS) in the vascular wall (Mullington et al., 2009), resulting in endothelial production of inflammatory markers (Chae, Lee, Rifai, & Ridker, 2001). ESS is the tangential stress derived from the friction of the flowing blood on the endothelial surface of the arterial wall (Chatzizisis et al., 2007). In sleep, endothelial markers drop to their lowest point in the day, coinciding with the aforementioned nocturnal dipping of blood pressure (Mullington et al., 2009). Consistently, increased endothelial activation has been demonstrated following sleep loss as demonstrated by increased circulating levels of endothelial cell activation markers, such as E-selectin or intercellular adhesion molecule-1, and microvascular dysfunction after one night of total sleep deprivation (Chae et al., 2001; Frey, Fleshner, & Wright, 2007). Thus, vascular shear stress, exacerbated by sleep-related change in blood pressure response, is a potential mechanism leading to the endothelial production of inflammatory mediators (e.g., Interleukin-6 [IL-6]) following reduced sleep duration, even in healthy subjects (Faraut et al., 2012), resulting in the production of measurable markers of inflammatory activity.

### *1.13.1. C-Reactive Protein*

Research suggests that these increased markers of inflammatory activity are valuable predictors of cardiovascular events even in healthy, asymptomatic men and women (Ridker, Cushman, Stampfer, Tracy, & Hennekens, 1997; Ridker, Hennekens, Buring, & Rifai, 2000) while recent research proposes that if there is a link between sleep deprivation and cardiovascular disease, it may be through changes in C-reactive protein (CRP) and other inflammatory markers (Van Leeuwen et al., 2009). CRP is a sensitive, nonspecific systemic marker of inflammation and is induced principally by the cytokine IL-6, during the acute phase response (the early response of an organism to infection and inflammation). Although CRP concentrations may increase many-fold in the acute-phase response, there is considerable evidence that CRP is present at low concentrations in asymptomatic individuals

and may reflect baseline activity of circulating cytokines (Herity, 2000). The determination of CRP concentrations in healthy individuals by a high-sensitivity assay (hs-CRP) has only recently become widely available (Rifai, Tracy, & Ridker, 1999).

CRP has also been shown to be stress reactive. Findings from Steptoe, Hamer and Chida (2007), in a meta-analysis of 30 studies, including over 1700 participants, examining the effects of acute psychological stress on circulating inflammatory factors in humans, demonstrated modest to robust effects for increases in inflammatory markers (including CRP and IL-6) following acute stress. Although IL-6 is thought to be predictive of the development of cardiovascular disease, CRP has an advantage in that it is more stable, with a longer half-life (Vigushin, Pepys, & Hawkins, 1993), is without diurnal rhythm (Meier-Ewert et al., 2001) and elevated serum CRP levels are associated with traditional cardiovascular risk factors (e.g., obesity; Buckley, Fu, Freeman, Rogers, & Helfand, 2009). For example, chronic inflammation is widely observed in obesity (Kershaw & Flier, 2004), itself associated with cardiovascular disease and increased morbidity and mortality (Poirier & Eckel, 2002), while both overweight (body mass index [BMI], 25-29.9 kg/m2) and obese (BMI ≥30 kg/m2) persons are more likely to have elevated CRP levels than their normal-weight counterparts (BMI < 25 kg/m2; Visser, Bouter, McQuillan, Wener, & Harris, 1999). Consistently, there is evidence for the presence of CRP in human adipose tissue (Calabro, Chang, Willerson, & Yeh, 2005) and growing evidence that adipose tissue can induce chronic low-grade inflammation by producing pro-inflammatory cytokines such as IL-6 (Bastard et al., 1999).

Moreover, while some epidemiological data suggest relatively moderate CRP prediction of CHD (Blankenberg & Yusuf, 2006; Danesh et al., 2004), several large, prospective, cohort studies have consistently reported that higher levels of CRP are associated with an increased risk for CVD, including MI, peripheral vascular disease, and

stroke (e.g., Koenig, Löwel, Baumert, & Meisinger, 2004). As a result, in the U.S., physicians are now recommended to measure hs-CRP in asymptomatic people with an intermediate risk of CHD to optimise their assessment of cardiovascular risk (Pearson et al., 2003), while the use of CRP measurement is becoming increasingly more common in U.K. clinical settings (Poole, Conway, & Currie, 2007). Further, while the current European guidelines on cardiovascular disease prevention in clinical practice recommend the use of hs-CRP in the risk assessment of individuals with a moderate CVD risk profile (Germano et al., 2012), recent reports from the Irish College of General Practitioners have found that screening of hs-CRP levels is not currently available at many hospital-based laboratories in Ireland (Kenny & Ríain, 2009).

CRP levels, commonly assessed using venipuncture, have recently been investigated through assay validation efforts using a hs-CRP measure in saliva, demonstrating moderate-to-strong associations between CRP measured in saliva and serum (Ouellet-Morin, Danese, Williams, & Arseneault, 2011). In addition, salivary CRP was associated with other associates of inflammation and synthesis of acute phase proteins (i.e., BMI, and serum levels of IL-6). Therefore, peripheral (i.e., salivary) CRP measures could offer an estimate of increases in systemic inflammation, resulting from stress-related acute phase activation, although as this marker has only recently been introduced in saliva (circa 2008; Salimetrics Europe Ltd.), this is an emerging area of investigation, requiring further exploration.

Existing literature of serum CRP and sleep, while limited, suggest that chronic partial sleep loss (5 nights of 4 hrs; Van Leeuwen et al., 2009) and (10 nights of 4.2 hrs; Meier-Ewert et al., 2004) increased serum CRP levels from baseline levels. However, as both of these studies reported accumulated, rather than per night averages, from an initial baseline period compared to 5 (Van Leeuwen et al., 2009) or 10 days (Meier-Ewert et al., 2004) thereafter, it remains uncertain as to the influence (if any) of more acute restricted sleep on

CRP level. Further, as noted in the aforementioned meta-analysis conducted by Steptoe et al. (2007), because the concentration of many circulating inflammatory markers appear to occur after a delay following acute stress, the time course of response has not been well established. As such, in addition to expanding the emerging literature base on salivary markers of CRP, the influence (if any) of a single night of acute sleep loss would help to elucidate the time course in CRP in response to stress, while further specifying effects linking states of acute sleep reduction with inflammatory response. As suggested by Faraut et al. (2012), although post-sleep loss alterations in biomarkers of inflammation are often small, such chronic sub-clinical shifts have been described as contributing to the pathogenesis of cardiovascular pathologies.

Finally, as noted previously in relation to HPA stress activity, the interactive effect of the inflammatory response and the HPA axis has been specifically highlighted by McEwen and Seeman as one of the physiological mechanisms leading to allostatic load (1999). For the immune system, adrenal steroids (i.e., glucocorticoids) promote allostasis together with catecholamines by prompting movement of immune cells to organs and tissues where they are needed to fight an infection or other challenge, while also modulating the expression of the hormones of the immune system, for examples the cytokines (McEwen et al., 1997). Some optimal levels of these mediators (i.e., cortisol) are required to maintain functional balance with the competing forces of the immune system, and the absence of sufficient levels of glucocorticoids allows other immune mediators to overreact, resulting in increased the risk of autoimmune and inflammatory disorders (Sternberg, 2001). Consistently, a lower cortisol response to psychological stress has also been demonstrated in patients with chronic inflammatory diseases, such as atopic dermatitis and rheumatoid arthritis (Buske-Kirschbaum, Geiben, Höllig, Morschhäuser, & Hellhammer, 2002; Chikanza, Petrou, Kingsley, Chrousos, & Panayi, 1992). Furthermore, animals with a genetically determined

hypo-responsiveness of the HPA axis are highly susceptible to autoimmune and inflammatory processes (Lechner et al., 1996). In specific relation to CRP, a significantly 'blunted' or decreased cortisol response to both physical and psychological stressors has been found to be associated with increased circulating levels of serum CRP in individuals with coronary artery disease (Nijm, Kristenson, Olsson, & Jonasson, 2007). Therefore, an inadequate response of the HPA axis and autonomic nervous system may be considered a type of allostatic load (McEwen & Seeman, 1999), in which the dysregulation of mediators, normally contained by adequate HPA and autonomic function, may become a primary, or contributory, factor in a disorder.

### *1.14. Inflammatory Processes: Summary*

Taken together, inflammatory activity, and associated measureable increases in levels of CRP, may be useful in predicting CHD as well as other vascular events, for example stroke and peripheral vascular disease. This has been validated by findings in a number of different populations, ranging from patients at low risk for CHD to those with overt CHD, including large prospective samples. Further, increases in CRP may go along with a number of negative cardiovascular developments, such as increased BMI, endothelial dysfunction, and decreased sleep duration (Nguyen & McLaughlin, 2002; Van Leeuwen et al., 2009). However, based on existing data relating serum CRP and sleep duration, it remains unclear as to the influence (if any) of a single night of restricted sleep on CRP level. With the recent introduction of a salivary CRP assay, a minimally invasive alternate measure of CRP (compared to serum assessment) is accessible. Further examination is therefore required to expand the emerging literature base on this novel marker of CRP, while additionally clarifying the association (if any) linking acute sleep reduction and CRP response.

*1.15. Sleep Loss: Empirical Conceptualisations*

As described by Grandner et al. (2010), both in the popular press (e.g., O'Connell, 2012) and within the scientific literature, synonymous terms (e.g., short sleep, sleep deprivation, sleep curtailment, and sleep restriction) are used interchangeably. As a result, such phrasing has been used to describe subjective and objective sleep duration, over both chosen and forced sleep opportunities, in both laboratory and home settings. It does not address how short the sleep is, what the shortness is relative to, and how it was determined. Thus, the empirical literature is confused by a proliferation of related terms, each of which connotes different methods and concepts, leading to confusion regarding interpretation of the data. In an attempt to put forth a set of working descriptors to provide clarity to the various terms used, bridging the gap between laboratory and epidemiological studies, Grandner et al. (2010) proposed a working lexicon relating to terms which have frequently appeared in the previous research.

Within this framework, and in keeping with the definition commonly utilised in many epidemiological accounts, short sleep is defined as normal (habitual) sleep time of 6 hrs or less. Empirical investigations of the effects of short sleep employ terms such as "sleep deprivation", defined as acute sleep curtailment (the deliberate shortening of the amount of time in which sleep is possible) in the laboratory setting. Such sleep curtailment can be either total (complete elimination of sleep for 24-hrs or more) or partial (some sleep is achieved). Further, this can be examined over both acute (i.e., a single night) or more chronic (i.e., several successive nights) experimental time frames. Sleep loss during only part of the night is one of the most common complaints by individuals who experience psychological or environmental stress and work pressure (Faraut et al., 2012), and as such, investigations examining partial sleep loss offer good ecological validity. Observation of such effects under acute conditions (i.e., one night of partial sleep loss), mirroring effects as might commonly be

encountered in natural contexts, avoids exposing participants to the burden involved in long-term sleep loss exposure, while still allowing extrapolation of effects occurring over the longer term, as suggested by effects associated with habitual short sleep durations (Guo et al., 2013). However, in terms of an optimal descriptor for scientific purposes, such working definitions lack acknowledgement of an individual's typical sleep time (enabling appropriate experimental manipulation of sleep duration to observe effects of short sleep) or effects which may be observed in non-laboratory contexts, both important in terms of understanding the effects of short sleep from an experimental perspective. Thus, for empirical purposes, further clarity may be denoted by the term "sleep restriction", defined as sleep deprivation (in or out of the laboratory), where some sleep is achieved, but where sleep duration is less than habitual sleep time.

### 1.16. Conclusions and Thesis Outline

Evidence suggests that sleep loss is a prevalent feature of contemporary society, while epidemiological data, relating subjective self-reported sleep duration to health, indicates that obtaining sufficient sleep is an important domain of healthy behaviour, implicating poor sleep as a predictor of cardiovascular risk. The experimental examination of the stress response (reactive to both the effects of sleep loss and additive effects of laboratory or ambulatory stressors), by means of observing activation of the classical stress systems (i.e., cardiovascular, neuroendocrine, and inflammatory responses), offers good research potential and clinically significant endpoints which align with the cardiovascular risks associated with short sleep. Using acute experimental manipulation of sleep duration, the present thesis aims to elucidate the potential physiological mechanisms underlying the relationship between sleep loss and cardiovascular health, examining stress system activation subsequent to sleep restriction.

Firstly, the influence of sleep loss on blood pressure response needs to be clarified. While some studies have reported that sleep loss produces modest increases in laboratory measures of blood pressure and/or HR, with some recent data to suggest that sleep-related increases in CVR may be evident in response to evaluative social stressors, the findings remain as yet inconclusive. Ambulatory cardiovascular monitoring can also be used to identify patterns of blood pressure response away from laboratory settings with research indicating significant shortcomings in the present knowledge regarding the effects of sleep loss on reactivity to "real" stressors, encountered in a natural context. Drawing on limitations in the present empirical data, the research addresses deficiencies in previous methodologies, utilising assessments of stress reactivity to naturally occurring ambulant stimuli. Ambulatory monitoring was additionally used to identify patterns of nocturnal blood pressure that may be relevant to health outcomes. Further, the research examined the hemodynamic variables underpinning the overall blood pressure response observed in the laboratory, as evidenced by change in CD (indicating degree of blood pressure change) and HP (measuring the nature the response; whether myocardial, vascular, or mixed) allowing closer scrutiny of the cardiovascular response to stress, and affording comparison with previous data revealing these variables to be sensitive to experimental sleep reduction in the laboratory.

Secondly, in order to advance understanding of how behavioural processes, such as reduced sleep duration, and biological processes, such as stress system functioning, interact to determine risk or resilience, it has been suggested that multiple measurements of stress-related biological processes be attained. While both SAM and HPA function are indicators of the body's response to stress, representing immediate and more prolonged activation respectively, and chronic dysfunction of these systems have been associated with health risk, associations between salivary biomarkers of these systems (sAA and CAR) and sleep loss, to date, have been both limited and inconsistent. For sAA, conflicting results, potentially as a

result of uncertain accuracy using self-reported sleep time, remain to be clarified before determination of sAA as a viable pathway denoting reduced sleep duration and SAM axis function. Cortisol awakening responses in relation to sleep loss are additionally uncertain, with recent data indicating a reduced CAR subsequent to sleep loss considered to be preliminary due to small sample sizes (i.e., total sample *N* of < 10 participants) and incomplete (i.e., single sample) CAR profiling. It was the present research's aim to therefore clarify the effect of verified sleep loss (by means of wrist actigraphy) on sAA as a purported non-invasive biomarker of SAM related stress reactivity, in addition to more rigorous (by means of complete salivary profiling) examination of CAR activity in a significantly larger sample than previously used.

Thirdly, CRP, a non-specific systemic marker of inflammation, is induced principally during the acute phase response (the early response of an organism to infection and inflammation); with previous data indicating elevated CRP levels associated with both acute stress and traditional cardiovascular risk factors (i.e., increased BMI). While chronic partial reduction of sleep duration has been shown to result in increased serum CRP levels, the effects of more acute sleep loss on CRP is unknown. The recent introduction of a salivary CRP assay provides researchers with a minimally invasive alternate measure of CRP (compared to serum assessment). Further examination is required to expand the emerging literature base on this novel marker of CRP, while further clarifying the association (if any) between acute sleep reduction and CRP response. Presented as a brief report, the present research additionally aimed to contribute to the emergent experimental literature relating to a novel marker of peripheral (e.g., salivary) systemic inflammation, as measured using salivary CRP, examined in association with acute experimental sleep restriction. A brief overview of the empirical research reported in the remainder of the thesis follows.

*1.16.1. Outline of General Method*

Following completion of a sleep diary over 7 nights in order to calculate habitual sleep time, participants presented for experimental testing across 3 consecutive days, on 2 consecutive weeks (see Figure 2). The project operationaslised sleep restriction largely as a within-subjects factor, counterbalanced across the sample. The sole caveat to this within-subjects treatment of sleep restriction was the between-subjects examination of sleep and CVR /sAA reactivity to laboratory social stress. This was due to the nature of the laboratory social stressor manipulation (i.e., the stressor precluded presentation on a repeated-measures basis using videoed confederates; see Study 1 and Study 2).

On Day 1, participant's weight and height were measured (BMI) before participants received instructions regarding the requisite procedures (rested or sleep restriction protocol to be implemented that night). Relevant materials were also supplied to the participant (i.e., saliva collection devices for CAR profiling and, if undergoing sleep restriction, actiwatch device to ensure adherence to this protocol). On Day 2, the laboratory session took place, including delivery of awakening saliva samples and assessments of cardiovascular/hemodynamic and sAA reactivity to social stress, CRP activation, and fatigue experience. After the laboratory session, participants were connected to an ABP monitor and provided with an activity diary for the monitoring of occurrences of ambulatory stress and social interaction. Salivary collection devices were supplied for CAR (recovery) estimation. On Day 3, participants retuned to the laboratory to return equipment. This was repeated one week later. Data collection occurred as part of a larger study undertaking experimental and cross-sectional analyses of sleep duration on blood pressure reactivity and hemodynamic profile in three separate cohorts; young, working, and older adults. Examining the younger adult cohort, pilot data for the present project (James & Gregg, 2004b), indicated that with an effect size of .40 (indicated by the pilot data), a level of significance of .05 (2-tailed) and

power of 80 per cent, a sample size of 128 was needed to detect similar effects of sleep restriction on hemodynamic variables of interest. Further details of these days, including partitioned sample sizes utilised in the individual investigations, are outlined below.

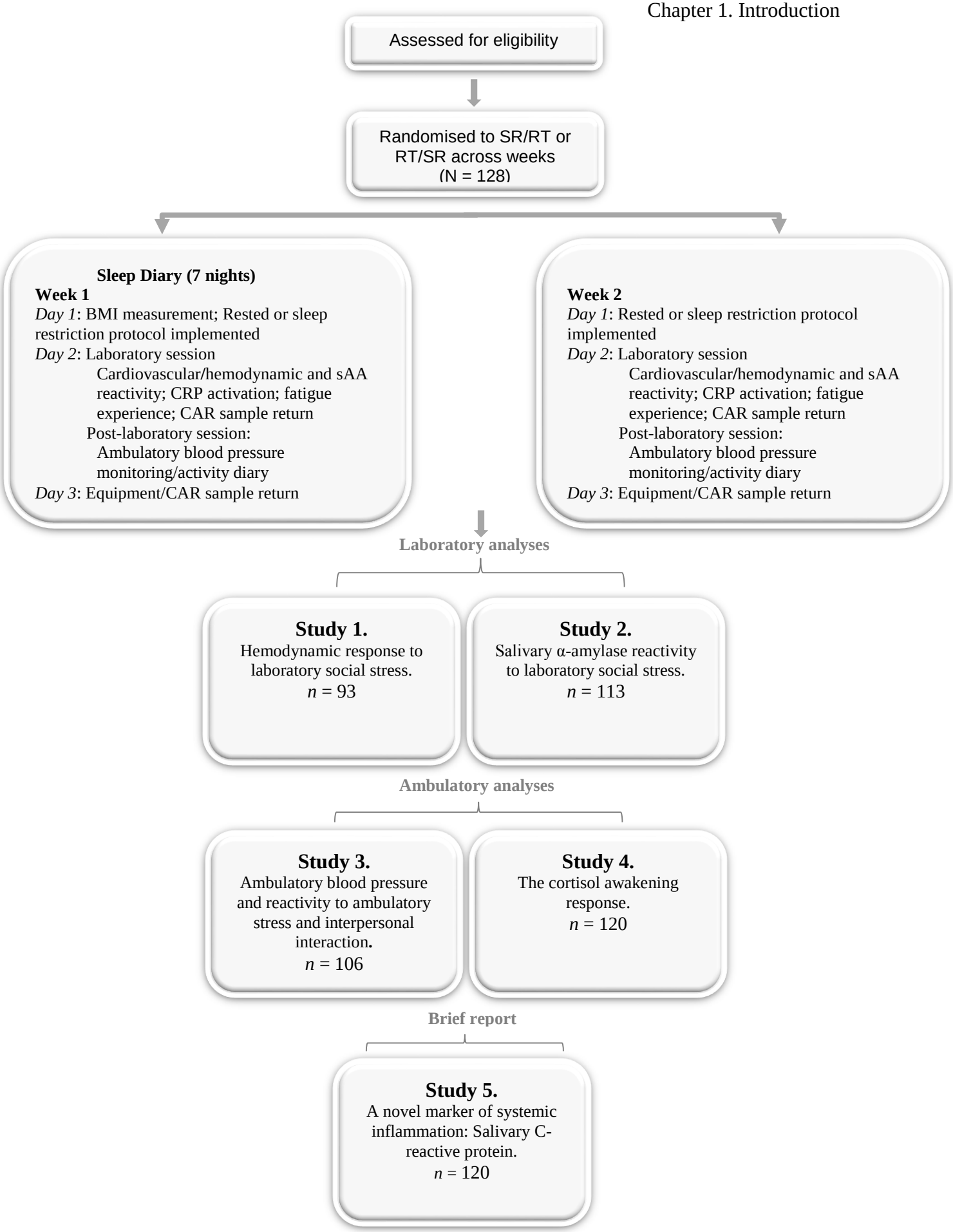


*Figure 2.* Overview of experimental procedures. CAR = Cortisol awakening response; CRP = C-reactive protein; sAA = Salivary α-amylase; SR = Sleep restricted; RT = Rested

**Study 1. Hemodynamic response to laboratory social stress: Effects of acute sleep restriction**

Study 1 (Chapter 2) examined laboratory CVR to social stress, including assessments of hemodynamic determinants of blood pressure, to determine if responsiveness to social stress was altered under conditions of experimental sleep restriction relative to when rested. Ninety three normotensive college students completed a sleep diary for seven nights, before random assignment to experimental group (rested or sleep restricted). For those in the sleep restricted group, participants were asked to go to bed at their usual times and to wake having obtained just 40 per cent of their usual night's sleep. To objectively monitor adherence to the sleep manipulation, participants were provided with a wrist activity monitor. Enhancing adherence to the procedure, at individual awakening times following sleep restriction, a text message was sent to the participant's mobile phone, as an added incentive to ensure scheduled awakening. For those in the rested group, participants were instructed to go to bed and wake at their usual times. Changes in overall blood pressure and hemodynamic variables in response to evaluative social stress (using video presentation) were compared between rested and sleep restricted groups. The primary aim of Study 1 was to examine laboratory CVR as a possible mechanism through which sleep restriction may be related to ill health. Using the same population of college students from which the sample in Study 1 was drawn (total data collection consisting of 128 participants); the subsequent studies addressed the remaining empirical inquiries, partitioned according to requirements of the particular outcome variable of interest.

**Study 2. Salivary α-amylase reactivity to laboratory social stress in an acute sleep restriction manipulation**

In order to assess possible additional physiological effects of laboratory social stress which may exist across stress systems, sAA response to social stress, as a measure of SAM system activity, was examined. The SAM axis releases the hormones EP and NE and denotes the instantaneous sympathetic response to stress. However, as only two of the previous examinations of sAA response following sleep loss exposure obtained objective assessment of sleep duration, further examinations of sAA response following verified short sleep durations is required. The design of Study 2 (Chapter 3) overlaps with that of Study 1, again using a between-subjects design, with participants divided into a sleep restricted (verified by actigraphy) or rested group. Screened for confounding medication usage, 113 college students provided saliva samples for the assessment of sAA response as they undertook laboratory social stress testing. The main aim of Study 2 was to examine whether sleep loss augmented sAA response to laboratory social stress relative to when rested. The majority of studies in this domain have used a standardised psychosocial stress test, the TSST (Kirschbaum, Pirke, & Hellhammer, 1993), to assess the usefulness of sAA as a biomarker of psychosocial induced stress. As a secondary aim, Study 2 sought to shed light on the sensitivity of the sAA response to a laboratory stress protocol (as used in Study 1), exposing participants to negative social evaluation, presented over video relay.

**Study 3. Ambulatory blood pressure and reactivity to ambulatory stress and interpersonal interaction: Effects of acute sleep restriction**

Study 3 (Chapter 4), extended the traditional CVR laboratory protocol, testing the potential for the findings of the previous laboratory based CVR observations in Study 1, to generalise to conditions outside of the laboratory setting. Further, few studies have measured how sleep loss affects response to naturalistic stressors, while periods of interpersonal contact have been suggested as a useful task for investigating CVR in the field. One hundred

and six normotensive college students underwent ABP and HR monitoring while engaging in everyday activities, comparing cardiovascular arousal during periods of naturally occurring high and low stress and interpersonal contact, whilst rested and sleep restricted. Examination of nocturnal blood pressure dipping was also conducted to explore if the habitual nocturnal reduction in blood pressure is associated with reduced sleep duration. While Study 1 and 2 operationalised sleep as a between-subjects variable (assessing responsivity to laboratory social stress when either rested or sleep restricted), Study 3 was completed within-subjects, with participants completing ABP monitoring over two 24-hr periods; once while rested and once while sleep restricted. The main aim of Study 3 was to examine whether sleep loss would influence ambulant cardiovascular responses to naturally occurring periods of stress and interpersonal contact.

**Study 4. The cortisol awakening response and acute sleep restriction**

In Study 4 (Chapter 5), in order to establish whether reduced sleep duration affects ambulant measures of HPA (i.e., awakening cortisol) activity, advancing the previous limited and incomplete testing of such effects, the awakening response of cortisol was examined. Relative to SAM axis activation (Study 2), the HPA axis controls the longer-term stress response. Following screening for associated medication usage, 120 college students were requested to provide four waking saliva samples, in their own homes, over the first 45 mins post-awakening, for the assessment of the CAR. These samples were provided following three sleep phases; (a) normal sleep duration (rested), (b) partial sleep duration (sleep restricted), and (c) recovery sleep on the first night post-sleep restriction (recovery). Cortisol assessments were completed within-subjects, with participants providing saliva samples on all three time-points. The main aim of Study 4 was to examine differences in CAR response as a function of sleep restriction.

**Study 5. --Brief Report-- Assessing the effects of acute sleep restriction on a novel marker of systemic inflammation: Salivary C-reactive protein**

The previous studies (1 and 3) examined the influence of sleep restriction, social stress, and periods of naturally occurring stress and interpersonal contact, on measures of laboratory and ambulatory CVR. Study 2 additionally examined levels of sAA following sleep loss, proposed to reflect SAM related activation, while Study 4 assessed measures of HPA activity, investigating sleep loss related change in the CAR. However, failure to obtain adequate amounts of sleep has also been suggested to promote low-level systemic inflammation, itself associated with cardiovascular risk. In Study 5 (Chapter 6), an exploratory investigation into the effect of sleep restriction on levels of a novel marker of low level systemic inflammation, CRP, detectable in saliva, was examined in 120 college students, screened for confounding medication use, offering a preliminary examination of both the validity of the marker and its ability to detect change associated with reduced sleep duration.

# Chapter 2. Study 1:

# HEMODYNAMIC RESPONSE TO LABORATORY SOCIAL STRESS: EFFECTS OF ACUTE SLEEP RESTRICTION[2]

## 2.1. INTRODUCTION

*Effects of sleep restriction on blood pressure*

Exaggerated CVR to psychological stress as measured in healthy adults is believed to lead to an increased risk of eventual cardiac disease (Kamarck & Lovallo, 2003) through a number of physiological mechanisms (Lovallo, 2005). Viewing sleep restriction as a potential source of psychophysiological stress (McEwen, 2006), several large prospective and epidemiological studies indicate that habitual sleep of insufficient duration is associated with negative cardiac health, suggesting short sleepers have an increased incidence of myocardial infarction (Ayas, White, Manson, et al., 2003; Ferrie et al., 2007) and all-cause mortality (Kripke, Garfinkel, Wingard, Klauber, & Marler, 2002) when compared with typical sleepers. At the population level, research suggests that the proportion of adult short sleepers (< 6 hrs per night) may be rising (Bin et al., 2012), while adequate sleep is increasingly becoming an important aspect of healthy behaviour (Colten & Altevogt, 2006; Grandner, 2012). However, the mediating mechanisms that may explain relationships between reduced sleep duration and cardiovascular ill-health remain unclear. For example, evidence of the effects of restricted sleep on hemodynamic variables examined in laboratory settings is inconsistent. While some studies have shown modest increases in blood pressure and/or HR (Kato et al., 2000; Muenter

[2] A manuscript based on material from this chapter has been accepted for publication in the *International Journal of Psychophysiology* with minor editorial modifications. This version, where appropriate, incorporates those revisions. O' Leary, É. D., Howard, S., Hughes, B. M., & James, J. E. (2013). An experimental test of blunting using sleep-restriction as an acute stressor in Type D and non-Type D women. *International Journal of Psychophysiology*, 90, 37-43.

et al., 2000), others have reported little or no effects (Meney et al., 1998; Miró et al., 2002; Smith & Maben, 1993).

*Underlying hemodynamic variables*

Stress-related changes in blood pressure and HR are cardiovascular indices frequently examined in the published literature. However, the underlying hemodynamic determinants of blood pressure have also received attention. A noteworthy aspect of previous findings is that effects of sleep restriction have been observed in relation to particular underlying hemodynamic determinants of blood pressure (James & Gregg, 2004b). It is known that changes in overall (SBP/DBP) blood pressure are underpinned by changes in CO, in TPR, or both (Turner et al., 1994). Reactivity patterns of CO and TPR have additionally been found to be stable over time for certain tasks (Kasprowicz et al., 1990), leading to the conclusion that individual differences in hemodynamic response patterns may be implicated in cardiovascular pathology (Gregg et al., 1999; Lawler et al., 2001).

As the relationship between CO and TPR is reciprocal, an increase in one parameter is usually accompanied by a relative decrease in the other (Guyton & Hall, 1997), with the effect that overall blood pressure changes can be interpreted as myocardial (if induced by CO) or vascular (if induced by TPR). However, previous methods used in the classifying of individuals as myocardial or vascular reactors have varied between studies, with previous methods failing to account for the reciprocal relationship between CO and TPR. In response to these shortcomings, Gregg et al. (2002) proposed a model which explains variations in blood pressure in terms of dynamic compensatory relationship between CO and TPR (James et al., 2012; James & Gregg, 2004b; Ottaviani et al., 2006).

A difficulty with previous approaches was that they confounded two separate concepts, CD and HP, which can be independently expressed. CD measures the extent to

which CO and TPR compensate, thereby indicating degree of change in blood pressure (increase, decrease, or no change). On the other hand, HP measures the nature the compensation, whether it is myocardial (CO predominates) or vascular (TPR predominates) or both (i.e., a "mixed response" involving equal amounts of change in CO and TPR). The validity of the model originally proposed (Gregg et al., 2002), has been subsequently confirmed (James & Gregg, 2004b; Ottaviani et al., 2006). As such, small or non-significant shifts in overall blood pressure can mask more vigorous change at the hemodynamic level, with the result that non-significant between-group differences in overall blood pressure (i.e., between rested and sleep restricted participants) may be characterised by significant between-group differences in hemodynamic patterning. The importance in examining such underlying processes is that HP appears to be implicated in the development of cardiovascular pathology. Specifically, it is believed that blood pressure changes characterised as vascular confer increased risk of disease (i.e., Obrist, 1982), while vascular changes have been previously reported in relation to sleep restriction (James & Gregg, 2004b).

Finally, there are some recent data to suggest that sleep-related increases in blood pressure may be observable following particular laboratory stress exposures, as evidenced by increased blood pressure responses to laboratory social evaluative stress following sleep loss (Franzen et al., 2011). While previous work has demonstrated augmentation to hemodynamic processes following sleep restriction in response to laboratory psychomotor and cognitive challenge and sustained vigilance (James & Gregg, 2004b), the effects of social stress exposure (in particular to a stressor high in evaluative threat) on HP/CD following sleep restriction is unknown.

Accordingly, the present study sought to examine the association between sleep restriction and laboratory measures of social stress, focussing on overall blood pressure measures and hemodynamic profile, in a sample of young healthy adults. While no specific

prediction was made regarding the effects of sleep restriction on overall blood pressure level (due to conflicting findings), it was hoped that examination of hemodynamic changes underling overall blood pressure response (i.e., HP and CD) would further elucidate inconsistencies in previous results.

## 2.2. METHODS

### 2.2.1. Design

The present study employed a mixed factorial design. The between-subjects factor was sleep group (rested or sleep restricted). Cardiovascular measures were recorded during baseline and stress periods, giving a within-subjects factor of period with two levels; baseline and social stress. Participants were met by the researcher at the laboratory on two consecutive days. On Day 1, participants were randomly assigned to experimental group (rested or sleep restricted). On Day 2, participants were greeted by the same researcher and completed laboratory testing.

### 2.2.2. Participants

Participants were 93 (71 female) university students (age 17 to 23 years; $M$ = 18.48 years, $SD$ = 0.96) with normal BMI ($M$ = 22.96, $SD$ = 2.67). All participants were physically healthy, tested as normotensive (resting blood pressure < 140/90 mmHg) and reported no history of heart disease. Recruitment of participants from a student population studying psychology comprising more than 70 per cent female, occurred via online and classroom announcements and was rewarded with course credit and financial reimbursement for expenses incurred over the course of the study (i.e., taxi transportation to the laboratory while sleep restricted). Participation was voluntary and participants held the right to withdraw at

any time. All procedures received ethical clearance from the institutional research ethics committee.

### 2.2.3. Materials and apparatus

*Fatigue.* Fatigue was measured using the Multidimensional Fatigue Inventory (MFI; Smets, Garssen, Bonke, & De Haes, 1995), covering multiple domains related to fatigue experience (general fatigue, physical fatigue, mental fatigue, reduced motivation, and reduced activity) with each of the subscales showing adequate to very good internal consistency (Shahid, Shen, & Shapiro, 2010; Smets et al., 1995). The MFI contains 20 statements (four items per subscale), with participants responding using a 7-point Likert scale. Scores range from 4-20, with a higher score indicating greater fatigue. Cronbach's α for the MFI subscales in the present sample ranged from .81 to .90, all indicative of good internal consistency.

*Cardiovascular assessment.* Beat-to-beat cardiovascular measures were recorded using a Finometer hemodynamic monitor (Finapres medical Systems BV, BT Arnheim, The Netherlands). An appropriately-sized finger cuff, attached to the participant's middle finger, is inflated to keep the arterial walls at a set diameter. An infrared photo-plethysmograph incorporated in the cuff detects changes in the diameter of the arterial wall. In accordance with the volume-clamp method first developed by Peňáz (1973), the volume of the arteries under the finger cuff, as observed by the photo-plethysmograph, is dynamically held constant by precisely-measured counter pressure in the finger cuff during blood pressure pulsations. The Finometer has been shown to achieve a standard of absolute blood pressure measurement meeting the validation criteria of the Association for the Advancement of Medical Instrumentation and the revised protocol of the British Hypertension Society (Guelen et al., 2003; Schutte, Huisman, van Rooyen, Oosthuizen, & Jerling, 2003).

### 2.2.4. Sleep restriction

In order to assess sleep duration prior to laboratory testing, participants completed a sleep diary for seven nights. Based on self-reported sleep and wake times, the average length of a night's sleep was computed for each participant. Participants in the sleep restricted group retired to bed in their own home at their usual bedtime, but set a bedside alarm to be awakened when they had completed just 40 per cent of their usual night's sleep. To enhance adherence to the procedure, on awakening following sleep restriction, a text message was sent to the participant's mobile phone, as an added incentive to ensure scheduled awakening. Those in the rested group were asked to go to bed and wake at their usual times. This sleep restriction protocol has been successfully implemented in previous studies (James & Gregg, 2004a; James, Gregg, Kane, & Harte, 2005).

To monitor adherence to the sleep manipulation, participants were provided with a wrist activity monitor (Actiwatch-Alert, Cambridge Neurotechnology Ltd, Cambridge, U.K.) previously shown to provide valid records of sleep activity (Coffield & Tryon, 2004). Participants activated the wrist monitor on awakening and wore the monitor continuously until they returned to their next laboratory session. The occurrence of 2 continuous mins of inactivity as recorded by the activity monitor triggered an alarm, which the participant had to deactivate manually. The monitor was removed by the researcher when the participant attended the laboratory session later on the same morning they were awakened early. The participant was deemed to have been awake provided activity occurred at least every 2 mins. Previous research using the same protocol indicated participant compliance in excess of 80 per cent (James & Gregg, 2004a).

### 2.2.5. Social stressor task

Participants were seated at a desk with a personal computer and informed that they would be required to complete a mental arithmetic task. In response to equations appearing on screen, the participant was required to click on the appropriate digits via an on-screen number grid using a mouse. In addition to being asked to answer the problems as quickly and accurately as possible, a progress bar to the left of screen was displayed, showing the time left to complete the task, emphasising time pressure demands. The task did not employ standardized flexibility (Hughes, 2001; Turner, 1994) in order to allow performance-related differences between sleep groups to be examined.

Participants were informed that a research confederate (whom they could see and hear on a nearby monitor and speaker) would observe them whilst they completed the task. Participants' attention was directed towards a video camera, orientated toward the participant, to enhance the plausibility of the context. In addition, participants were informed that they would receive feedback on their performance during the task, from this confederate. The feedback was designed to enhance the participant's perceived level of negative social evaluation (i.e., commentary centred on the participants' speed and accuracy relative to other participants, such as 'Okay, looking at your progress here, most participants have performed slightly better than you at this point'). Participants were led to believe that the confederate was in an adjoining room when in reality the monitor displayed a video recording of a gender-matched research confederate, as used by Thorsteinsson, James, and Gregg (1998) in a manipulation of video-relayed social support. The confederate was in the same age-range of the participant (under 25 years) and were trained to portray a non-accepting and critical manner as outlined by Gruenewald et al. (2004).

### 2.2.6. Procedure

At the laboratory session, participants were seated at a desk and the Finometer finger cuff was attached to the middle finger of the non-dominant hand. Participants were given approximately 20 mins to acclimatize to the laboratory while psychometric measures were completed. Following acclimatisation, resting cardiovascular measures were taken during a formal 10 min baseline phase. Neutral reading material was supplied during baseline in order to promote relaxation and establish a true cardiovascular baseline by countering ruminated-related arousal (Jennings, Kamarck, Stewart, Eddy, & Johnson, 1992). Following baseline, participants were given instruction on the laboratory stressor. The mental arithmetic task (combined with video-relayed evaluation of performance) lasted 6 mins, throughout which hemodynamic responses were measured continuously using the Finometer. Following completion of the task, participants used a 10-point Likert scale to rate how stressful, enjoyable, and difficult they found the task to be.

## 2.3. RESULTS

### 2.3.1. Overview of analyses

Mean levels of SBP, DBP, and HR were computed for both periods of the experiment; namely, baseline and social stress. Internal reliability consistency for each mean cardiovascular variable was excellent, with Cronbach's $\alpha > .96$ for both phases on all cardiovascular parameters.

Mixed factorial analysis of variance (ANOVA) was conducted for each cardiovascular parameter (SBP, DBP, and HR), using the between-subjects factor, sleep (rested vs. restricted), and the within-subjects factor, period (baseline, social stress). Independent samples *t*-tests were conducted to examine differences in task performance and post-stress ratings, when rested and sleep restricted.

Effects of gender differences on blood pressure reactivity, when rested and sleep restricted, were examined by means of mixed factorial ANOVA, using the between-subjects factors, sleep and gender, and the within-subjects factor, period. Main effects for gender were indicated for DBP *(p* = .027) and HR (*p* = .009), with lower DBP and higher HR seen in women compared to men. However, no significant interactions of gender with either sleep or period were observed (all *p*s > .114), in accordance with effects previously reported (James & Gregg, 2004b).

Examination of the hemodynamic changes underlying observed blood pressure changes was based on the quantification of hemodynamic patterning as proposed by Gregg et al. (2002). That is, composite scores representing HP and CD were computed; the former indicating the degree to which blood pressure changes are attributable to increases in either CO or TPR, and the latter indicating the magnitude of homeostatic regulation between CO and TPR. If a blood pressure response is mainly attributable to increased CO it is considered myocardial and if it is attributable to increased TPR it is considered vascular. In this way, the extent to which CO and TPR compensate, indicating degree of change in blood pressure (increase, decrease, or no change) and nature of the reciprocal changes in CO and TPR (representing a myocardial, vascular, or mixed response) can be measured and tested for statistical significance.

For one sample and independent *t*-tests, Bonferroni correction was applied to multiple comparisons. Data were tested for normal distribution and homogeneity of variance using a Kolmogorov - Smirnov and Levene's test before statistical procedures were applied. ANOVA effect sizes are presented as partial $\eta^2$. Partial $\eta^2$ rather than simple is recommended for ANOVA designs with multiple independent variables (Tabachnick & Fidell, 1989), and values of .04, .25, and .64, are taken as indicating small, medium, and large effects, respectively (Cohen, 1992).

### 2.3.2. Adherence to sleep manipulation

Sleep diary data indicated that participants reported experiencing a mean of 517 mins (8h and 37mins, *SD* = 80 mins) of sleep per night. Sleep amount was determined individually according to habitual sleep time. This meant that, on average, participants in the restricted group were to receive a mean of 207 mins (3h and 27mins, *SD* = 32 mins), on restricted nights. This represented 40 per cent of their usual sleep time according to the sleep diary data. Actigraphy records indicated high levels of adherence to the sleep restriction protocol. Activity levels, monitored on the evening prior to the restricted night to ensure participants maintained wakefulness until their usual bedtime, indicated the average percentage of time participants were awake during the period to be 93.57 per cent (*SD* = 11.73). Equally high (*M* = 392.94, *SD* = 151.73) evening activity scores per 2 min epoch recorded (a total score of ≥ 80 per epoch designating an epoch as being "awake") also substantiated protocol adherence during the pre-sleep evening phase. During the restriction vigil (following normal bedtime and awakening on receipt of 40 per cent usual sleep duration), the mean percentage of time participants were awake was 84.52 per cent (*SD* = 19.94). The mean activity score per 2 min epoch was 244.45 (*SD* = 127.59). Both percentage time awake and activity score indicated substantial compliance to the sleep restriction protocol.

### 2.3.3. Manipulation Checks

*2.3.3.1. Self-reported fatigue.* To confirm adherence to the sleep restriction protocol (and compare relative levels of fatigue in the rested group), a series of independent samples *t*-tests were conducted for each of the MFI fatigue subscales. Independent *t*-test revealed significantly higher general fatigue reported when sleep restricted (*M* = 14.74, *SD* = 2.95) compared to rested (*M* = 9.65, *SD* = 2.71; $t(87) = 8.47$, $p < .001$). For the remaining four MFI subscales (mental fatigue, physical fatigue, reduced activity, and reduced motivation),

significantly greater sleep loss related fatigue was again observed, demonstrating significantly greater fatigue under sleep restriction compared to rested (all *p*s < .007).

2.3.3.2. *Task Engagement.* Independent samples *t*-tests indicated effects for sleep on post-task ratings of how stressful the task was found to be, such that the task was rated as significantly more stressful under sleep restriction ($M$ = 6.53, $SD$ = 2.25) compared to rested ($M$ = 5.23, $SD$ = 2.23; $t(87) = 2.74$, $p = .007$). The task was reported to be more difficult under sleep restriction ($M$ = 7.42, $SD$ = 1.82) compared to rested ($M$ = 6.32, $SD$ = 2.43) but only marginally so, after Bonferroni correction was applied ($p = .051$). There were no effects for sleep on ratings of enjoyableness, indicating that sleep did not influence participant's perceptions of how enjoyable the task was.

Independent *t*-tests revealed no differences in the number of problems answered correctly, incorrectly, or left unanswered (all *p*s >.33), when rested and sleep restricted.

2.3.3.3. *Confirmation of reactivity.* Mixed ANOVA confirmed a main effect for period on SBP, $F(1, 91) = 355.70$, $p < .001$, partial $\eta^2 = .796$, DBP, $F(1, 91) = 322.11$, $p < .001$, partial $\eta^2 = .785$, and HR, $F(1, 91) = 36.13$, $p < .001$, partial $\eta^2 = .284$, with task levels greater than baseline levels on all parameters. These main effects indicate the success of the stressor in eliciting increased reactivity under conditions of social stress across the cardiovascular parameters recorded. Means (with *SD*s) for cardiovascular measures during baseline and social stress, as a function of sleep group, are shown in Table 1.

Table 1

*Means (with* SD*s) for cardiovascular measures across experimental groups*

| Variable | Rested | | | | Variable | Sleep Restricted | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Baseline | | Task | | | Baseline | | Task | |
| | Mean | *SD* | Mean | *SD* | | Mean | *SD* | Mean | *SD* |
| SBP[a] | 119.57 | 1.32 | 153.50 | 2.21 | SBP[a] | 121.12 | 1.42 | 154.19 | 3.15 |
| DBP[a] | 74.66 | 1.05 | 97.03 | 1.60 | DBP[a] | 74.84 | 0.88 | 98.18 | 2.19 |
| HR[b] | 77.50 | 1.74 | 82.81 | 1.65 | HR[b] | 78.91 | 1.72 | 82.56 | 1.86 |

a mmHg.

b bpm.

*2.3.4. Cardiovascular reactivity and sleep restriction*

Mixed ANOVA revealed no significant variation in patterns of SBP, DBP, or HR reactivity to social stress across sleep groups, as indicated by no main effects for sleep (all *p*s >.65) or sleep × period interactions (all *p*s > .27; see Table 2). Reactivity profiles for SBP and DBP are illustrated in Figure 2.

Table 2

*Summary of mixed-model ANOVA (*F *values) for Sleep and Period*

| Source | *df* | SBP | DBP | HR |
|---|---|---|---|---|
| Sleep | 1, 91 | <1 | <1 | 1.25 |
| Period | 1, 91 | 355.7** | 332.11** | 36.13** |
| Sleep × Period | 1, 91 | <1 | <1 | <1 |

Period: baseline and social stress. Sleep: rested versus restricted.

** *P* < .001

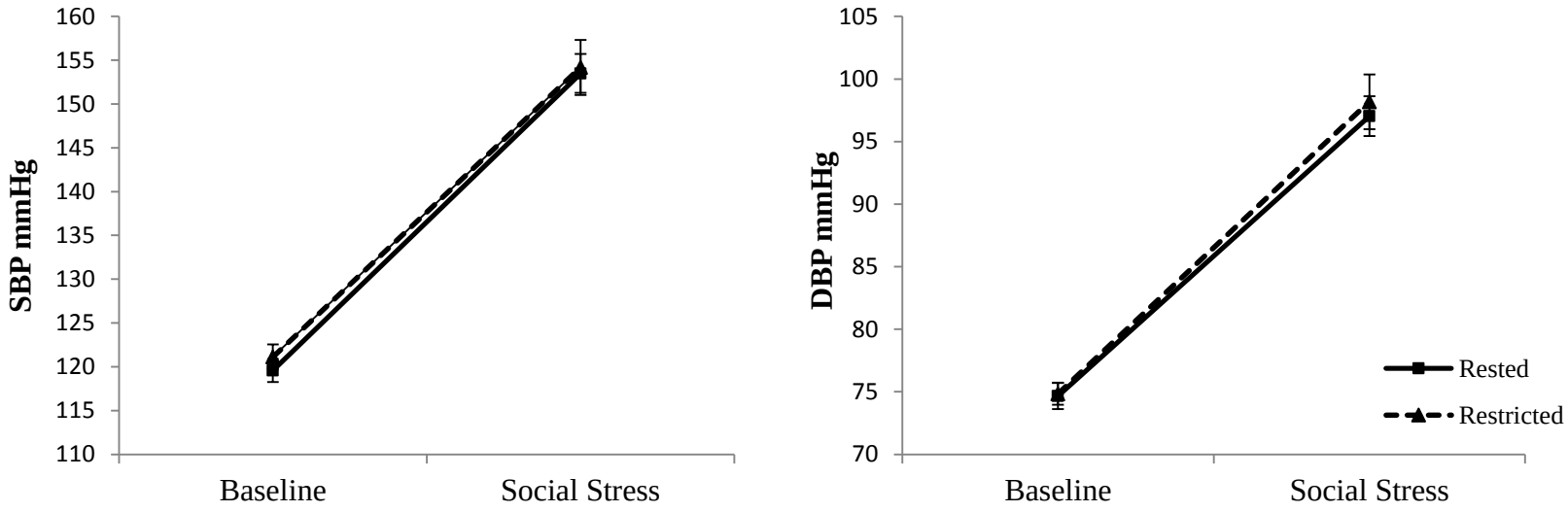


*Figure 3.* Non-significant variation in patterns of systolic blood pressure (SBP) and diastolic blood pressure (DBP) reactivity to laboratory social stress across sleep groups. Error bars denote standard errors of the mean

2.3.5. *Hemodynamic Profile*

Based on the method delineated by Gregg et al. (2002), and subsequently reviewed by James et al. (2012), values for HP and CD were computed for each group, with values returned on a quasi-standard scale with a hypothesised mean of 0 and *SD* close to 1.

Those in the restricted group had a mean HP of .086 (*SD* = .18) and a mean CD of .081 (SD = .04), compared to the rested group with a mean HP of .032 (*SD* = .14) and a mean CD of .086 (*SD* = .03).

One-sample *t*-tests to examine if the groups differed significantly from 0 indicated that both rested and restricted groups both demonstrated significant change in CD, $t(46) = 18.04$, $p < .001$, for rested, and, $t(45) = 12.92$, $p < .001$, for restricted, which is consistent with the main effects for period on blood pressure changes in response to laboratory social stress as evidenced in Figure 2. For HP, while the rested group did not significantly change from 0, $t(46) = 1.56$, $p = .125$, the restricted group demonstrated significant change, $t(45)= 3.11$, $p = .003$. The positive *t*-values on HP scores, signalling an increase from 0, is indicative of a vascular response to the social stress task, with a marked vascular response (highly significant positive score) observed in the sleep restricted group, confirming effects found by James and Gregg (2004b), as illustrated in Figure 3.

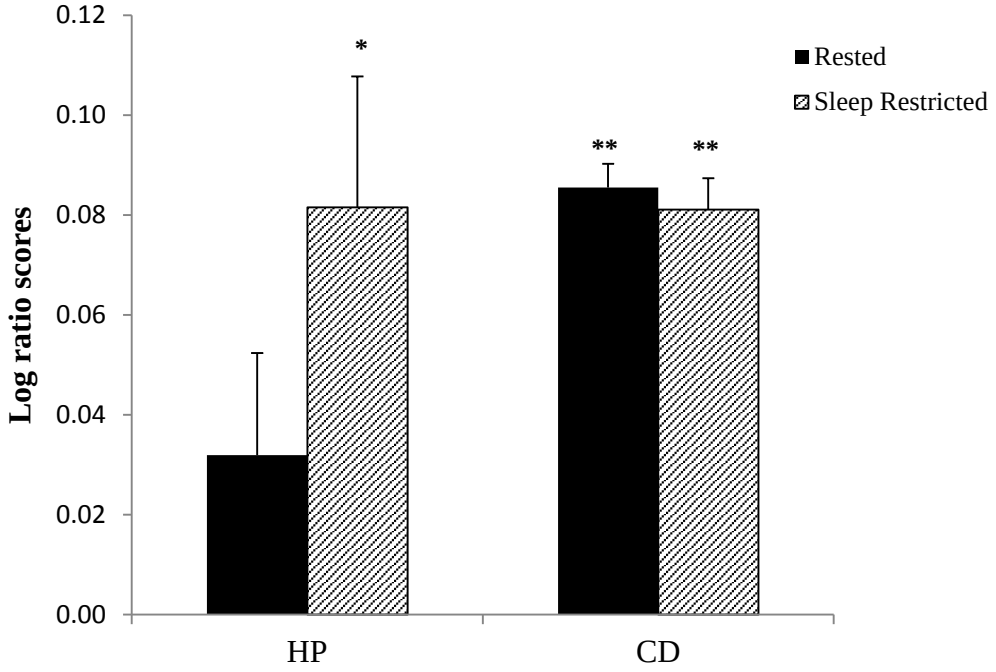


*Figure 4.* Main effects (expressed as log ratio scores) for compensation deficit (CD) and hemodynamic profile (HP) during laboratory social stress, per sleep group, * $p < .05$; ** $p < .001$. Error bars denote standard errors of the mean

## 2.4. DISCUSSION

The present study examined hemodynamic response to an acute evaluative social stress task amongst a sample of young healthy adults, and found that sleep restriction had no significant effect on overall measures of CVR (SBP, DBP, or HR). Conversely, sleep restriction was observed to produce a marked response on HP, indicating a significant vascular effect.

The potential importance of HP as part of the process in the determination of cardiovascular risk is illustrated in the present study by the observed effects of restricted sleep. Examination of overall blood pressure measures, classically utilised in the stress reactivity literature, revealed blood pressure and HR response to stress was unaffected by the

induced sleep restriction. However, the profile of hemodynamic effects brought about by sleep restriction was markedly vascular. The current findings corroborate previous effects reported by James and Gregg (2004b), who also found a vascular HP following sleep restriction, in response to a number of laboratory stressor (e.g., reaction time, mental arithmetic, memory and vigilance tasks) in a sample of 96 healthy male and female adults. The present results further extend that finding to laboratory social stress involving social evaluative threat.

These findings are important as they suggest (together with previous data) that some null changes in blood pressure (i.e., as previously noted following sleep loss) may be characterised by differential patterns of underlying hemodynamic functioning. As the effect was found to be vascular (as opposed to more adaptive myocardial), the findings may suggest that vascular responses, and their inducement by increased peripheral resistance (considered to be maladaptive, in particular in relation to development of established [i.e., chronic] hypertension; Mayet & Hughes, 2003), may, over continued periods of restricted sleep, lead to increased risk to cardiovascular health.

A further consideration of importance is the potential significance of the laboratory task chosen. The current stressor involved high levels of negative social evaluation (in comparison to more standard stressor tasks) which may have interacted with known associations between "active" and "passive" stressors and hemodynamic responses. The terms "myocardial" and "vascular" have been previously used as a method to distinguish hemodynamic responses to stimulation of β- and α-adrenergic receptors, respectively. In situations with little or no control opportunity, or "passive coping" conditions, the cardiovascular system appears to be primarily under α-adrenergic control (Allen, Obrist, Sherwood, & Growell, 2007; Sherwood, Dolan, & Light, 1990) resulting in enhanced vascular resistance (Gramer, 2006). The stressor used in the present study afforded a low

level of control over receipt of ongoing negative evaluation and in so doing, created a more passive (compared to active) coping situation. Consistently, the present stressor was seen to induce a vascular profile when both rested and (markedly significant) when sleep restricted. Such observations provide verification of vascular task responding in accordance with previous results for tasks involving more passive coping mechanisms. As such, the observed effects, in addition to being a function of stressor type, reflect unique effects of a vascular response individually associated with sleep restriction.

The experimental manipulation of sleep duration in the present study offers a strength over previous examinations of sleep related effects on hemodynamic response. However, while objective data was obtained to identify adherence to the sleep restriction protocol, those individuals in the rested group may not have received a complete night's rest as directed. This lack of objective measurement of sleep quality in the rested group prior to the laboratory session is a potential weakness. Nevertheless, self-reported fatigue data confirmed that those in the sleep restricted group were significantly more fatigued, across multiple domains of functioning as measured using the MFI, than the rested group. Issues around expectation bias aside, this offers some evidence supporting the validity of the rested protocol.

Findings from Franzen et al. (2011) suggested that sleep-related increases in overall blood pressure may be evident in social stress exposures to social-evaluative threat, based on their observation of increases in SBP in response to evaluative social stress following sleep loss. However, these reported effects were observed following total (25-26 hrs awake), rather than partial, sleep restriction (Franzen et al., 2011). To obtain ecologically valid results, the present study used partial (40 per cent of usual sleep duration), rather than total, restriction of sleep duration in order to closely mirror the acute reductions in sleep duration commonly observed in everyday experience (Faraut et al., 2012). As such, further insight into blood pressure reactivity to social stress and interactions with sleep might in future be derived from

greater variance in sleep duration. Additionally, testing social stress exposure in alternate (i.e., ambulatory) settings, would enable investigation of how sleep-related CVR to social stress may operate differentially, across contexts (as outlined below).

In sum, while epidemiological data indicate that chronic sleep loss contributes to the development of increased risk to cardiovascular health, experimental testing of such effects on hemodynamic variables has resulted in inconsistent results. In the present study blood pressure was found to be unaffected by sleep restriction. However, in addition to corroborating previous findings of a vascular HP in response to sleep restriction, the present study extends such effects to social stress reactivity involving social evaluative threat. That the effect was vascular, rather than myocardial, may support the apparent adverse effects of restricted sleep on cardiovascular health. The data from such work suggests that altered patterns of HP may underlie apparent null overall blood pressure responses to sleep loss, suggesting a possible mechanism by which sleep restriction, over time, may become related to cardiovascular disease pathogenesis.

What the present study did not address is whether sleep restriction evokes additional cardiovascular responsivity to stress in a non-laboratory context or effects which may exist across physiological systems. Using the same population of young adults from which the present studies sample was drawn, the following chapters address these issues. The next chapter examines the effects of sleep restriction on a salivary marker of stress reactivity, sAA, in response to laboratory social stress. Further, Chapter 4 utilises ambulatory blood pressure monitoring comparing cardiovascular arousal during periods of naturally occurring high and low stress and interpersonal contact, whilst rested and sleep restricted.

# Chapter 3. Study 2:

# SALIVARY α-AMYLASE REACTIVITY TO LABORATORY SOCIAL STRESS IN AN ACUTE SLEEP RESTRICTION MANIPULATION[3]

## 3.1. INTRODUCTION

*What is salivary α-amylase?*

In addition to activation of cardiovascular responses, arousal to stressful experience evokes an associative chain of neuroendocrine and nervous system reactions. While a main focus of research in this domain has been on HPA axis reactivity and salivary cortisol, there is a relative lack of systematic evidence regarding salivary markers of SAM system activity (Strahler, Mueller, Rosenloecher, Kirschbaum, & Rohleder, 2010). Multi-biomarker assessments have become increasingly popular among psychophysiological researchers as a way of linking behavioural, environmental, and social factors to an individual's health and well-being (Zethelius et al., 2008). As a substance that can be relatively easily and cost-effectively assessed, a growing number of studies suggest that sAA may serve as a surrogate marker of SAM activity (for reviews see Granger et al., 2006; Nater & Rohleder, 2009a; Schumacher, Kirschbaum, Fydrich, & Ströhle). Saliva sampling has the advantage that it is comparatively less intrusive than other sampling methods, enabling relatively stress-free multiple assessments. Non-invasiveness has been proposed as an important requirement in stress research (Gröschl, 2008) in order to control for the confounding effects of additional stress induced by intrusive sampling techniques such as venepuncture. sAA may therefore be assayed as a comparatively non-invasive index of the SAM system compared to established

[3] A manuscript based on this chapter has been submitted for publication.

sympathetic markers (e.g., plasma concentrations of the catecholamines norepinephrine [NE] or epinephrine [EP]; Thoma et al., 2012).

The potential relationship between sAA and psychological stress was first mooted in the context of experimentation into the effects of hyperbaric exposure in the late 1970's (Gilman et al., 1979). However, it was not until Chatterton et al. (1996) reported increases in sAA to a variety of psychophysiological stressful conditions that attention was again drawn to sAA as a marker of the stress response. Since that time, marked increases in sAA activity as a result of acute stress have been observed in studies examining a wide variety of stressful contexts, including pre-examination and pre-skydive stress (Bosch et al., 1996; Chatterton, Vogelsong, Lu, & Hudgens, 1997), critical life events/daily stress (Bosch et al., 1998), as well as exposure to negative emotional pictures (van Stegeren, Rohleder, Everaerd, & Wolf, 2006) and video (Bosch, de Geus, Veerman, Hoogstraten, & Amerongen, 2003; Takai et al., 2004).

*Why look at sleep restriction and sAA?*

More recent findings have begun to emerge which suggest sAA may also be a useful marker in the context of sleep research (Nater & Rohleder, 2009a), especially in relation to the possible sensitivity of sAA to sleep loss (Seugnet et al., 2006). However, amongst the small number of studies that have reported on sleep-related variables and sAA response, findings have been inconsistent. For example, non-stress dependent (resting) levels of sAA (measured after awakening) in healthy young adults were found to be unrelated to self-reported awakening time, sleep duration, or sleep quality (Nater et al., 2007). Alternatively, others have shown that when sleep duration is experimentally manipulated, the greater the sleep duration prior to a morning assessment of sAA (no sleep, 3h sleep, 7h sleep), the lower the observed α-amylase concentration (Figueiro & Rea, 2011). Poor sleep efficiency (but not

duration) has also been associated with higher sAA activity resulting from social stress (Trier Social Stress Test for Children; TSST-C) in 8-year-old children (Räikkönen et al., 2010).

As only two of these previous examinations (Räikkönen et al., 2010; Seugnet et al., 2006) obtained objective assessment of sleep duration, previous inconsistencies may be as a result of uncertain accuracy based upon self-reported sleep time (Lauderdale et al., 2008), specifying further examinations of sAA responses following verified short sleep durations is required. To date, no research has combined these two research spheres, examining social stress related change in sAA, while experimentally manipulating sleep duration. In order to advance understanding of how biological and behavioural processes interact to determine risk or resilience, it has been suggested that multiple measurements of stress-related biological processes be attained (Kario & Shimada, 2004; Ohkubo et al., 2002). A significant limitation of research in this area is that HPA and SAM axes reactivity are rarely assessed simultaneously (Rifai & Ridker, 2001). The SAM axis releases the hormones EP and NE and promotes the immediate 'fight-flight' response to stress, whilst the HPA axis controls the longer-term stress response, principally through the glucocorticoid cortisol. Whereas cortisol typically peaks approximately 15 mins after exposure to stress, before gradually returning to baseline, alpha-amylase has a near immediate stress-response and returns to baseline approximately 10 mins after stress exposure. This asymmetry in response pattern is consistent with the faster reactivity of the SNS compared to the HPA axis (Gordis et al., 2006; Takai et al., 2004). Therefore, examination of concurrent actions (i.e., reduced sleep duration) across systems permits a more thorough understanding of the physiological correlates of sleep restriction than examination of activity in single systems. Investigations are therefore needed to clarify the effect of objectively measured sleep loss on sAA response as a purported biomarker of SAM related stress reactivity, in addition to interactions (if any) with social stress reactivity.

Turning specifically to the relationship between sAA and social stress, the majority of studies in this domain have used a standardised psychosocial stress test, the aforementioned TSST (Kirschbaum et al., 1993). The TSST (comprising a mock job interview and a mental arithmetic task, both performed in front of a live audience) has generally been found to elicit marked increases in both subjective and physiological stress responses. For example, Nater et al. (2006; 2005) found that the TSST led to significant increases in sAA relative to a rest condition, and this finding has been replicated in studies involving healthy children (Granger et al., 2006), adolescents (Gordis et al., 2006), and adults (Maruyama et al., 2012). In all of these studies, the participant was challenged by evaluative comments from audience members who, as part of the standard TSST laboratory structure, were physically present in the laboratory.

While such research demonstrates the effectiveness of the TSST in its ability to reliably induce stress responding, other laboratory manipulations have been less successful. For example, Vedhara et al. (2010) failed to find physiological reactivity (HR, SBP, and cortisol) in response to their laboratory social stress manipulation. These researchers suggested that there may have been a loss of audience evaluative strength due to appraisal being delivered remotely (via video) rather than using the physical presence of others for evaluation. They further suggested that their manipulation may have been unsuccessful due the fact that there was a failure to demonstrate "explicit evidence of 'negative' social evaluation" (p. 197). Alternatively, findings from Thorsteinsson et al. (1998) in a manipulation of video-relayed social support, found that a pre-recorded "live" video feed of a laboratory confederate, affording standardisation of characteristics otherwise potentially prone to poor control and bias (i.e., verbal and non-verbal communication), was successful in attenuating HR and cortisol stress responses. In this context, the level of automation provided by video technology might enable more scope and precision than live presentation for

manipulating aspects of laboratory-based psychosocial stressors and observing effects on sAA responsivity.

Accordingly, the present analysis sought to examine sAA response, in a sample of healthy young adults. A primary aim of the present study was to examine sAA response following a period of acute sleep restriction, verified by wrist actigraphy, compared to a rested group. Incorporating methodological considerations from both Thorsteinsson et al. (1998) and Vedhara et al. (2010), a secondary aim of sought to shed light on the sensitivity of sAA response to a laboratory stress protocol, exposing participants to negative social evaluation, presented by video relay (as outlined in Study 1).

## 3.2. METHODS

### 3.2.1. Design

The design of Study 2 overlaps with that of Study 1, again employing a mixed factorial design. The between-subjects factor was sleep group (rested or sleep restricted). sAA measures were recorded during baseline and stress periods, giving a within-subjects factor of period with two levels; baseline and social stress. Sleep restriction (sleep diary and actigraph sleep assessment) and laboratory social stress (mental arithmetic with video-relayed negative evaluation) procedures were completed by participants as outlined in Chapter 2.

### 3.2.2. Participants

As discussed by Nater and Rohleder (2009b) sAA release is believed to be stimulated by activation of β-adrenergic receptors and reduced by blockade of these receptors. Consequently, participants were screened for adrenergic agonist/antagonist usage, and this resulted in the exclusion of six participants due to reported use of salbutamol for treatment of bronchospasm. All remaining participants were physically healthy and refrained from

smoking, eating, and drinking beverages (with the exception of water) for at least 1 hr prior to testing. The final sample consisted of 113 (84 female) university students (age 17 to 22 years; *M* = 18.39 years, *SD* = .87) with normal BMI (*M* = 22.92, *SD* = 2.68).

Recruitment of participants to the study, participant incentives, and associated ethical approval of procedures, were as described in Chapter 2.

### 3.2.3. Materials and apparatus

*Fatigue.* Fatigue was again measured using the MFI (Smets et al., 1995), covering pertinent domains related to fatigue experience; general fatigue, physical fatigue, mental fatigue, reduced motivation, and reduced activity. Cronbach's α for the MFI subscales in the present sample ranged from .78 to .90, indicative of good internal consistency.

*Salivary sampling and biochemical analyses.* Unstimulated whole saliva was collected using the passive drool technique as recommended for sAA sampling (Bosch, Veerman, de Geus, & Proctor, 2011; Rohleder & Nater, 2009). Participants accumulated secreted saliva in their mouth for 2 mins while being monitored by the researcher, before depositing the sample to a collection device (Sarstedt, Nümbrecht, Germany). Samples were stored at −20 °C after completion of the laboratory session until biochemical analysis took place. All samples were assayed for sAA using a commercially available kinetic reaction assay (Salimetrics, State College, PA., U.S.A.). Intra-assay coefficient of variation (CV) computed for the mean of nine replicate tests was 13.4 per cent. On the day of testing, samples were brought to room temperature, centrifuged at 3,000 revolutions per min (rpm) for 15 mins, and the clear top-phase of the sample was pipetted into appropriate test tubes. The assay employs a chromogenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose. The enzymatic action of sAA on this substrate yields 2-cholor-p-nitrophenol, which can be spectrophotometrically measured at 405 nanometers (nm) using a standard laboratory plate

reader. The amount of sAA activity present in the sample is directly proportional to the increase (over a 2 min period) in absorbance at 405 nm. The Salimetrics substrate was heated to 37 °C in a preheated microtiter plate incubator for 20 mins. Samples were diluted (1:200) with a diluent (phosphate buffered solution containing a non-mercury preservative). Eight microliters (μL) of prediluted controls (for assay calibration), as well as each diluted sample, were transferred to a 96-well microtiter plate. Three-hundred and twenty μL of the preheated substrate were added to each well with a multichannel pipette. The plate was placed in a microtiter plate mixer and mixed at 500–600 rpm for 1 min, before being transferred to a microplate reader and read at 450 nm. The plate was then mixed at 500 – 600 rpm for 2 mins and again read at 450 nm. Results were computed in units per millilitre (u/ml*)* of sAA using the formula: [Absorbance difference per min × total assay volume (.328 ml) × dilution factor (200)]/[millimolar absorptivity of 2-chloro-p-nitrophenol (12.9) × sample volume (0.008 ml) × light path (.97)]. All samples were assayed by the researcher on-site in a bioassay laboratory.

### 3.2.4. Procedure

After random assignment to experimental group (sleep restriction or rested) on Day 1, participants presented to the laboratory the following morning (Day 2) for social stress testing. Participants acclimatised to the laboratory for approximately 20 mins while psychometric measures were completed. A resting saliva sample was then taken by the participant using the aforementioned methods. Participants were given instruction on the laboratory stressor, consisting of mental arithmetic task, combined with video-relayed evaluation of performance (see Chapter 2). Following completion of the task, participants provided a second saliva sample, before completing Likert scale ratings of how stressful, enjoyable, and difficult they found the stress task to be.

## 3.3. RESULTS

### 3.3.1. Overview of analyses

sAA data typically do not show normal distributions, and are positively skewed (Rohleder & Nater, 2009). Accordingly, due to positively skewed distributions in the present sample, sAA values were log-transformed to approximate normal distributions in accordance with previous α-amylase data transformations (Nater et al., 2007). Data were tested for normal distribution and homogeneity of variance using a Kolmogorov - Smirnov and Levene's test before statistical procedures were applied. The concentration of α-amylase in saliva was expressed in u/ml.

Logarithmically transformed levels of sAA were utilised for both periods of the experiment; namely, baseline and social stress. Previous data indicate that neither basal sAA activity or acute sAA response to different stressors differs between the men and women (Rohleder & Nater, 2009). However, there have been some reports suggesting men tend may show a smaller diurnal sAA increase over the course of the day (Nater et al., 2007; Wingenfeld et al., 2010). An initial mixed ANOVA was run to examine the effect of gender on sAA response, using the within-subjects factor period (baseline, social stress) and the between-subjects factors sleep (rested vs. sleep restricted) and gender (male vs. female), which indicated that gender did not result in any significant main or interaction effects on sAA level (all *p*s >.11).

To inspect sAA response to social stress while rested and restricted, a mixed factorial ANOVA was conducted, using the between-subjects factor, sleep (rested vs. restricted), and the within-subjects factor, period (baseline, social stress). Additional independent samples *t*-tests, applying Bonferroni correction when appropriate, were conducted to examine differences in task performance and post-stress ratings, when rested and sleep restricted.

Figures and tables present untransformed sAA values for illustrative purposes. As previously, ANOVA effect sizes are presented as partial $\eta^2$.

### 3.3.2. Adherence to sleep manipulation

Compliance with the sleep manipulation protocol, as assessed using wrist actigraphy, indicated high levels of participant adherence. In the current sample, activity levels, monitored on the evening prior to the restricted night to ensure participants maintained wakefulness until their usual bedtime, indicated the average percentage of time participants were awake during the evening prior to the sleep restriction period to be 92.31 per cent (*SD* = 12.25). Equally high (*M* = 378.99, *SD* = 151.68) evening activity scores were recorded (a total score of ≥ 80 designating an epoch as being "awake"). During the restriction vigil (following normal bedtime and awakening on receipt of 40 per cent usual sleep duration), the mean percentage of time participants were awake was 85.19 per cent (*SD* = 18.08). The mean activity score per 2 min epoch was 239.50 (*SD* = 123.00). Both percentage time awake and activity score indicated substantial compliance to the sleep restriction protocol.

### 3.3.3. Manipulation Checks

*3.3.3.1. Self-reported fatigue.* To confirm adherence to the sleep restriction protocol, while also comparing relative levels of fatigue in the rested group, a series of independent samples *t*-tests were conducted for each of the MFI fatigue subscales. Independent *t*-test revealed significantly higher general fatigue reported when sleep restricted (*M* = 14.94, *SD* = 2.83) compared to rested (*M* = 9.39, *SD* = 2.81; $t(105) = 10.18$, $p < .001$). For the remaining four MFI subscales (mental fatigue, physical fatigue, reduced activity, and reduced motivation), significantly greater fatigue under sleep loss was again observed, demonstrating significantly greater fatigue under sleep restriction compared to rested (all *p*s < .001).

*3.3.3.2. Task Engagement.* Independent samples *t*-tests indicated effects for sleep on post-task ratings of how stressful the task was found to be, such that the task was rated as significantly more stressful under sleep restriction ($M$ = 6.64, $SD$ = 2.25) compared to rested ($M$ = 5.52, $SD$ = 2.32; $t(101) = 2.47$, $p = .015$). There were no effects for sleep on ratings of enjoyableness or difficulty, indicating that sleep did not influence participant's perceptions of how difficult or enjoyable the task was. Independent *t*-tests revealed no differences in the number of problems answered correctly, incorrectly, or left unanswered (all *p*s >.29), when rested and sleep restricted.

3.3.4. Confirmation of sAA reactivity.

Mixed ANOVA confirmed a main effect for period, $F(1, 103) = 68.24$, $p < .001$, partial $\eta^2 = .399$, with task levels greater than baseline levels, in both rested and sleep restricted groups. These main effects indicate the success of the stressor in eliciting increased sAA reactivity under conditions of video-relayed evaluative social stress. Means (with *SD*s) for sAA activity during baseline and social stress, as a function of sleep group, are shown in Table 3.

Table 3

*Means (with* SD*s) for salivary α-amylase (*s*AA) activity by sleep group*

| | Rested | | Sleep Restricted | |
|---|---|---|---|---|
| | Mean | *SD* | Mean | *SD* |
| Baseline | 40.26 | 30.86 | 71.58 | 83.13 |
| Social Stress | 83.65 | 81.58 | 97.70 | 100.48 |

Note: Untransformed α-amylase values presented in units per millilitre (u/ml)

A sleep × period interaction, $F(1,103) = 4.04$, $p = .047$, partial $\eta^2 = .038$, indicated that sAA increased more from baseline levels in the rested group compared to the sleep restricted group. However, as can be seen in Figure 4, this interaction effect was driven by significantly higher baseline levels of sAA under sleep restriction compared to rest. No main effects for sleep were found on sAA across the study ($p = .10$). See Table 4 for summary of ANOVA effects.

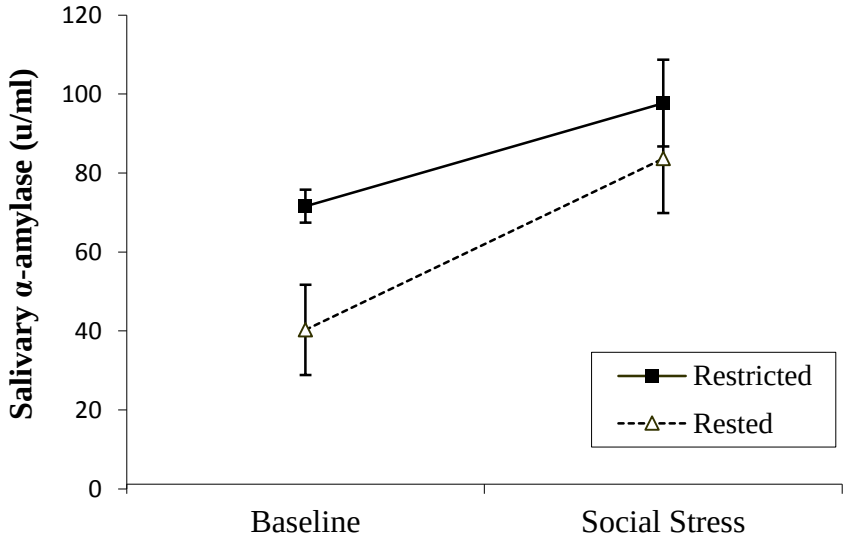


*Figure 5.* Sleep × period interaction on salivary α-amylase (sAA) activity, under rested and restricted conditions. Error bars denote standard errors of the mean

Table 4

*Summary table of mixed ANOVA results for salivary α-amylase (sAA)*

| Source | Sums of squares | *df* | Mean Square | *F* | Partial $\eta^2$ |
|---|---|---|---|---|---|
| Sleep | < 1 | 1, 103 | < 1 | 2.75 | .026 |
| Period | 3.12 | 1, 103 | 3.12 | 68.24** | .399 |
| Sleep × Period | < 1 | 1, 103 | < 1 | 4.06* | .038 |

Period: baseline and social stress. Sleep: rested versus restricted.

* $p < .05$ ** $p < .001$

## 3.4. DISCUSSION

The present study examined sAA response to a social stressor involving video-relayed negative evaluation, under rested or sleep restricted conditions, in a sample of healthy young adults. Most notably, one night of partial sleep restriction was associated with significantly increased basal (i.e., resting) sAA activity. The results also demonstrate significantly increased sAA activity immediately following an acute evaluative stressor presented by video relay.

In addition to increased self-reported fatigue, significantly higher basal (and higher stress-phase) levels of sAA activity were identified in the sleep restriction compared to the rested group. These findings support the results of Figueiro and Rea (2011) who found that increasing increments of sleep duration resulted in lower resting sAA levels. Basal sAA activity shows diurnal oscillation, with decreased levels post-awakening, gradually increasing to peak levels in late afternoon (Rohleder et al., 2004). However, this would not have confounded present findings because laboratory sessions were conducted at the same time of day (morning) following random allocation to sleep group across all participants.

Nater et al. (2007) found that basal amylase after awakening was unrelated to participant self-report of awakening time, sleep duration, or sleep quality. Examining sleep duration specifically, one night of partial sleep restriction has been implicated in the nocturnal regulation of sympathetic activity (via increases in plasma EP and NE; Irwin et al., 1999), and Seugnet et al. (2006) identified significantly higher basal sAA activity after 28h of waking in a small sample of healthy adults ($N$ = 9). Notably, previous effects were observed following unverified sleep duration (Nater et al., 2007) resulting in methodological uncertainty (Lauderdale et al., 2008), or involved small sample size (Seugnet et al., 2006). Further, plasma assessment may be prone to confounding effects of increased stress associated with measurement method (i.e., venepuncture; Meeran et al., 1993). Using objectively verified short sleep duration, the present study is the first to show that basal sAA activity is increased following partial sleep restriction (to 40 per cent of usual sleep time) for one night, in a sizable sample of healthy young adults. In addition to corroborating previous findings of increased sympathetic activity following partial sleep loss identified in plasma (e.g., Irwin et al., 1999), these data further support the potential usefulness of the sAA analyte as a purported non-invasive index of the SAM system, helping to rule out the confounding effects of additional stress induced by techniques such as blood draw.

van Stegeren et al. (2006) observed increased sAA activity as a result of anticipatory anxiety caused by an upcoming fMRI investigation, while over the same period, the researchers did not observe an increase in the cardiac markers obtained (SBP, DBP, and HR), which they feel "could suggest that sAA is more sensitive to subtle psychological stress than blood pressure or HR" (p. 141). Consistent with such a hypothesis, the present evidence of basal sAA increases under sleep restriction may also demonstrate effects on autonomic homeostasis in the absence of overall cardiovascular change (as evidenced by no acute

reactive effect of sleep restriction on overall cardiovascular parameters [SBP, DBP, and HR] with observable effects only on underlying regulatory vascular processes in Chapter 2).

Present findings reveal no difference in sAA response to social stress under sleep restriction compared to when rested. This observation is consistent with the findings of Räikkönen et al. (2010), who found that sAA responses to social stress in children (TSST-C) did not vary significantly according to sleep duration. However, these researchers did observe that the lower the children's sleep efficiency, the higher the overall level of sAA across the entire TSST-C protocol. The present study similarly observed raised (baseline and stress) levels of sAA across the complete social stress protocol under sleep restriction (though not reaching statistical significance post-stress), with the methodological advantage of experimentally manipulating sleep duration. While there are few previous studies linking sleep pattern with sAA reactivity, present findings, together with previous findings (Figueiro & Rea, 2011; Räikkönen et al., 2010), indicate a general upregulation of sAA activity following poor sleep, reinforcing the sympathetic neurohormonal implications of poorer sleep. As the SAM axis is designed for an immediate stress response, relative to activation of the HPA axis, after the onset of a stressor (Deak, 2007; O'Connor, O'Halloran, & Shanahan, 2000), the effects observed here suggest activation of the first-phase neuroendocrine stress response following acute reduction in sleep duration.

This study also contributes new knowledge concerning sAA and reactivity to social stress. Specifically, present results demonstrated a significant increase in sAA in response to an acute social stress protocol, where the primary social evaluative element was presented by virtual (i.e., video-relayed) observers. Incorporating experimental considerations from Thorsteinsson et al. (1998) and Vedhara et al. (2010), these results support the use of video presentation as a potentially more precise and cost-efficient way of examining social stress effects than is achievable using a live audience. Such a methodology affords greater

standardisation of verbal instruction to the participant, in addition to gender, gestures, facial expressions, and other physical and nonverbal characteristics of the person(s) providing the evaluation. Regarding the specific nature of the feedback provided, previous laboratory sAA social stress (TSST) investigations have involved an implicit perception of the potential for negative evaluation. Present findings substantiate the fact that explicit negative feedback results in increased sAA activity. However, as there was no control (positive feedback/no feedback) condition, the findings regarding the precise effects of negative feedback on sAA require further clarification.

Present findings contrast (on observed physiological reactivity) with findings from Vedhara et al. (2010) who reported that salivary cortisol, HR, and SBP responses to carbon dioxide stress were not affected by social evaluation using a video audience. However, unlike the Vedhara et al. manipulation, the present study exposed participants to a continuous "live" feed of the evaluative confederate via the installed monitor. This enabled visual contact with the video confederate throughout the stressor, in addition to the receipt of on-going standardised feedback (as used in the Thorsteinsson et al., 1998 manipulation), resulting in enhanced evaluative stress, and eliciting the observed sAA reactivity in the present study.

While the present study provides evidence that partial reductions in sleep duration can effect change in basal amylase enzymatic activity, a number of considerations should be noted. While empirical data and assay supplier recommendations at the time of data collection for the present study indicated that sAA concentrations are not affected by salivary flow rates (see Rohleder, Wolf, Maldonado, & Kirschbaum, 2006), current recommendations advise examination of the secretion rate for sAA (i.e., the output as units per minute [u/min]) in addition to sAA concentration (u/mL) when using sAA as a marker of nervous activity (Bosch et al., 2011). While stimulation of salivary proteins is generally ascribed to sympathetic stimulation, stimulation of saliva flow rate is mainly mediated by

parasympathetic nerves (Anderson et al., 1984; Garrett, 1987). It has been suggested that sAA concentration may reflect the combined effect of salivary flow rate (largely parasympathetic) alongside protein secretion (which, in sympathetically activated glands, is largely sympathetic). Thus, until the question of flow-rate dependency for sAA is further examined, future investigations utilising sAA are advised to record salivary flow rates in addition to sAA concentration.

The weight of accumulated evidence would suggest that sAA is a sensitive marker of acute stress. However, when considering the physiological implications of sAA (and changes therein), Bosch et al. (2011) advised caution in the interpretation of sAA activity exclusively as a measure of SNS activity. Instead, they suggested that sAA release from the salivary glands (primarily the parotid and minor palatine glands) may reflect a mixture of both sympathetic and concurrent parasympathetic activity. Indeed, some data suggest no association when plasma catecholamines (established markers of sympathetic activity), NE and EP, are measured simultaneously with sAA levels (Nater et al., 2006). However, previous findings have demonstrated significant correlations between $\alpha$-amylase and both of these catecholamines (NE and EP) in a variety of stressful conditions (Chatterton et al., 1996), indicating an association between sAA and activity of the SAM system. Further, pharmacological manipulation of the SAM system has also underscored the role of $\alpha$-amylase activity as an indicator of sympathetic activity. Application of the β-adrenergic receptor blocker propranolol was successful in reducing sAA activity in unstimulated whole saliva (Nederfors & Dahlöf, 1992) as well reducing stress-induced sAA increases (Nater & Rohleder, 2009b; van Stegeren et al., 2006). Recent findings (in contrast to previous findings from the same laboratory), now suggest that during both pharmacological challenge or social stress (TSST), sAA is related to NE and EP in blood (Nater et al., 2012). Such effects have been further independently verified (in relation to NE) by others. For a large sample ($N$ = 66)

of healthy participants, sAA, NE, and EP increased significantly in response to acute laboratory social stress (TSST), with sAA stress responses significantly predicting stress responses in NE (Thoma et al., 2012). While further investigation is warranted to establish if increase in sAA reflects stress-dependent sympathetic activity, or involves concurrent parasympathetic stimulation, existing findings point to sAA as an important biomarker of autonomic SAM function in both acute and chronic stress contexts, and as an identifier of adaptation to stressful experience (Nater et al., 2006; Nater & Rohleder, 2009b).

Significantly increased basal levels of sAA activity following one night of verified partial sleep restriction were observed in the present study, contributing to the limited previous data linking sleep pattern with sAA response. Present results also corroborate previous findings of sAA sensitivity to laboratory stress, though the main laboratory analogues that have been used to date may offer less operational precision and cost-effectiveness than video-relayed alternatives. As SAM axis activation is designed for an immediate response to threat (see review by Dickerson & Kemeny, 2004), such data suggest changes in the first phase stress response, evidenced through SAM related sAA activity, due to acute reduction in sleep duration. On the other hand, HPA axis activation (e.g., cortisol response) is a relatively more prolonged hormonal response that is observed approximately 15 – 20 min following stressor onset.

To further inform interpretation of such effects regarding longer-term neuroendocrine stress responses (and observe ambulatory salivary physiological responsivity in relation to sleep restriction), Chapter 5 will examine sleep related activation of the HPA axis, as evidenced by the awakening response in cortisol, assessed in the home setting. In advance of this, the next chapter further explores the cardiovascular effects of sleep restriction, examined previously in relation to laboratory stress in Chapter 2, utilising 24-hr ambulatory blood pressure and HR monitoring.

# Chapter 4. Study 3:

# AMBULATORY BLOOD PRESSURE AND REACTIVITY TO AMBULATORY STRESS AND INTERPERSONAL INTERACTION: EFFECTS OF ACUTE SLEEP RESTRICTION

## 4.1. INTRODUCTION

*Why look at sleep restriction and ambulatory blood pressure?*

Among the possible mechanisms linking sleep disturbances and cardiovascular disease, ABP assessment may offer significant advantages over laboratory measurements. For example, a recent review documented the superiority of ambulatory over clinic blood pressure measurements in the prediction of major cardiovascular events (Verdecchia, Angeli, Gattobigio, & Porcellati, 2003). In addition to providing a profile of cardiovascular activity away from laboratory settings, thereby providing a representation of blood pressure response in non-artificial settings, ambulant monitoring also typically involves repeated assessment, increasing reliability of observed effects. ABP monitoring has also become a frequently used method of assessing the generalisability of laboratory-based CVR to real-life settings (e.g., Ottaviani et al., 2006).

Amongst the limited number of studies that have examined the effects of sleep restriction using ABP monitoring, findings have been inconsistent. While some data support cardiovascular increases (SBP and DBP) following sleep restriction (Tochikubo et al., 1996), others show a mixture of both increases and decreases. Lusardi et al. (1996) found that following a period of reduced sleep (43 per cent of usual sleep time) in young normotensive participants, ABP monitoring revealed decreased SBP and DBP during the nocturnal period, in addition to increased SBP and HR post-awakening, compared to a night of full rest. However, an important issue regarding previous inconsistent results relates to measurement

strategy. Ambulatory monitoring allows examination of blood pressure and HR responses during daily activities, thereby allowing assessment of reactivity to ambulant stimuli (Pickering, 1996).

Few studies have measured how sleep loss affects response to naturalistic stressors (Meerlo et al., 2008). Interpersonal contact has been suggested as a useful task for investigating CVR in the field (Bastard et al., 1999; Holt-Lunstad et al., 2009). For example, using a population of undergraduate students (comparable to the sample used in the present research, with a similar mean age) Lehman and Conley (2010) found that momentary reports of evaluative stress during naturally occurring interactions were associated with concurrent increases in both SBP and DBP. As sleep loss has been shown to increase negative emotionality surrounding interpersonal interaction (Kahn-Greene et al., 2006), an examination of periods of stress and interpersonal contact while rested and sleep restricted, and resulting effects on ABP, is required. This is an issue of importance, identifying whether sleep restriction affects not only mean daytime/nighttime ABP activity, but also reactivity to ambulatory stimuli (Meerlo et al., 2008).

*Nocturnal blood pressure dipping*

In normotensive individuals, sleep is associated with reduced blood pressure, referred to as the "dipping" phenomenon, whereby SBP and DBP may decline by 10 per cent to 20 per cent (compared to awake levels) during sleep (Rafey, 2009). Further, evidence suggests that individuals who fall outside this range, that is, who fail to show lower nighttime blood pressure of at least 10 per cent , or greater than 20 per cent, relative to daytime levels, are at higher risk for cardiovascular morbidity and mortality than those with a more typical dipping pattern (Dolan et al., 2005; O'Brien, 2010; Verdecchia, Angeli, Borgioni, Gattobigio, & Reboldi, 2007).

Dipping pattern could be influenced by inadequate sleep, and associated sleep disorders (Matthews et al., 2008). Amongst studies of normotensive and hypertensive men and women, non-dippers had more night time (and less daytime) movement as measured by actigraphy than did dippers (Herity, 2000; Leary et al., 2000). Schillaci et al. (2007) also reported that individuals who reported longer duration of sleep also had greater blood pressure dipping from day to night. However, rather than a binary conception of dipping versus non-dipping, with the former associated with the most favourable health outcomes, Kario et al. (2004) additionally identify a second potential high risk group among dippers; the sub-group of "extreme dippers", characterising individuals who have an exaggerated nocturnal blood pressure fall. For example, data suggest that among elderly hypertensive patients, extreme dippers have more marked cerebrovascular damage than dippers (Rifai & Ridker, 2001) and increased arterial stiffness in normotensive individuals with risk components of the Metabolic Syndrome (Epstein & Ross, 1999). To the extent that non-dipping is associated with more movement during sleep, and longer self-reported sleep duration, it is important to understand potential factors accounting for such results (i.e., associations of non-dipping with substantiated sleep restriction, in addition to association [if any] with a counterpoised extreme dipping nocturnal profile).

There are several benefits of examining ABP compared to clinic and laboratory assessments, including increased reliability and a stronger approximation of cardiovascular risk (Ouchi et al., 2003; Pickering et al., 2005). Further, whether laboratory responses generalise to the challenges of daily living has implications for understanding the potential mechanisms linking cardiovascular reactivity with ill health. Therefore, the present study complements the pervious laboratory cardiovascular investigation (Chapter 2), affording increased ecological validity in an examination of sleep loss and associated cardiovascular function across contexts.

The present work sought to examine the effects of one night of actigraphy-verified partial sleep restriction on measures of ABP and HR, compared to one night of full rest, in a sample of young adults. Data from the previous (limited) investigations of ABP and experimental manipulation of sleep duration remain inconsistent, while there is additional uncertainty surrounding the extent to which sleep restriction may affect reactivity to periods of ambulatory stress and interpersonal contact. As such, the present study compared cardiovascular arousal during periods of high and low stress and interpersonal contact, whilst rested and sleep restricted. Finally, examination of nocturnal blood pressure dipping was also conducted to explore to explore the hypothesis that nocturnal reduction in blood pressure is associated sleep duration, accounting for abnormalities in habitual nocturnal hemodynamic balance.

## 4.2. METHODS

### 4.2.1. Design

After completion of laboratory measures on Day 2 (Chapters 2 & 3), ambulatory assessment of participants' blood pressure and HR was undertaken. Participants were also requested to complete an activity diary at every ABP measurement time-point (on cuff inflation). While Studies 1 and 2 had mixed factor designs, Study 3 was completed within-subjects, with participants completing ABP monitoring over two 24-hr periods; once while rested and once while sleep restricted (participants being randomly assigned to experimental condition [rested or sleep restricted] on Day 1). Sleep restriction procedures (sleep diary and actigraph sleep assessment) were completed by participants as outlined in Chapter 2.

### 4.2.2. Participants

Participants were 106 (82 female) university students (age 17 to 24 years; $M$ = 18.52 years, $SD$ = 1.13) with normal BMI ($M$ = 22.98, $SD$ = 2.69). All participants were physically

healthy, tested as normotensive (resting blood pressure < 140/90 mmHg) and reported no history of heart disease. Recruitment of participants to the study, participant incentives, and associated ethical approval of procedures, were as described in Chapter 2.

### 4.2.3. Materials and apparatus

*Ambulatory blood pressure monitoring.* Ambulatory measurement of SBP, DBP, and HR were assessed using a Suntech Oscar II ABP monitor (SunTech Medical, Ltd., Oxfordshire, U.K.). The Oscar II unit is a small, non-invasive, blood pressure and HR monitoring device, which is worn by the patient either on a waist belt or shoulder strap and is connected to a cuff, which is affixed around the non-dominant upper arm. Blood pressure is measured by the Oscar II using the oscillometric method (Bonnafoux, 1996), which analyses pulse waves collected from the cuff during constricted blood flow. The monitor was set to randomly take a reading approximately every 20 mins during the day and every 45 mins during the night. Blood pressure measurements determined by the Oscar II have been shown to be equivalent to those obtained by a trained observer using the cuff/stethoscope auscultation method, and validated for clinical use by the International Protocol of the European Society of Hypertension and British Hypertension Society (Goodwin, Bilous, Winship, Finn, & Jones, 2007).

*Activity diary.* A paper-and-pencil ambulatory monitoring diary was used to collect information at the time of cuff inflation. Participants were required to take note of the time at which the measurement occurred, in addition rating elements of social interaction and stress, with each item rated on a 1- to 4- point Likert scale. One item assessed perceived stress (rated "not at all" to "high") and one item assessed interpersonal contact (rated "low" to "high"). The criterion for interpersonal contact was defined as any activity in which participants were mutually engaged with another individual (Holt-Lunstad et al., 2009).

### 4.2.4. Procedure

On completion of the laboratory elements (studies 1 and 2), participants were briefed on the procedures required for the ambulatory portion of the study. As part of orientation to the ambulatory monitoring, participants were instructed that when the blood pressure cuff began to inflate, movement should be kept to a minimum until the cuff was completely deflated. Participants were asked to complete the activity diary as soon as possible after the cuff had deflated. To ensure that the participants understood how to complete the activity diary (and confirm correct cuff placement) a practice measure was completed in the presence of the researcher. Once one valid ABP reading and required activity diary items had been successfully recorded, the participant was requested to wear the monitor throughout the rest of the day as they went about their usual activities, before retiring to bed at their normal bedtime. The participant was requested to return the assessment materials to the laboratory the following day, after a full 24-hrs of ambulatory monitoring had been completed.

### 4.2.5. Data screening and reduction

*4.2.5.1. Outlier and error detection.* The Oscar II utilises a number of event codes (EC) that signify problems with the estimation of an ambulatory cardiovascular assessment at a particular time-point. Based on previous handling of ambulatory test codes (see Holt-Lunstad et al., 2009; Kamarck et al., 1998) readings were eliminated if they were associated with an erratic oscillometric signal, signifying movement during a reading or when a reading exceeded the measurement time limit (120 seconds), signifying air leak or insufficient cuff tightness (EC 2 and 4 respectively; see ABP manual, Suntech Medical Instruments, 2011). Each ABP record was additionally screened for outliers associated with artifactual values, using the criteria proposed by Marler, Jacob, Lechoczky, and Shapiro (2006). Both awake and asleep SBP and DBP values were excluded from analysis if a) SBP < 70 or > 250 mmHg,

(b) DBP < 45 or > 150 mmHg, and (c) SBP/DBP < (1.065+[.00125 × DBP]) or > 3.0. This resulted in 56 sleep restricted and 58 rested SBP and DBP records being eliminated from waking ABP readings. The same criteria were imposed on sleeping ABP values, resulting in 118 sleep restricted and 113 rested SBP and DBP records being eliminated. Heart-rate values < 40 bpm or > 200 bpm were also eliminated (Kamarck et al., 1998). This resulted in two rested and three sleep restricted waking values and four rested sleeping HR values being eliminated.

Altogether, from the remaining readings, valid measures of awake ambulatory SBP, DBP, and HR readings were obtained in 12,678 cases when sleep restricted and 12,807 cases when rested. For sleep measures, valid readings were obtained in 3,399 cases when sleep restricted and 3,420 when rested.

In accordance with previous examinations of ABP and diary records (Luecken, Kraft, Appelhans, & Enders, 2009) the expected number of records varied per participant depending on individual (habitual) sleep time. Therefore, calculations were based on predetermined cut-offs prior to analysis relating to a daytime reference period of approximately 13 hrs of recording (38 expected records) and a nighttime reference period of approximately 8 hrs 15 mins of recording (11 expected records).

*4.2.5.2. ABP dependent variables.* Each ABP outcome variable was computed separately for SBP, DBP, and HR. Excellent internal reliability consistency for each mean ABP measure was observed, when both rested and sleep restricted (Cronbach's $\alpha > .93$ for daytime measures [$n = 38$]; $\alpha > .75$ for nighttime measures [$n = 11$] ). Outcome variables were as follows:

(1) 24-hr ABP, defined as the average of all of the valid ABP readings obtained across both daytime- and nighttime- reference periods.

(2) Daytime ABP, defined as the average of all of the valid ABP readings obtained during reported waking hours.

(3) Nighttime ABP, defined as the average of all of the valid ABP readings during reported sleeping hours.

(4) As time of day (Wallace, Park, Zakutansky, Lehmkuhl, & Jastremski, 2005) and changes to aspects of the psychosocial environment (Steptoe, 2000) may affect ABP profile over the course of the day, daytime recordings were further divided into day (21 expected records) vs. evening (17 expected records).

(5) Percentage of SBP and DBP dipping was computed by subtracting the sleeping average from the daytime average, dividing by the daytime average and then multiplying by 100 (Bishop, Pek, & Ngau, 2006). While this criterion is the one most commonly used in the literature on nocturnal dipping, variations on this calculation have also been used (Holt-Lunstad et al., 2009).

(6) As an additional measure, nocturnal BP dipping was calculated by means of a daytime-nighttime change score, taking the average daytime readings and subtracting the average nighttime readings. Thus, higher scores indicate greater dipping.

(7) Finally, mean levels of self-reported stress and social contact were computed for each sleep phase (rested, sleep restricted) and were further categorised into high versus low on each variable, based on a median split within each sleep phase.

## 4.3. RESULTS

### 4.3.1. Overview of statistical analyses

In order to examine effects of sleep restriction on ABP, data for each cardiovascular parameter was subjected to paired sample *t*-tests, conducted on mean 24-hr ABP when rested and sleep restricted. These were followed by separate repeated-measures ANOVA,

examining sleep and time, using the within-subjects factors, sleep (rested, sleep restricted) and time (daytime, nighttime); before examining time further, using the within-subjects factors, sleep (rested, sleep restricted) and time (day, evening, nighttime).

Differences in stress and social contact were examined using paired samples *t*-tests, conducted on mean levels of stress and contact when rested and sleep restricted. As measures of stress and contact were assessed during waking hours only, reactivity to high and low levels of stress and social contact was assessed comparing daytime ABP (rested and sleep restricted) by means of a series of between-subject ANOVAs, using the between-subjects factors stress (high vs. low) and social contact (high vs. low). Waking hours were further reduced into day and evening ABP (rested and sleep restricted), and examined by mixed factorial ANOVA, using the within-subjects factor time (day, evening) and the between-subjects factors of stress and social contact.

Finally, SBP and DBP percentage of nocturnal dipping and daytime-nighttime change score were examined using paired samples *t*-tests, in conjunction with between-subjects ANOVA, examining association of nocturnal dipping with high versus low stress and social contact, comparing rested and sleep restricted conditions.

Effects of gender differences in ABP were examined using a mixed factorial ANOVA, with the within-subjects factor sleep (rested, sleep restricted) and the between-subjects factor gender (male vs. female); which revealed no significant interactions with sleep across, 24-hr SBP ($p$ = .972), DBP ($p$ = .972), or HR ($p$ = .914).

Data were tested for normal distribution and homogeneity of variance using a Kolmogorov - Smirnov and Levene's test before statistical procedures were applied. All reported results were corrected by the Greenhouse-Geisser procedure where appropriate (violation of sphericity assumption) and Bonferroni correction applied to multiple *t*-test

comparisons. Slight variations in degrees of freedom across conditions reflect occasional missing data. As previously, for all analyses, ANOVA effect sizes are presented as partial $\eta^2$.

### 4.3.3. Adherence to sleep manipulation

Compliance with the sleep manipulation protocol, as assessed using wrist actigraphy, indicated high levels of participant adherence. In the current sample, activity levels monitored on the evening prior to the restricted night to ensure participants maintained wakefulness until their usual bedtime, indicated the average percentage of time participants were awake during the evening prior to the sleep restriction period to be 91.92 per cent (*SD* = 12.44). Equally high (*M* = 382.54, *SD* = 156.56) evening activity scores were recorded (a total score of ≥ 80 designating an epoch as being "awake"). During the restriction vigil (following normal bedtime and awakening on receipt of 40 per cent usual sleep duration), the mean percentage of time participants were awake was 84.48 per cent (*SD* = 17.69). The mean activity score per 2 min epoch was 231.50 (*SD* = 120.94). Both percentage time awake and activity score indicated substantial compliance to the sleep restriction protocol.

### 4.3.4. Acute sleep restriction and ABP

Paired samples *t*-tests indicated that there was no effect of sleep restriction on mean 24-hr SBP ($p = .870$), DBP ($p = .154$), or HR ($p = .208$), when compared to rested. Mean levels of SBP, DBP, and HR as a function of time and SBP and DBP as a function of nocturnal dipping, following rested and sleep restricted conditions, are shown in Table 5.

Examining daytime and nighttime ABP levels, the ANOVA revealed main effects for time on both SBP, $F(1, 97) = 406.99$, $p < .001$, partial $\eta^2 = .808$, and HR, $F(1, 97) = 484.53$, $p < .001$, partial $\eta^2 = .833$, with the expected diurnal pattern of higher levels during the daytime

compared to nighttime. No main effects for sleep, or sleep × time interactions were observed (*p*s >.43).

For DBP, a main effect for time was again observed, $F(1, 97) = 1040.55$, $p < .001$, partial $\eta^2 = .915$, confirming the pattern of higher daytime to nighttime levels. A further sleep × time interaction was additionally observed, $F(1, 97) = 4.95$, $p = .028$, partial $\eta^2 = .049$, with lower DBP at night when sleep restricted compared to rested, as illustrated in Figure 5. No main effect for sleep was observed on DBP ($p = .292$).

Examining time further, across day, evening, and nighttime ABP levels; on SBP and HR, main effects for time were again observed, with contrasts revealing that both day and evening levels were significantly higher than nighttime (all *p*s < .001). No main effects for sleep or sleep × time interactions were observed (all *p*s > .417).

For DBP, a main effect for time, with higher day and evening levels compared to nighttime was observed, $F(2, 186) = 427.44$, $p < .001$, partial $\eta^2 = .821$. The sleep × time interaction approached significance, $F(2, 186) = 3.02$, $p = .051$, partial $\eta^2 = .031$, with contrasts confirming lower DBP at nighttime compared to day, under sleep restriction compared to rested, $F(1, 93) = 6.78$, $p = .011$, partial $\eta^2 = .068$, with no difference between evening and nighttime ($p = .192$) or day and evening ($p = .285$). It would appear, therefore, that the effect of lower nighttime DBP under sleep restriction is influenced by a steeper decline in DBP level from day (compared to evening) DBP level, to nighttime, as illustrated in Figure 6.

Table 5

*Means (with* SDs*) for cardiovascular parameters by sleep condition*

| Variable | Rested | | Sleep Restricted | |
|---|---|---|---|---|
| | Mean | *SD* | Mean | *SD* |
| SBP 24-Hr (mmHg) | 117.41 | 10.65 | 117.51 | 9.28 |
| DBP 24-Hr (mmHg) | 64.33 | 5.49 | 63.70 | 5.42 |
| HR 24-Hr (bpm) | 71.91 | 8.02 | 71.20 | 8.44 |
| SBP Daytime (mmHg) | 124.85 | 9.20 | 125.08 | 9.85 |
| DBP Daytime (mmHg) | 71.07 | 5.76 | 71.32 | 6.73 |
| HR Daytime (bpm) | 78.50 | 8.63 | 77.66 | 9.78 |
| SBP Nighttime (mmHg) | 109.41 | 13.79 | 109.91 | 10.81 |
| DBP Nighttime (mmHg) | 57.18 | 6.25 | 56.10 | 5.55 |
| HR Nighttime (bpm) | 65.00 | 8.71 | 64.82 | 8.92 |
| SBP Nocturnal Dipping[a] | 12.33 | 8.38 | 12.10 | 6.70 |
| DBP Nocturnal Dipping[a] | 19.43 | 7.21 | 21.28 | 6.87 |
| SBP Daytime-Nighttime change score[b] | 15.30 | 10.19 | 15.29 | 8.91 |
| DBP Daytime-Nighttime change score[b] | 13.88 | 5.44 | 15.39 | 5.66 |

a Percentage of SBP/DBP dipping computed by subtracting sleeping average from the daytime average, dividing by the daytime average and multiplying by 100

b SBP/DBP daytime-nighttime change scores computed by subtracting average nighttime readings from average daytime readings

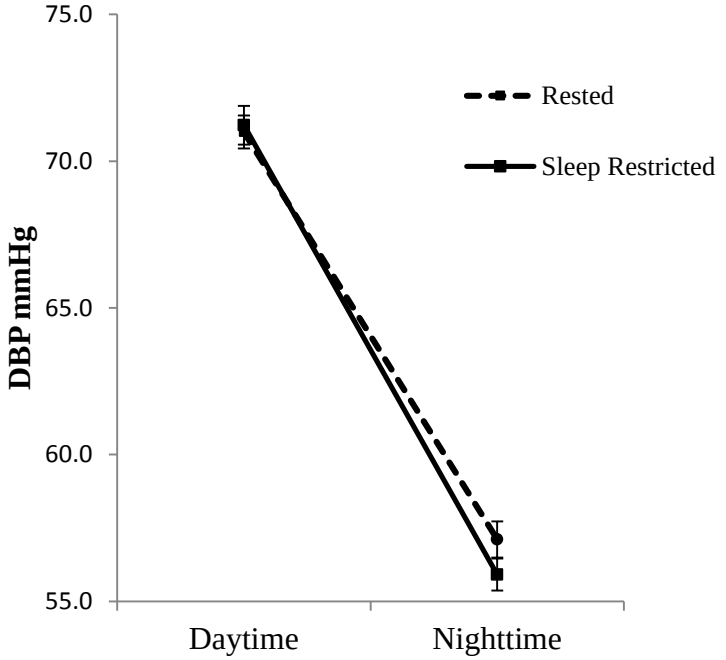


*Figure 6.* Change in diastolic blood pressure (DBP) across daytime and nighttime by sleep condition. Error bars denote standard errors of the mean

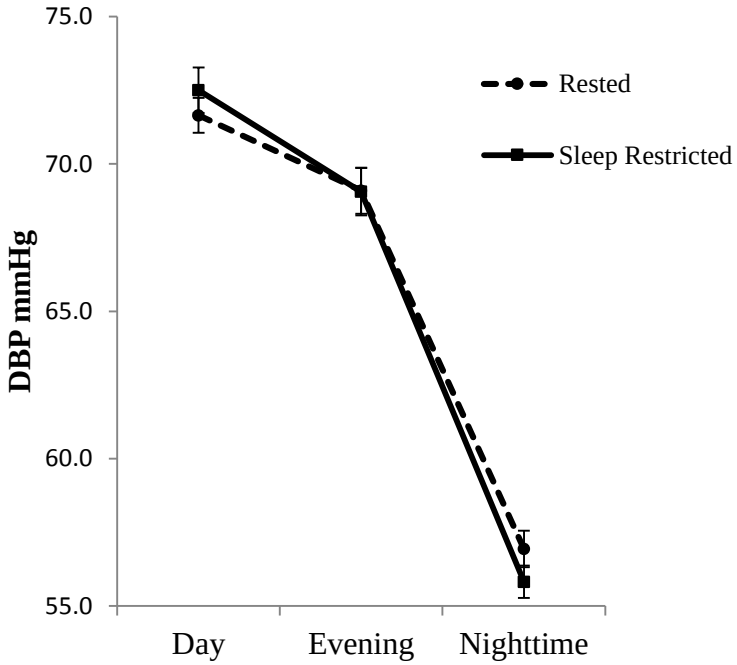


*Figure 7.* Change in diastolic blood pressure (DBP) across day, evening, and nighttime by sleep condition. Error bars denote standard errors of the mean

4.3.5. Stress, interpersonal contact, and ABP

Paired samples *t*-tests on levels of self-reported ambulatory stress and interpersonal contact revealed that mean levels of stress while sleep restricted were significantly higher (*M* = 1.40, *SD* = .43) than when rested (*M* = 1.29, *SD* = .33; $t(99) = 2.93$, $p = .004$). Interpersonal contact did not differ across sleep restricted (*M* = 2.20, *SD* = .54) and rested (*M* = 2.17, *SD* = .62) conditions, $t(99) = .336$, $p = .738$.

Examining daytime SBP and DBP when rested, the ANOVA revealed no main effects for interpersonal contact under rested conditions on either SBP or DBP (*p*s > .374), with no main effect for stress or stress × interpersonal contact interactions (*p*s > .424). However, for daytime SBP and DBP when sleep restricted, the ANOVA revealed main effects for interpersonal contact on both SBP, $F(1, 97) = 4.99$, $p = .028$, partial $\eta^2 = .049$, and DBP, $F(1, 97) = 5.19$, $p = .025$, partial $\eta^2 = .051$, indicating higher blood pressure reactivity under conditions of high compared to low interpersonal contact when sleep restricted. No main effects for stress, or stress × interpersonal contact interactions were observed (*p*s > .156). These ANOVA results suggest SBP and DBP reactivity to high compared to low interpersonal contact when sleep restricted but not when rested. For HR, no main effects for interpersonal contact or stress, or stress × interpersonal contact interactions were noted, when either sleep restricted or rested (*p*s > .109).

Examining time further, inspecting day versus evening SBP and DBP when sleep restricted, main effects for time, on both SBP, $F(1, 92) = 9.30$, $p = .003$, partial $\eta^2 = .092$, and DBP, $F(1, 92) = 19.21$, $p < .001$, partial $\eta^2 = .173$, with higher levels during the day compared to evening were observed. Main effects for interpersonal contact, on SBP, $F(1, 92) = 5.43$, $p = .022$, partial $\eta^2 = .056$, and DBP, $F(1, 92) = 6.53$, $p = .012$, partial $\eta^2 = .066$, were again indicated, with higher reactivity to high compared to low interpersonal contact, observed when sleep restricted. Trends toward time × interpersonal contact interactions were also

noted on SBP, $F(1, 92) = 3.47$, $p = .066$, partial $\eta^2 = .036$, and on DBP, $F(1, 92) = 3.95$, $p = .050$, partial $\eta^2 = .041$, indicating higher SBP and DBP under high interpersonal contact during the day compared to evening. No main or interaction effects for stress were observed on either SBP or DBP (all *p*s > .077). Examining SBP and DBP when rested, while there were main effects for time on SBP, $F(1, 97) = 8.11$, $p = .005$, partial $\eta^2 = .072$, and on DBP, $F(1, 97) = 13.74$, $p < .001$, partial $\eta^2 = .124$, with higher levels during the day compared to evening, no main effects for stress or interpersonal contact, or interactions with time were observed (all *p*s >.12). Overall, both SBP and DBP show greater reactivity to high compared to low interpersonal contact (with trends toward higher reactivity during the day compared to evening), when sleep restricted compared to when rested, as illustrated in Figure 7.

For HR, while there were main effects for time when both sleep restricted, $F(1, 92) = 19.87$, $p < .001$, partial $\eta^2 = .178$, and rested, $F(1, 97) = 28.55$, $p < .001$, partial $\eta^2 = .227$, with higher day levels compared to evening, no other main or interaction effects were observed (all *p*s > .116).

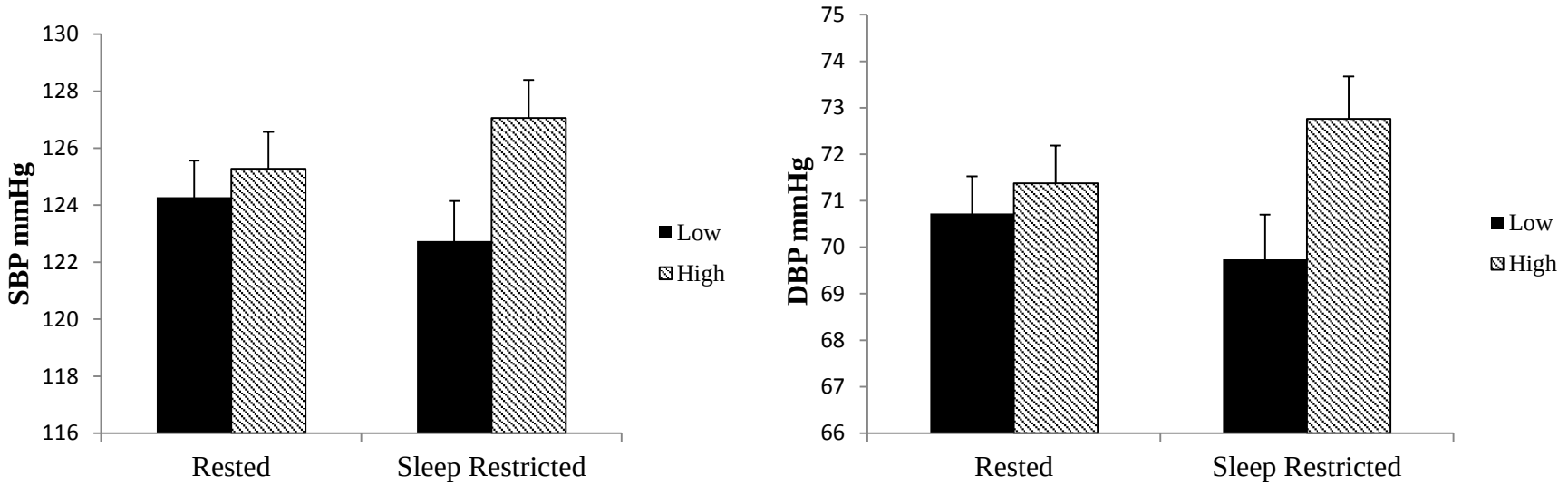


*Figure 8.* Significant main effects for interpersonal contact (low versus high) on ambulatory systolic blood pressure (SBP) and diastolic blood pressure (DBP) by sleep condition. Error bars denote standard errors of the mean

### 4.3.6. Nocturnal dipping

Paired samples *t*-tests were conducted on SBP and DBP percentage of nocturnal blood pressure dipping and daytime-nighttime change score, comparing rested and restricted conditions. The *t*-tests revealed no difference between rested and restricted conditions in percentage dip or daytime-nighttime change score on SBP (*p*s >.648) or on DBP, when adjusted for multiple comparisons (*p*s >.112).

Examining the effect of stress and interpersonal contact on nocturnal dipping, between-subjects ANOVA revealed no significant main or interaction effects with stress or interpersonal contact on percentage of either SBP or DBP dipping (all *p*s > .12), or SBP change score (all *p*s > .29), when either rested or sleep restricted.

The ANOVA examining DBP change score, when rested, revealed no significant main or interaction effects for either stress or interpersonal contact (all *p*s > .47). However, when sleep restricted, a significant main effect of interpersonal contact was observed on DBP change score, $F(1, 99) = 4.55$, $p = .035$, partial $\eta^2 = .045$, which indicated significantly greater DBP dipping amongst those who reported high ($M = 16.48$, $SD = 6.23$) compared to low ($M = 14.07$, $SD = 4.81$) interpersonal contact, when sleep restricted. No main effects for stress or stress × interpersonal contact interaction was observed (*p*s > .23).

## 4.4. DISCUSSION

To contribute to the limited experimental examinations of ABP following controlled reduction of sleep duration, the present study aimed to explore if one night of partial sleep restriction would affect ambulant measures of cardiovascular response over the succeeding 24-hrs. The results suggest that while mean blood pressure level was unchanged across daytime (and 24-hr) periods, evidence of reduced nighttime DBP when sleep restricted was revealed. Moreover, based on the prediction that cardiovascular function related to everyday

experiences may reveal insights not accessible using a laboratory setting (Kamarck, Debski, & Manuck, 2000), the present findings indicate SBP and DBP change associated with high (compared to low) naturally occurring social interactions when participants were sleep restricted compared to when rested. These findings further implicate sleep restriction in affecting altered cardiovascular activity, indicating involvement of both nocturnal blood pressure and response to ambulant interpersonal interactions.

Although previous studies have examined associations between sleep loss and cardiovascular response to laboratory stress (e.g., James & Gregg, 2004b; Kato et al., 2000), also examined in association with laboratory social stress in Chapter 2, a shortcoming of the present state of knowledge is that few studies have examined sleep related reactivity to "real" stimuli (Meerlo et al., 2008). This omission is considered an issue of key importance to understand whether stress-related responses (i.e., cardiovascular) function differently under conditions of restricted sleep in natural contexts. As interpersonal contact has been suggested as a useful task for investigating CVR in field settings (due to its association with increases in blood pressure and HR; Kario & Shimada, 2004), the current study examined the effects of CVR to naturally occurring interpersonal interactions, while rested and sleep restricted.

The present results affirm that occurrences of high (compared to low) levels of interpersonal contact throughout the day were associated with increased SBP and DBP blood pressure responses, when sleep restricted but not when rested. These findings support previous findings of increased cardiovascular arousal during periods of high daily social interaction (i.e., interpersonal interactions relative to non-communicative work activities; Brondolo, Karlin, Alexander, Bobrow, & Schwartz, 1999) and further suggest that such arousal is augmented under conditions of acute reduced sleep duration relative to when rested. Notable trends toward higher reactivity to high social contact during day compared to

evening periods further suggest that such effects may be more pronounced in daytime interactions (i.e., work/study) in comparison to later day (i.e., home) social activity.

In addition, the potential clinical significance of the observed effects is noteworthy. Exposure to psychological stress and related physiological responses measured during daily experience, such as ABP, predict CVD risk (Kamarck et al., 2005). Scrutiny of Figure 7 indicates that the effects of reactivity to high interpersonal contact for SBP and DBP involved discrepancies in the region of 2 to 3 mmHg between the rested and sleep restricted phase. While such discrepancies do not include direct clinical risk in individual cases, population statistics of the relationship between blood pressure level and rates of cardiovascular diseases (i.e., Kario, Schwartz, & Pickering, 2000) suggest that a swing of 2 to 4 mmHg in usual blood pressure would be expected to reduce premature death from CHD by 14 per cent and from stroke by 20 per cent (James, 2004). For example, it is thought that reductions in the region of 2 to 3 mmHg in the population distribution of blood pressure would result in effects equal to the cumulative benefits achieved by anti-hypertensive treatment (Imai, Ohkubo, Tsuji, Satoh, & Hisamichi, 1999; Kamarck et al., 2000). The interpersonal contact examined in the present study is considered analogous to the common social contact in settings in which "usual" blood pressure is assessed. Thus, the present findings suggest that sleep restriction could contribute to deviations of this magnitude in large sections of the population who experience regular partial sleep curtailment, and so may prove to be of important epidemiological/cardiovascular health related significance in population terms.

Because the influence of mental and physical activity is minimal during sleep, nocturnal blood pressure is thought to provide more accurate information (in comparison to daytime levels) concerning basal ambulatory cardiovascular activity (Imai et al., 1999). In this nocturnal period, evidence of reduced nighttime DBP when sleep restricted compared to when rested was revealed by the current findings. These data are consistent with previous

investigations utilising experimental acute sleep curtailment. Lusardi et al. (1996) reported reduced DBP (and SBP) nocturnal blood pressure before awakening in normotensive young adults, following approximately 40 per cent of normal sleep duration, which they attributed to a compensatory effect of an increase in the deeper forms of non-REM sleep, as a result of sleep restriction. A marked nocturnal fall in blood pressure has been attributed to increased cardiovascular health risks amongst current at risk individuals, for example increased risk for nonfatal stroke and myocardial ischemia in hypertensive individuals (Kario & Shimada, 2004; O'Brien, 2010). However, such associations are evidenced by substantial increases in nocturnal blood pressure dipping pattern (a change from day to night of >20 per cent) or "extreme dipping", as opposed to nocturnal blood pressure level alone (Kario et al., 2000).

Examining blood pressure dipping pattern, whereas dipping status was relatively unchanged for SBP in the current study, the trends observed for DBP dipping (both percentage dip and change score) indicate an upward shift in levels of dipping when sleep restricted compared to rested. Indeed, the mean percentage of DBP dipping while sleep restricted reached levels attributable to marginal "extreme" dipping, indicating nocturnal blood pressure fall of approximately 21 per cent in the current sample. Consistently, Kario et al. (2000) also found, in a sample of hypertensive participants, that sleep DBP (and SBP) was significantly lower amongst extreme dippers when compared to dippers. Thus, dipping status appeared to be determined more by the nighttime than by the daytime blood pressure. Further, regarding the interaction of dipping with measures of interpersonal contact, increased interpersonal interactions were associated with significantly higher DBP change score (but not dipping percentage), again observed when sleep restricted compared to when rested. Taken together, the significant reduction in mean nighttime DBP (consistent with Lusardi et al., 1996), and trends toward increases in DBP dipping (both independently and in combination with high levels of contact) while sleep restricted, may be indicative of a pattern

of sleep loss related lowering of nocturnal DBP, to levels which have previous been reported to be associated with negative cardiovascular outcome amongst at risk individuals. Consistent with the suggestion that the nocturnal period may be important for the representation of basal cardiovascular function (due to low physical and/or psychological concomitants) such effects may pinpoint reduced sleep duration in altered nighttime basal ambulatory DBP control. However, as the relative change between rested and sleep restricted phases (both DBP dipping measures) was moderately low, inferences related to such proposed effects are speculative.

Tochikubo et al. (1996) reported increases in overall ABP following a reported night of reduced sleep duration in the range of 3 to 6 mm Hg, for DBP and SBP, respectively. However, such findings were observed in a restricted number ($N$ = 18) of male participants. Further, there was no verifiable observation or objective evidence of the reduced sleep duration recorded. In the present analysis, average SBP, DBP, and HR were unchanged by sleep restriction across daytime (and 24-hr) periods, mirroring the findings of unchanged overall measures of laboratory cardiovascular response following actigraph verified sleep loss outlined in Chapter 2. While mean levels of reported stress was significantly higher when sleep restricted compared to rested, ABP was unaffected by ratings of self-reported stress, when either rested or sleep restricted. This finding contrasts with some previous findings which observed increased cardiovascular response to instances of high stress and interpersonal interactions (Lehman & Conley, 2010). However, underlying such observations, specific characteristics of the reported stress (i.e., anxiety over others' specific evaluative reactions during social interactions) appeared to have a bearing on response strength. It is therefore conceivable that such variables may have influenced the specific interaction of ambulant stress and cardiovascular response similarly in the current sample.

While high (compared to low) interpersonal contact was associated with increased SBP and DBP response when sleep restricted compared to when rested, there is considerable methodological literature examining and demonstrating negative consequences of dichotomisation procedures to transform continuous variables into dichotomous variables, possibly leading to an increase in Type II errors and loss of potentially valuable information (MacCallum et al., 2002; Royston, Altman, & Sauerbrei, 2006) and favouring the use of regression methods on undichotomised variables. Nevertheless, binary classification is common in research examining cardiovascular clinical outcomes (e.g., hypertensive status, Altman & Royston, 2006), and facilitates dichotomisation of independent variables prior to conducting ANOVA analyses.

Finally, structural qualities (i.e., interpersonal relationships) regarding the interactions examined in the present study are unknown. Previous findings (Holt-Lunstad, Uchino, Smith, Olson-Cerny, & Nealey-Moore, 2003) found interactions with ambivalent others (i.e., strangers) to be associated with higher ABP compared to interactions with family members and spouses, which were associated with lower responses. As such, further ambulatory physiological examinations that include examination of aspects of social relationships across daily interpersonal interactions, when both rested and sleep restricted, could be revealing.

In summary, the present study highlights the potential role that sleep restriction may have in negatively affecting both nocturnal, and social interaction reactive, ambulatory cardiovascular indices, in ways that have been implicated in the etiology of heightening risk for cardiovascular related disease. In doing so, the present study highlights a possible mechanism, associated with increased reactivity to daily interaction, through which reduced sleep duration may, over time, become negatively associated with cardiac health. Such effects further supplement previous cardiovascular findings of increased laboratory vascular hemodynamic responses to social stress under conditions of sleep restriction observed in

Chapter 2. As well as elucidating the cardiovascular plausibility of sleep restriction as a disease-linked behaviour, the findings highlight the importance of related laboratory and ambulatory cardiovascular assessments, allowing increased ecological validity in the examination of sleep loss and associated effects on cardiovascular control.

# Chapter 5. Study 4:

# THE CORTISOL AWAKENING RESPONSE AND ACUTE SLEEP RESTRICTION

## 5.1. INTRODUCTION

*What is the cortisol awakening response?*

As outlined in Chapter 1, when a situation is interpreted as being stressful, it triggers the activation of the HPA axis, whereby neurons in the hypothalamus release a hormone called CRH. The release of CRH triggers release of associated hormones (ACTH and AVP) from the pituitary gland. When ACTH is released, it travels in the blood to the adrenal glands in the kidneys, triggering the secretion of a primary stress hormone; the glucocorticoid, cortisol.

The secretion of cortisol from the adrenal glands follows a diurnal cycle (with a circadian peak in the morning, which gradually declines from late afternoon to a trough in the early nocturnal period), in addition to a profound increase after awakening. This increase, a phenomenon termed the CAR, appears to be a distinct feature of the HPA axis, superimposed upon the typical circadian rhythmicity of cortisol secretion. Since the introduction of the CAR as an index of adrenocortical activity (Pruessner et al., 1997; Schmidt-Reinwald et al., 1999), it has become a valuable biological marker for the assessment of HPA axis regulation in ambulatory settings (Fries, Dettenborn, & Kirschbaum, 2009). Although its precise mechanism of action is not yet fully understood, the CAR is thought to reflect anticipation of the metabolic and postural challenges of starting a new day (Kunz-Ebrecht, Kirschbaum, Marmot, & Steptoe, 2004; McEwen, 2006), thereby providing a physiological ‘‘boost’’ for anticipated demands based on individual prior experience (Adam, 2006).

The awakening response in cortisol has attracted attention from researchers for a number of reasons. The CAR does not require laboratory conditions or administration of

exogenous agents/stressors; rather awakening itself is a consistent, recurring, and strong stimulus for HPA activity (Wilhelm et al., 2007). It has additionally been noted that the CAR is under somewhat independent control from cortisol output during the remainder of the day, with associations between the CAR and cortisol sampled later in the day being low (Edwards, Clow, et al., 2001; Schmidt-Reinwald et al., 1999). Finally, the magnitude of the CAR appears to be associated with psychosocial factors and health in potentially significant ways, suggesting that it may be a distinctive indicator of HPA function and dysfunction (Chida & Steptoe, 2009).

*Why look at the CAR and sleep restriction?*

Results regarding the effects of sleep-related factors including waking time and sleep duration on the CAR have been inconsistent. Waking time has been studied frequently. While some studies reported that the CAR was unrelated to waking time (Pruessner et al., 1997) others have reported a larger CAR in individuals waking up earlier in the morning (Edwards, Evans, Hucklebridge, & Clow, 2001; Kudielka & Kirschbaum, 2003). As noted by Gribbin et al. (2011), interpretation of the existing data on the effects of sleep-related factors and the CAR is complicated by the fact that many of the previous findings have been based upon correlational and/or quasi-experimental studies with subjective measurement of sleep (bedtime, wake time, and sleep duration) or incomplete control over potential confounds (Backhaus, Junghanns, & Hohagen, 2004; Federenko et al., 2004; Pruessner et al., 1997; Waye, Clow, Edwards, Hucklebridge, & Rylander, 2003; Wust et al., 2000). As a result, uncertainty exists about the relationship of the CAR to sleep behaviours.

The possible influence of sleep duration on the cortisol rise after awakening was investigated by Pruessner et al. (1997) and Federenko et al. (2004) who reported no relation between self-reported hours of sleep during the previous night and the CAR. Others have

found a larger CAR in individuals with a shorter sleep length (Schlotz, Hellhammer, Schulz, & Stone, 2004). Wüst et al. (2000) also describe a slightly larger CAR in participants with short sleep durations, though, as this accounted for < 1 per cent of the variance in cortisol level after awakening, it was discounted. However, as the sleep measures in these pervious investigations have relied upon self-report (and did not assess sleep latency and waking periods during the night) they do not reflect actual sleep time, but rather time in bed. This methodological difference may (in part) account for the inconsistent effects of sleep duration on the cortisol response to awakening.

Two recent findings stand out in this regard. Using laboratory polysomnography verified sleep duration, Wu et al. (2008) revealed that short-term sleep restriction in healthy men (3 hrs of sleep/night for four consecutive days) resulted in a significant decrease in morning (one sample, 7:00 a.m.) cortisol level compared to baseline, as measured in serum samples. Further, using actigraph-measured sleep duration in participants own homes, Gribbin et al. (2011) found that one night of shortened sleep duration in 2- to 4-year-old children had the effect of dampening overall cortisol secretion during the first 45 mins post-awakening. However, these findings may be considered preliminary due to small sample sizes ($N$ = 10 and seven, respectively) and incomplete (see Clow et al., 2010) CAR profiling (i.e., single morning sample in serum; Wu et al., 2008). Further, whether effects observed by Gribbin et al. (2011) would be observable in an older sample (i.e., young adults) is unknown, and the novelty of this finding warrants further investigation.

Inconsistencies in findings concerning awakening cortisol level and sleep-related parameters (e.g., sleep duration), and the limited extent of that research, indicate the need for further investigation. The present study sought to examine the association between acute sleep restriction and the awakening response of cortisol in a sample of young adults. Following periods of rested, restricted, and recovery sleep, four salivary samples were

provided by participants in their own homes in order to assess CAR over the first 45 mins post-awakening.

## 5.2. METHODS

### 5.2.1. Design

Participants were requested to provide four waking saliva samples, in their own homes, for the assessment of the CAR. These samples were provided, on awakening, following three sleep phases; (a) normal sleep duration (rested), (b) partial sleep loss (sleep restricted), and (c) recovery sleep on the first night post-sleep restriction (recovery). Cortisol assessments were completed within-subjects, with participants providing saliva samples on all three time-points. Sleep restriction procedures were completed as outlined in Chapter 2.

### 5.2.2. Participants

As previously, participants were drawn from the same population of participants outlined in Chapters 2 – 4. Inclusion criteria for Study 4 were no endocrine or immune disorders, not taking glucocorticoid medication, and no periodontal disease, following previous examinations of CAR in young adults (Heaney, Phillips, & Carroll, 2012). The final sample consisted of 120 (88 female) university students (age 17 to 24 years; $M$ = 18.49 years, $SD$ = 1.02). All participants were physically healthy, with normal BMI ($M$ = 22.78, $SD$ = 2.55). Recruitment of participants to the study, participant incentives, and associated ethical approval of procedures, were as described in Chapter 2.

### 5.2.3. Materials and apparatus

*Activity diary.* To assess compliance, participants were requested to complete CAR relevant sections in the provided ambulatory activity diary (see Chapter 4). Participants

reported individual awakening times, and the time at which they provided the samples. To enhance adherence to the procedure, on awakening following sleep restriction, a text message was sent to the participants' mobile phone, as a reminder to begin the CAR sampling. From the final total salivary sample (924 saliva samples), according to the diary self-reports, 80.5 per cent were taken on time in the rested phase, 79.2 per cent in the sleep restricted phase, and 81.8 per cent in the recovery phase. These compliance rates are consistent with previous research, for example, Heaney et al. (2012), who reported a compliance rate of 79 per cent.

*Salivary biochemical analyses*. Participants stored completed saliva samples in their home refrigerator until returning to the laboratory. As per examination of α-amylase (outlined in Chapter 3), saliva samples were stored in the laboratory at −20 °C before assay. For quantitative determination of salivary cortisol, the Salimetrics High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit (Salimetrics Ltd., U.K.) was used in the testing of all unknowns (individual participant samples), with inter- and intra- assay coefficients of variation between duplicate repeats of < 16 and 10 per cent, respectively. The lower limit of sensitivity for the assay was < 0.003 μg/dL. Cortisol in standards and unknowns competes with cortisol linked to horseradish peroxidase for antibody binding sites. After incubation, unbound components are washed away. Bound cortisol peroxidase is measured by the reaction of the peroxidase enzyme on the substrate tetramethylbenzidine. This reaction produces a blue colour. A yellow colour is formed after stopping the reaction with sulfuric acid. Optical density is read on a standard plate reader at 450 nm. The amount of cortisol peroxidase detected, as measured by the intensity of colour, is inversely proportional to the amount of cortisol present. All samples were assayed by a researcher assistant on-site in a bioassay laboratory.

5.2.4. Procedure

Twelve polypropylene collection vials (Sarstedt, Nümbrecht, Germany; four vials for each testing day), were provided to participants for the provision of CAR samples, in their own homes, on awakening from sleep. Participants completed three separate sets of samples; rested, sleep restricted, and recovery (see above). Participants were instructed to collect four saliva samples during the first 45 mins after awakening on each of the three test days. The first sample was provided immediately after awakening, followed by samples 15, 30, and 45 mins thereafter (0 min, +15 min, +30 min, +45 min). It was stressed to participants that the first sample was to be provided immediately after awakening, in order to avoid a potentially confounding effect of a delay (> 15 mins) between the time of awakening and start of the CAR assessment (Kudielka, Broderick, & Kirschbaum, 2003). To avoid contamination of saliva with blood, participants were instructed to not brush their teeth before completing the saliva samples. Additionally, eating and drinking beverages containing caffeine or fruit juices were not allowed until after the final CAR saliva sample was provided. Samples were stored in participants' home refrigerators before being transported back to the laboratory.

## 5.3. RESULTS

5.3.1. Overview of statistical analyses

Repeated measures ANOVA were conducted to reveal possible time (0 min, +15 min, +30 min, +45 min) and sleep (rested, sleep restricted, recovery) effects on the CAR. Area under the total response curve with respect to the ground ($AUC_G$), for each sleep phase, was calculated using the trapezoid formula following Pruessner, Kirschbaum, Meinlschmid, and Hellhammer (2003). $AUC_G$ is the total area under the curve (cumulative cortisol secretion) of all measurements, and takes into account both sensitivity (the difference between the single measurements from each other) and intensity (the distance of these measures from ground;

Fekedulegn et al., 2007). Mean increase in cortisol (MnInc) over the CAR period was also calculated, for each sleep phase (MnInc; Awakening cortisol at (15 min + 30 min + 45 min )/3 – awakening cortisol at 0 min), following Wust et al. (2000). As all four samples were required for CAR estimation, missing data (missing sample or insufficient saliva) was found in $n$ = 43 participants, resulting in a total sample of $N$ = 77.

The effects of a number of demographic and health-related factors with putative influence on the CAR response have been inconsistent (for reviews see Almeida, Piazza, & Stawski, 2009; Fries et al., 2009). Nevertheless, the potential explanatory effect of such factors is thought to be important in CAR analyses (Walker, O'Connor, Schaefer, Talbot, & Hendrickx, 2011). Among the possible confounds to the CAR, smoking, gender, and use of oral contraceptives have previously been reported to account for approximately 1 – 4 per cent of the total variance observed (Pruessner et al., 1997; Wust et al., 2000). An initial set of mixed factorial ANOVAs were run to examine the effect of these potential confounds on (a) overall CAR (b) $AUG_G$, and (c) MnInc, across sleep phases. Both, gender (19 males) and oral contraceptive use (11.7 per cent of total sample) did not result in any significant main or interaction effects on cortisol outcome variables and were therefore ignored in the final analyses. However, main effects for smoking were indicated on the overall CAR ($p$ = .007), $AUC_G$ ($p$ = .018) and MnInc ($p$ = .016). ANOVA analyses were re-run on a sub-sample of participants, excluding smokers. Significant findings reported in the main analyses were maintained across all outcome variables (all $p$s < .05); therefore smokers (14.3 per cent of total sample) were included in the final analyses.

Investigating the influence of awakening time on CAR response, with previous research suggesting that more pronounced CAR responses are observable in early awakeners compared to later awakeners (Edwards, Evans, et al., 2001), a sub-sample of participants were classified as either “early” or “later” awakeners (based on median-split), if they

demonstrated a consistent waking pattern across both the rested and recovery mornings (*n* = 43; waking time range: 7am to 10:30am and 7am to 11:45am, respectively). ANOVA analyses revealed that awakening time did not result in any main or interaction effects across the cortisol outcome variables (overall CAR, MnInc and $AUC_G$; all *p*s >.18).

As CAR provision took place in the home setting (as opposed to monitored provision of sAA samples in Chapter 3) the potential effects of participant non-compliance with the CAR sampling procedure was also examined and controlled for as follows: In line with previous research, non-compliance was defined as a temporal delay of > 15 mins between the time of awakening and the provision of the first saliva sample (DeSantis, Adam, Mendelsohn, & Doane, 2010; Okun et al., 2010; Wolfram, Bellingrath, & Kudielka, 2011). To examine the effect of non-compliance, cortisol increases between compliant and non-compliant participants per sleep phase (rested, sleep restricted, recovery), were compared, and examined using independent samples *t*-tests. Additionally, the potential impact of the time delay on the cortisol increase in non-compliant participants was further examined using ANCOVA (delay expressed in mins and introduced as a continuous covariate in the ANOVA outlined below).

To examine the effect of sleep phase on the CAR, a repeated measures ANOVA, comprising the within-subject factor sleep (rested, sleep restricted, recovery) and time (0 min, +15 min, +30 min, +45 min) was conducted. Additional, one-way repeated measures ANOVA, using the within-subjects factor sleep, were conducted to examine effects on the dependent variables MnInc and $AUC_G$.

Data were tested for normal distribution and homogeneity of variance using a Kolmogorov - Smirnov and Levene's test before statistical procedures were applied. The concentration of cortisol in saliva was expressed as milligrams/decilitre (ug/dl). Cortisol values were log-transformed due to positively skewed scores before statistical analysis

(figures and tables present untransformed values for illustrative purposes) and Bonferroni correction applied to multiple *t*-test comparisons. All reported results were corrected by the Greenhouse-Geisser procedure where appropriate (violation of sphericity assumption). Slight variations in degrees of freedom across groups reflect occasional missing data or insufficient saliva for analysis, as per pervious research (Heaney et al., 2011). As previously, for all analyses, ANOVA effect sizes are presented as partial $\eta^2$.

### 5.3.2. Adherence to sleep manipulation

Compliance with the sleep manipulation protocol, as assessed using wrist actigraphy, indicated high levels of participant adherence. In the current sample, activity levels, monitored on the evening prior to the restricted night to ensure participants maintained wakefulness until their usual bedtime, indicated the average percentage of time participants were awake during the evening prior to the sleep restriction period to be 92.92 per cent (*SD* = 11.34). Equally high (*M* = 378.77, *SD* = 131.38) evening activity scores were recorded (a total score of ≥ 80 designating an epoch as being "awake"). During the restriction vigil (following normal bedtime and awakening on receipt of 40 per cent usual sleep duration), the mean percentage of time participants were awake was 85.67 per cent (*SD* = 15.17). The mean activity score per 2 min epoch was 247.48 (*SD* = 115.21). Both percentage time awake and activity score indicated substantial compliance to the sleep restriction protocol

### 5.3.3. Non-compliance analyses

Non-compliance with the saliva sampling schedule, as defined above, was indicated in 3.8 per cent of the collected samples; for three participants during the sleep restriction phase, four participants during the rested phase, and two participants during the recovery phase. Independent samples *t*-tests revealed that cortisol increase (MnInc scores) did not

significantly differ between compliant and non-compliant participants (all *p*s > 0.17). Additionally, mins of delay as a continuous variable had no significant impact on the course of the CAR, in any of the sleep phases (interaction effects for time × mins of delay; all *p*s > 0.25).

### 5.3.4. Awakening salivary cortisol responses

Mean levels (with *SD*s) of all CAR parameters following rested, sleep restricted, and recovery phases are shown in Table 6. Corresponding with previous findings demonstrating the typical course of the CAR, within-subjects ANOVA indicated a main effect for time, demonstrating a significant increase of salivary cortisol levels after awakening, $F(2.49, 188.87) = 35.04$, $p < .001$, partial $\eta^2 = .316$ (see Table 7).

A main effect for sleep was also observed, $F(1.56, 119.71) = 135.09$, $p < .001$, partial $\eta^2 = .640$. Pairwise comparisons revealed that the CAR following sleep restriction was significantly lower than following the rested ($p < .001$) and recovery ($p < .001$) phases, while the CAR between rested and recovery phases did not significantly differ ($p = .066$), as illustrated in Figure 8. The interaction of sleep × time was not significant ($p = .339$).

Table 6

*Means (with* SDs*) for salivary cortisol measures across experimental phases*

| | Rested | | Sleep Restricted | | Recovery | |
|---|---|---|---|---|---|---|
| | Mean | *SD* | Mean | *SD* | Mean | *SD* |
| $CAR_0$ | 0.29 | 0.19 | 0.09 | 0.07 | 0.33 | 0.27 |
| $CAR_{+15}$ | 0.40 | 0.37 | 0.12 | 0.10 | 0.43 | 0.25 |
| $CAR_{+30}$ | 0.41 | 0.29 | 0.16 | 0.16 | 0.46 | 0.30 |
| $CAR_{+45}$ | 0.45 | 0.34 | 0.17 | 0.17 | 0.45 | 0.25 |
| MnInc | 0.98 | 0.80 | 0.37 | 0.37 | 1.00 | 0.59 |
| $AUC_G$ | 17.76 | 11.85 | 6.26 | 5.15 | 19.19 | 9.26 |

Notes: Cortisol outcomes presented in milligrams/decilitre (ug/dl). $CAR_{0\text{-}45}$ represent cortisol over the first 45 mins post-awakening. MnInc = mean increase in awakening cortisol ([15 min + 30 min + 45 min]/3 – awakening cortisol at 0 min). $AUC_G$ = area under the curve (AUC) with respect to the ground

Table 7

*Summary of repeated-measures ANOVA (*F *values) for Sleep and Time*

| Source | *df* | $CAR_{0\text{-}45}$ | *df* | MnInc | *df* | $AUC_G$ |
|---|---|---|---|---|---|---|
| Sleep | 1.56, 119.71 | 135.09** | 1.85, 140.91 | 56.88** | 1.51, 114.30 | 129.72** |
| Time | 2.49, 188.87 | 35.04** | | | | |
| Sleep × Time | 4.51, 342.48 | 1.14 | | | | |

Sleep: rested, sleep restricted, recovery. Time: 0 min, +15 min, +30 min, +45 min.

** $p < .001$

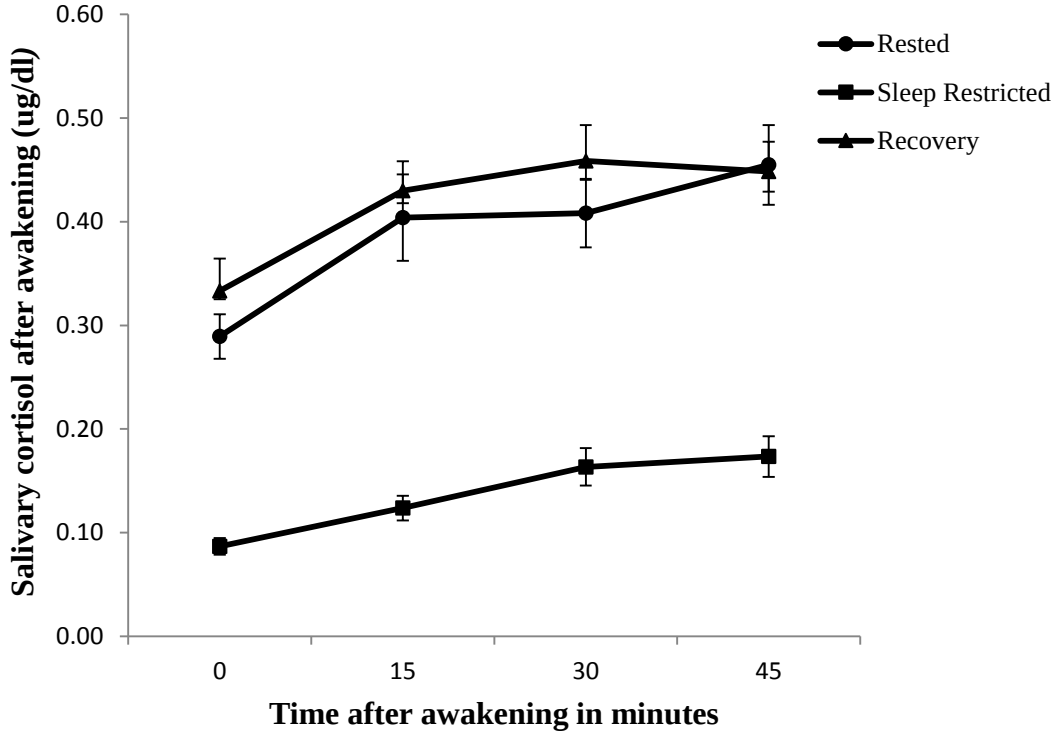


*Figure 9.* Morning cortisol awakening response profiles (error bars denote standard error of the mean) across three sleep phases. Error bars denote standard errors of the mean

5.3.5. Mean increase in cortisol and area under the curve

One-way within-subjects ANOVA conducted on MnInc scores, showed a main effect for sleep, indicating a significant difference in cortisol increases across the sleep phases, $F(1.85, 140.91) = 56.88$, $p < .001$, partial $\eta^2 = .428$. Paired samples *t*-tests revealed that cortisol increase was significantly lower following sleep restriction compared to rested, $t(76) = 8.37$, $p < .001$, and recovery, $t(76) = 8.88$, $p < .001$. There was no difference in cortisol increase between rested and recovery phases, $t(76) = 1.60$, $p = .228$.

Examining the cumulative cortisol secretion over the four CAR time-points, defined as $AUC_G$, the ANOVA revealed a main effect for sleep, indicating significant differences across sleep phases, $F(1.51, 114.30) = 129.72$, $p < .001$, partial $\eta^2 = .631$. Paired samples *t*-

tests revealed a significantly smaller area under the curve following sleep restriction compared to both rested, $t(76) = 10.41$, $p < .001$, and recovery phases, $t(76) = 15.93$, $p < .001$. There was no difference (when corrected for multiple comparisons) in $AUC_G$ between rested and recovery phases, ($p = .069$), as illustrated in Figure 9.

## 5.4. DISCUSSION

The present study aimed to extend previous investigations of sleep duration and cortisol, by investigating the CAR of young adults in the first 45 mins post-awakening, across three distinct sleep phases; rested, sleep restricted, and recovery. Inconsistencies in findings concerning CAR response and sleep-related parameters (e.g., sleep duration), and the incomplete extent (i.e., small sample sizes and incomplete CAR assessment) of that research indicated the need for further investigation. The present data affirm pervious findings of a dampened CAR post-sleep restriction and extends this observed patterning to young adults. Moreover, this lower response was also evident when examining the dynamic change in cortisol over the 45 min sampling phases, with smaller increases in the CAR and smaller total cortisol secretion observed post-sleep restriction when compared to both rested and recovery phases. These results held after controlling for potential confounds to the CAR such as sampling non-compliance.

These findings further implicate reduced sleep duration in the etiology of variation in the CAR. The present data corroborate the recent findings by Wu et al. (2008) and Gribbin et al (2011), both observing a reduced CAR following short-term sleep restriction. Moreover, present results confirm these earlier findings in a larger sample, including methodological refinement over previous investigations, using a more comprehensive (four sample) waking CAR profile, in addition to actigraph verification of sleep duration.

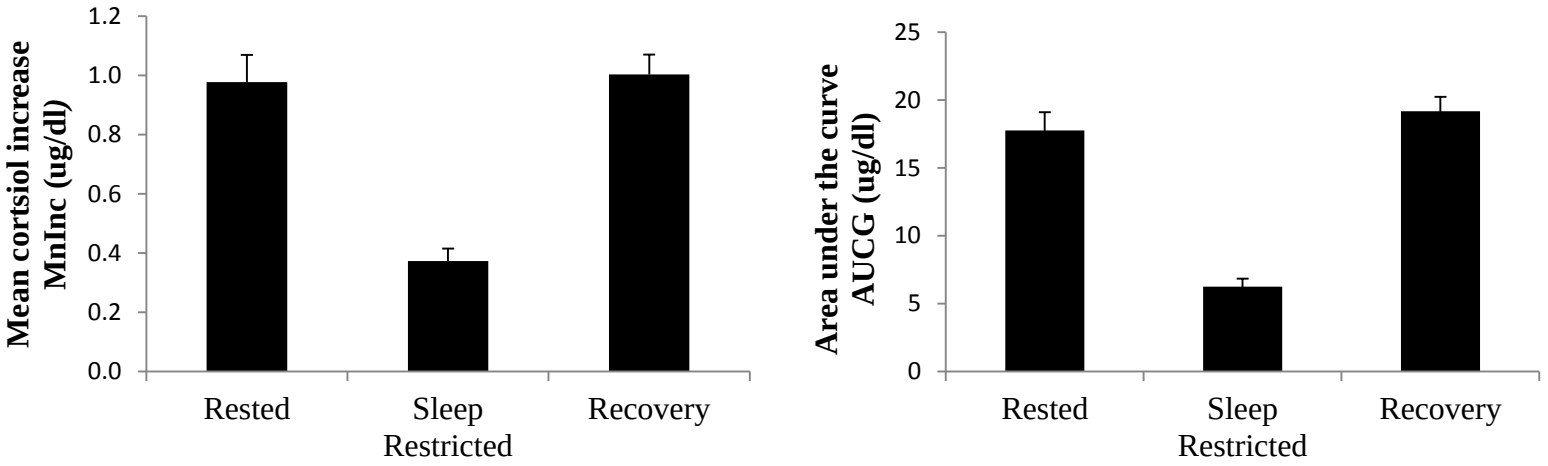


*Figure 10.* Mean increase (MnInc) and area under the curve ($AUC_G$) in cortisol awakening response (CAR), per sleep phase. Error bars denote standard errors of the mean

While these effects on the CAR are attributable to short-reductions in sleep duration, further data suggest similar trends with total sleep deprivation (26 hrs; Figueiro & Rea, 2011). Consistently, Späth-Schwalbe et al. (1992) also reported higher releases of cortisol after long sleep durations as compared to short sleep, measured using polysomnography. Others have also reported associations between poor sleep and subsequent reduced cortisol reactivity to acute stress using both subjective (Capaldi, Handwerger, Richardson, & Stroud, 2005) and objective (Wright, Valdimarsdottir, Erblich, & Bovbjerg, 2007) measures of sleep quality.

Previous findings of associations between psychosocial factors and the CAR indicate that fatigue, burnout, and exhaustion are characterised by a reduced CAR (Chida & Steptoe, 2009). In addition, in many disorders including cardiovascular, autoimmune, and psychiatric disease (among others), a blunted CAR has been observed when comparing patients with healthy controls (Kudielka & Kirschbaum, 2003). For example, a reduced CAR has been described in patients with chronic pain (Geiss, Varadi, Steinbach, Bauer, & Anton, 1997), patients with systemic hypertension (Wirtz et al., 2007) and women with high intima-media thickness, an indicator for early, non-symptomatic atherosclerosis (Hurwitz Eller, Netterstrom, & Marie Hansen, 2001). Additionally, for several psychiatric conditions a reduced CAR has been described, such as in posttraumatic stress disorder (Wessa, Rohleder, Kirschbaum, & Flor, 2006), chronic fatigue syndrome (Roberts, Wessely, Chalder, Papadopoulos, & Cleare, 2004), or sleep disorder (i.e., cortisol after awakening was significantly decreased in primary insomnia; Backhaus et al., 2004).

In an attempt to delineate common underlying pathways linking such negaive health states to a reduced CAR, one possible explanation is that in such disorders (i.e., burnout and fatigue), sleep patterns are seriously disrupted (Ekstedt et al., 2006). The CAR is thought to be due to two to four secretory bursts over the period after waking (Chida & Steptoe, 2009).

If sleep patterns are disturbed, perhaps affected individuals become aroused to levels nearer to consciousness (i.e., lighter sleep) before awakening. The secretory bursts may then be distributed over a longer time period, so the CAR itself is smaller (Chida & Steptoe, 2009).

Notably, the idea that sleep processes may be (one of) the primary determinants of CAR disruption underlying disorders associated with CAR inhibition, may be consistent with the increasing numbers of studies producing conflicting results for depression and the CAR (Chida & Steptoe, 2009). Depression has been previously reported to be related to both to increased (Bhagwagar, Hafizi, & Cowen, 2005; Pruessner, Hellhammer, Pruessner, & Lupien, 2003) and reduced (Ellenbogen, Hodgins, Walker, Couture, & Adam, 2006; Stetler & Miller, 2005) CAR profiles. As sleep disturbance is one of the main characteristics of patients with affective disorders (Ivanenko, McLaughlin Crabtree, & Gozal, 2005), this may account for some of the failures to obtain consistent results in previous investigations. For example, Huber et al. (2006) reported a blunted CAR in depressed as compared to non-depressed patients. However, the quality or duration of sleep in the depressed group may have differed from that in the non-depressed group, as they state "the depressed patients might have woken up spontaneously earlier and have slept only superficially thereafter, thus explaining the observed (reduced) changes in the CAR" (p. 903). Together with the present (and previous recent experimental) findings of reduced awakening cortisol responses associated with sleep restriction, related findings observed in groups where sleep patterns are known to be disrupted may mutually contribute toward an understanding of a potential underlying pathway implicating sleep disruption in the etiology of a reduced CAR profile.

The current study focused on the relationship of the CAR to sleep duration, finding dampened CAR after sleep restriction relative to rested and recovery conditions. The degree to which these effects are physiologically related to health states remains to be determined. However, it has been hypothesised that blunted HPA-axis response (i.e., to awakening)

increases the vulnerability for the development of certain disorders (de Rooij, in press). Support for this hypothesis comes from a study in a strain of rats selected for their blunted HPA-axis response to stress (Cohen et al., 2006). Compared to wild type rats (as well as to rats with a hyper-responsive HPA system), the rats exhibiting lowered HPA-axis responses showed enhanced and more extreme anxiety-like behaviours in response to stressors.

The concept that blunted stress reactivity may have negative health consequences in itself also appears to match part of the allostatic load model, which was put forward by McEwen and Seeman (1999). Allostatic load represents the 'wear and tear' the body experiences when repeated allostatic processes, aimed at maintaining physiological stability, are activated in response to stress. There are several conditions that lead to allostatic load and one of them is an inadequate response by some allostatic systems triggering compensatory increases in others. McEwen and Seeman highlight the example of cortisol secretion failing to increase in response to stress, resulting in the secretion of inflammatory cytokines (which are normally counter regulated by cortisol). This, in turn, may affect health as an enhanced inflammatory response, associated with a range of negative health consequences.

While awakening time in the current study did not reveal any significant effects on CAR response, this may have been due to a limited average waking range (participants waking within 4 hrs, 6 mins of one another) across rested and recovery mornings, when compared to previous investigations of waking time (e.g., participants waking within 5 hrs of one another; Federenko et al., 2004). Nevertheless, if we compare early and late awakeners using the sleep restricted (waking time 0215 – 0515 hrs) compared to the rested (0700 – 1030 hrs) mornings, rather than observe a higher CAR in the early awakeners as found in previous investigations, we observed the opposite. This suggests that the effects that were observed, rather than being a confound of early awakening, are due to a separate process (i.e., reduced sleep duration). The CAR has previously been reported to be more pronounced following

morning light exposure in healthy participants (Thorn, Hucklebridge, Esgate, Evans, & Clow, 2004). As a caveat, light exposure was not controlled in the present study, resulting in the possiblity that participants received differing levels of initial light exposure across sleep phases.

Overall, these data, expanding on previous preliminary data, indicate that acute sleep loss is related to morning stimulation of the HPA-axis and, in particular, dampening of morning cortisol levels. Further, relative to the more immediate stress response denoted by SAM system activation, the observed CAR profile suggests alteration to cortisol response persisting up to 45 mins post-stimulus (awakening). While such lowering of the CAR may be indicative of sleep loss related negative health states (e.g., Chida & Steptoe, 2009), as suggested by McEwen and Seeman (1999), if this reduction of the CAR pertains to a specifically maladaptive reciprocal pathway, there may be an observable compensatory upregulation of inflammatory processes. The next chapter presents an exploratory investigation into whether sleep restriction influences a systemic marker of inflammation, when compared to rest, as measured in saliva.

# Chapter 6. Study 5:

## --BRIEF REPORT--

## ASSESSING THE EFFECTS OF ACUTE SLEEP RESTRICTION ON A NOVEL MARKER OF SYSTEMIC INFLAMMATION: SALIVARY C-REACTIVE PROTEIN

### 6.1. INTRODUCTION

Chapters 2 and 4 examined the influence of sleep restriction, social stress, and periods of naturally occuring stress and interpersonal contact, on measures of laboratory and ambulatory CVR. Chapter 3 additionally outlined findings indicating that partial sleep loss induced significantly increased resting basal levels of sAA, proposed to reflect SAM related activation. Further, Chapter 5 demonstrated lower awakening cortisol responses following sleep loss. However, failure to obtain adequate amounts of sleep has also been suggested to promote low-level systemic inflammation, itself associated with cardiovascular risk (Faraut et al., 2012).

Both epidemiological and clinical studies have shown consistent relationships between markers of inflammation and risk of future cardiovascular events (Freeman et al., 2002; Koenig et al., 2004; Pai et al., 2004). Moreover, the evidence that low-grade local and systemic inflammation occurs in all stages of atherogenesis (de Boer, van der Wal, & Becker, 2000; Hansson, 2005) and is associated with endothelial shear stresses (Chae et al., 2001), has led to the discovery of a number of novel independent predictors of cardiovascular risk. Among these emerging biomarkers, leukocyte count, IL-6, Myeloperoxidase, and levels of CRP are all increased in healthy humans after experimental sleep deprivation (Dinges et al., 1994; Faraut et al., 2011; Irwin, Wang, Campomayor, Collado-Hidalgo, & Cole, 2006; Meier-Ewert et al., 2004). Although these effects are often small, such chronic sub-clinical

shifts have been described as contributing to cardiovascular pathogenesis (Faraut et al., 2012).

CRP, a hepatocyte protein, is induced principally during the "acute phase response" to infection and inflammation, with synthesis of CRP in the liver being largely controlled by IL-6 (and also by tumor necrosis factor-alpha and IL-1; Castell et al., 2005), and CRP production thought to reflect the activity of these cytokines, particularly IL-6 (Herity, 2000). The discovery of large amounts of CRP in the serum of patients during the acute phase of pneumococcal pneumonia in 1930 focused interest on the plasma protein changes that accompany inflammatory states. CRP and other plasma proteins whose concentrations rose significantly under such circumstances were accordingly referred to as acute-phase proteins (APPs; Epstein, Gabay, & Kushner, 1999). Although CRP concentrations may increase many-fold in the acute-phase response, there is evidence that CRP is present at low concentrations in asymptomatic individuals and may reflect baseline activity of circulating cytokines (Epstein & Ross, 1999; Herity, 2000). These levels were found to be associated with future risk for the development of CVD, even in healthy, asymptomatic men (Ridker et al., 1997) and women (Ridker, 2001; Ridker et al., 2000). As a result, in the U.S., physicians are now recommended to measure hs-CRP in asymptomatic individuals with an intermediate risk of CHD to optimise their assessment of cardiovascular risk (Pearson et al., 2003). Further, despite current European guidelines on cardiovascular disease prevention in clinical practice recommending the use of hs-CRP in the risk assessment of individuals with a moderate CVD risk profile (Germano et al., 2012), recent reports from the Irish College of General Practitioners have found that screening of hs-CRP levels is not currently available at many hospital-based laboratories in Ireland (Kenny & Ríain, 2009).

CRP has also been shown to be stress reactive. Findings from Steptoe, Hamer and Chida (2007), in a meta-analysis of 30 studies, including over 1700 participants, examining

the effects of acute psychological stress on circulating inflammatory factors in humans, demonstrated modest to robust effects for increases in inflammatory markers (including CRP and IL-6) following acute stress. Although IL-6 is thought to be predictive of the development of cardiovascular disease, CRP has an advantage in that it is more stable, with a longer half-life (Vigushin et al., 1993), is without diurnal rhythm (Meier-Ewert et al., 2001), and elevated serum CRP levels are associated with traditional cardiovascular risk factors (e.g., obesity; Buckley et al., 2009). For example, chronic inflammation is widely observed in obesity (Kershaw & Flier, 2004), itself associated with cardiovascular disease and increased morbidity and mortality (Poirier & Eckel, 2002), while both overweight (BMI 25-29.9 kg/m2) and obese (BMI > or = 30 kg/m2) persons are more likely to have elevated CRP levels than their normal-weight counterparts (BMI < 25 kg/m2; Visser et al., 1999). Consistently, there is evidence for the presence of CRP in human adipose tissue (Calabro et al., 2005) and growing evidence that adipose tissue can induce chronic low-grade inflammation by producing pro-inflammatory cytokines such as IL-6 (Bastard et al., 1999).

Existing literature of CRP measured in blood and its association to sleeping behaviours suggest that both total and partial sleep loss has the effect of increasing serum CRP level. Meier-Ewert et al. (2004) measured both total (88 continuous hrs awake) and partial (10 nights of 4.2 hrs sleep) sleep loss in 10 healthy adults, and found that both conditions increased basal concentrations of CRP in healthy volunteers. Similarly, research by Van Leeuwen et al. (2009) found that in a sample of 13 healthy young men, 5 nights of 4 hrs sleep per night, had the effect of increasing serum CRP significantly after sleep restriction compared to baseline levels. However, as both of these studies reported accumulated, rather than per night, averages, from an initial baseline period compared to 5 (Van Leeuwen et al., 2009) or 10 days (Meier-Ewert et al., 2004) thereafter, it remains unclear as to the influence (if any) of a single night of partial sleep restriction on CRP level. This may be an important

consideration, as the concentration of many circulating inflammatory markers appear to occur after a delay following acute stress, the time course of response of inflammatory markers such as CRP has not been well established (Steptoe et al., 2007).

Inflammation levels are commonly measured through venepuncture. As such, serum CRP has become known as the gold standard measurement of low-grade inflammation and associated prediction of future cardiovascular disease risk. However, as noted previously, venipuncture is a comparatively invasive procedure, placing considerable burden on research participants and requiring skilled phlebotomy personnel. In contrast, collecting oral fluids is generally stress- and pain-free and therefore less burdensome for participants, and as evidenced from previous results, it allows for both laboratory (Study 2) and home (Study 4) self-sample collection. Several immune markers can be detected and assessed in oral fluids, including APPs such as CRP (Pfaffe, Cooper-White, Beyerlein, Kostner, & Punyadeera, 2011), and with the introduction of the salivary CRP assay, commercially available since November 2008 (Salimetrics Europe Ltd.), a minimally invasive alternate measure of CRP (compared to serum assessment) is accessible.

Electronic searches conducted in Medline, PsycINFO, Sciencedirect, and PubMed, using ("salivary CRP") or ("salivary c reactive protein") search terms, resulted in 50 matches. Amongst the emerging studies which have investigated the association between circulating and salivary CRP levels, utilising samples of healthy adults, varying results have been reported. Evidence suggests that CRP levels in oral fluids (oral fluid being a mixture of saliva and oral mucosal transudate) show strong positive correlations with levels in circulation (Megson, Fitzsimmons, Dharmapatni, & Mark Bartold, 2010). However, when measured using salivary fluids, some studies have reported no significant association between CRP in saliva and blood (Dillon et al., 2010; Kopanczyk et al., 2010). In contrast, recent findings from Ouellet-Morin et al. (2011) demonstrated moderate-to-strong associations between CRP

measured in saliva and serum, in a sample of healthy adults, screened for infectious, immune, and salivary gland disorders. In addition, salivary CRP was associated with another associate of inflammation and synthesis of APPs (i.e., BMI).

The present study aimed to extend the literature investigating a novel marker of low level systemic inflammation, by offering a brief preliminary (single CRP sample assessment) investigation into the effect of one night of partial sleep restriction on levels of CRP detectable in saliva, in a sample of young healthy adults.

## 6.2. METHODS

### 6.2.1. Design

As with Study 3 and 4, the present study was completed using a within-subjects design, with participants providing a single saliva sample on two occasions, once while rested and again while sleep restricted (participants being randomly assigned to experimental condition [rested or sleep restricted]). These saliva samples were used for the determination of CRP level. Sleep restriction procedures were completed by participants as outlined in Chapter 2.

### 6.2.2. Participants

All participants had normal BMI (ranging from 16.53 to 29.49, *M* = 22.95, *SD* = 2.63), and none reported immune or salivary gland disorders. Two participants reported taking antibiotic medication (CRP Means of 2303 and 2484.75 picogram/millilitre [pg/ml]) and one participant reported taking medication for gingivitis (CRP Mean of 3093.95 pg/ml). As salivary CRP levels for these three participants were within the normal expected range for healthy young adults (*M* = 1293.28, *SEM* = 140.61, range 113.69 – 6131.40 pg/ml), they were retained in the analyses (in accordance with previous procedures; Ouellet-Morin et al.,

2011). All remaining participants were physically healthy and refrained from smoking, eating, and drinking beverages (with the exception of water) for at least 1 hr prior to testing. The final sample consisted of 120 university students, age 17 to 24 years ($M$ = 18.50 years, $SD$ = 1.08).

Recruitment of participants to the study, participant incentives, and associated ethical approval of procedures, were as described in Chapter 2.

### 6.2.3. Materials and apparatus

*High-sensitivity salivary CRP*. As per previous studies, samples were stored frozen at −20 °C in the laboratory following collection. Samples were subsequently batched and shipped on dry-ice overnight to Salimetrics Laboratories (Suffolk, U.K.). On the day of testing, salivary CRP was determined in duplicates by enzyme-linked immunoassay with the Salivary C-Reactive Protein ELISA Kit (Salimetrics, 12/09/2010). Saliva samples were thawed to room temperature, centrifuged at 3000 rpm for 15 mins to remove mucins, and diluted (1:10) in a phosphate buffered solution before assay. Samples were then transferred (50 µl) to microtitre plate wells pre-coated with mouse anti-CRP antibodies alongside with the standards and controls. Detection antibodies (goat anti-human CRP antibodies linked to horseradish peroxidise) were diluted (1:250) in a phosphate buffered solution, the resulting solution was added to each well (150 µl) and incubated at room temperature for 2 hrs mixing constantly (500 rpm). The wells were washed four times using a wash solution containing detergents. Tetramethylbenzidine (200 µl) was added to each well for colour development prior to sealing and the incubation of the plate in the dark and at room temperature for 30 mins mixing constantly. A stop solution (sulfuric acid; 50 µl) was added before the plates were mixed on a plate rotator for 3 mins and placed on a standard microplate reader at 450 nm (within 10 mins of the addition of the stop solution). The average optical density

values the control and unknown duplicates were plotted against the standard curve made according to the following standard concentrations: 93.8, 187.5, 375, 750, 1500 and 3000 pg/ml. The assay had a lower limit of sensitivity of 10 pg/ml. CRP assays were run in duplicates and coefficient of variation for the difference optical density between the duplicate repeats was 1.64 per cent.

### 6.2.4. Procedure

Data collection took place between 08:00 and 12:30 in the research laboratory. While at rest, participants were invited to rinse their mouth with water before accumulating secreted saliva in their mouth for 2 mins, while being monitored by the researcher. Samples were deposited into a polypropylene collection device (Sarstedt, Nümbrecht, Germany) using the passive drool technique (as detailed in Chapter 2). Samples were stored (at −20 °C) after completion of the laboratory session, until shipment for biochemical analysis took place.

## 6.3. RESULTS

### 6.3.1. Overview of statistical analyses

Due to insufficient specimen volume, CRP level could not be determined in 21 samples. One sample was found to have a CRP concentration above the upper limit of sensitivity for the assay, and was excluded. Due to low saliva volume (with the resulting volume reflecting potential contamination from sediment present in the saliva) 19 samples with low volumes were excluded. A further 28 rested and 30 sleep restricted salivary CRP values exceeded 2 *SD* from the mean reported in healthy adults by the manufacturer (2 SD = 6163.46 pg/ml; Salimetrics, 12/09/2010) and were eliminated from the rest of the analyses, as suggested by previous treatment of CRP concentrations (Ouellet-Morin et al., 2011). This resulted in a total sample of 74 when rested and 75 when sleep restricted. After exclusion,

salivary concentrations ranged from 1369.05 to 5802.50 pg/ml when rested and from 1326.65 to 6111.1 pg/ml when sleep restricted.

In accordance with previous examinations of CRP in saliva, an initial mixed-factorial ANOVA was conducted to examine the effect of a reported potential confound to CRP level, namely smoking (Anderson et al., 1984; Garrett, 1987). For the ANOVA, the within-subjects factor was sleep (rested, sleep restricted) and the between-subjects factor was smoking (smokers vs. non-smokers). The results indicated that smoking did not result in any significant main or interaction effects on CRP in the current sample (13.3 per cent of total sample; *p*s >.485), and was therefore ignored in remaining analyses.

Previous validation analyses for the salivary CRP assay suggested an association between salivary CRP and BMI (a known correlate of systemic inflammation; Ouellet-Morin et al., 2011). To inspect change in CRP when rested and sleep restricted, while controlling for the effects of BMI, repeated measures analysis of covariance (ANCOVA) was conducted, using the within-subjects factor sleep (rested, sleep restricted) and BMI entered as a continuous covariate. Pearson's product moment correlation coefficients were used to explore any BMI main effects, using BMI continuous scoring as the predictor variable (following; Ouellet-Morin et al., 2011).

An inverse transformation was applied to correct for skewed distribution. Data were tested for normal distribution and homogeneity of variance using a Kolmogorov - Smirnov before statistical procedures were applied. The concentration of CRP in saliva was expressed as pg/ml.

Figures and mean values are presented in untransformed CRP values for illustrative purposes. ANOVA effect sizes are presented as partial $\eta^2$. For correlation analyses, effect sizes are presented as *r*, with values of .10, .25, and .37 taken as representing small, medium, and large effect sizes respectively (Cohen, 1988, 1992).

### 6.3.2. Adherence to sleep manipulation

Compliance with the sleep manipulation protocol, as assessed using wrist actigraphy, again indicated high levels of participant adherence in the current sample. Activity levels, monitored on the evening prior to the restricted night to ensure participants maintained wakefulness until their usual bedtime, indicated the average percentage of time participants were awake during the evening prior to the sleep restriction period to be 92.56 per cent (*SD* = 11.92). Equally high (*M* = 381.39, *SD* = 151.63) evening activity scores were recorded (a total score of ≥ 80 designating an epoch as being "awake"). During the restriction vigil (following normal bedtime and awakening on receipt of 40 per cent usual sleep duration), the mean percentage of time participants were awake was 84.86 per cent (*SD* = 17.86). The mean activity score per 2 min epoch was 236.77 (*SD* = 121.32). Both percentage time awake and activity score indicated substantial compliance to the sleep restriction protocol.

### 6.3.3. Salivary CRP and sleep condition

Assessing within-subjects differences in CRP level, the ANCOVA confirmed a main effect for BMI, $F(1, 54) = 6.90$, $p = .011$, partial $\eta^2 = .113$, indicating an association of BMI to salivary CRP level, which is consistent with previous assay validation findings using the salivary CRP measure. No main effects for sleep, $F(1, 54) = .775$, $p = .383$, or sleep × BMI interaction $F(1, 54) = .616$, $p = .436$, were observed, as illustrated in Figure 10.

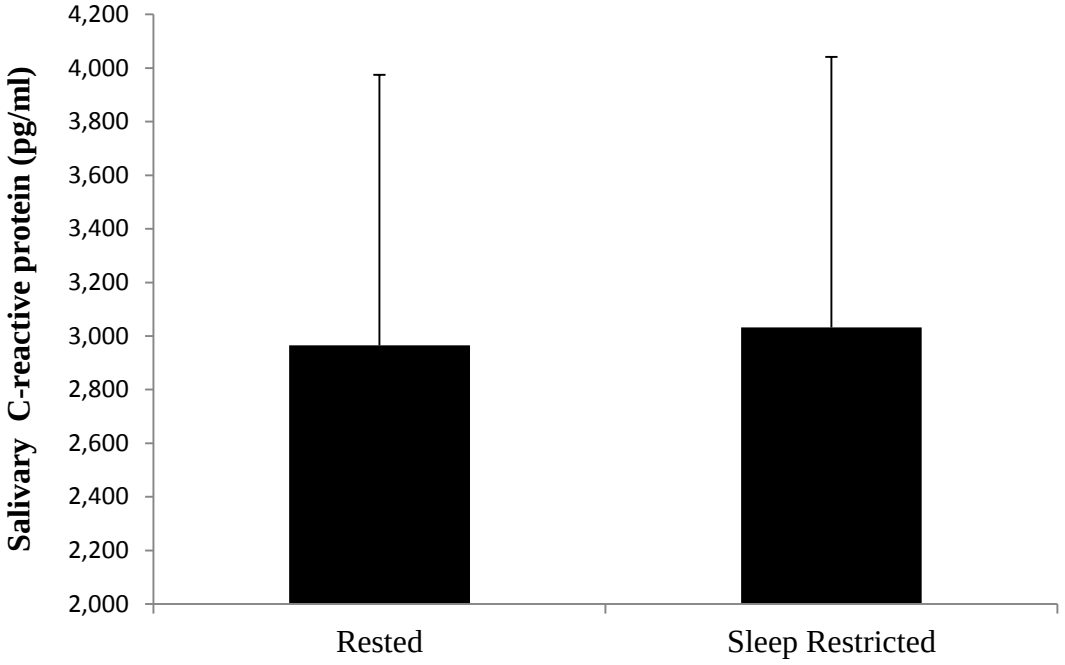


*Figure 11.* Salivary C-reactive protien (CRP) levels for rested and sleep restricted conditions. Error bars denote standard errors of the mean

6.3.4. Salivary CRP and BMI

To further examine the relationship of BMI and CRP level, replicating previous examinations of associations between continuous measures of salivary CRP and BMI (Ouellet-Morin et al., 2011), Pearson's correlations were conducted on BMI and CRP level, when rested and sleep restricted. Correlations revealed CRP level to be significantly positively correlated with BMI, when sleep restricted ($r$ = +.234, $p$ = .043) but not when rested ($r$ = +.195, $p$ = .096), as illustrated in Figure 11.

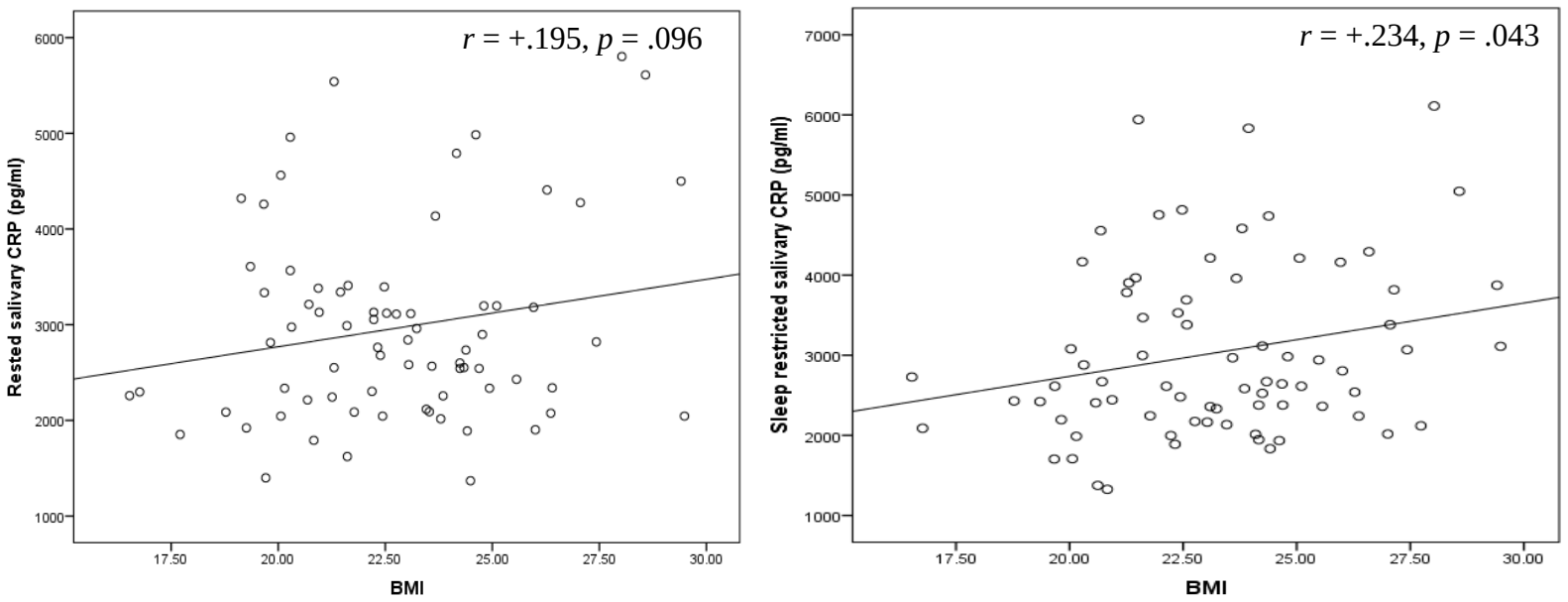


*Figure 12.* Scatterplots reflecting correlations of salivary C-reactive protein (CRP) level and body mass index (BMI), by sleep condition

## 6.4. DISCUSSION

Since inflammation is believed to have a role in the pathogenesis of cardiovascular events, measurement of markers of inflammation has been proposed as a method to improve the prediction of the risk of such events. The present study aimed to contribute to the limited experimental literature relating to a novel marker of systemic inflammation, as measured using salivary CRP. In relation to the time course of salivary CRP, one night of partial sleep restriction did not significantly alter CRP level. However, higher BMI was significantly positively associated with increased CRP response when sleep restricted but not when rested. These results are consistent with previous findings of salivary CRP associations with BMI, a known correlate of systemic inflammation.

The positive trend between BMI and salivary CRP level, when rested, is consistent with salivary CRP assay validation findings of Ouellet-Morin et al. (2011), who also found that this association followed the same direction in a sample of healthy adults, in addition to aligning with previous findings indicating that higher BMIs (as well as central obesity) are associated with higher levels of serum CRP (e.g., Kao et al., 2009). Further, this trend held despite a more limited variability in BMI in the current sample relative to previous studies (i.e., BMI range of 18.29 to 38.35 reported by Ouellet-Morin et al., 2011). Moreover, the positive trend identified between BMI and CRP level became significant under conditions of sleep restriction compared to when rested. Such findings suggest the possibility that when sleep restricted, known correlates of systemic inflammation (i.e., increased BMI) may become more salient, combining with conditions of reduced sleep to influence CRP response. Experimental sleep loss has been shown to acutely elevate pro-inflammatory cytokine levels including CRP and IL-6 (Haack et al., 2007; Meier-Ewert et al., 2001) and there is additional evidence for the presence of CRP in human adipose tissue (Ouchi et al., 2003). Further, data also suggest that adipose tissue can induce chronic low-grade inflammation by producing

pro-inflammatory cytokines such as IL-6 as well as CRP (Bastard et al., 1999). As such, a certain degree of inflammatory process in subjects with higher BMI is suspected (Kao et al., 2009) while the current study further suggests a mutual relationship between BMI and conditions of reduced sleep (known to increase serum inflammatory markers) influencing CRP detectable in saliva.

In Chapter 5, awakening cortisol response was seen to be significantly lower in the sleep restricted condition relative to both rested and recovery conditions (consistent with recent experimental findings using controlled sleep restriction), whereas in the present study, significant associations between higher BMI and increased CRP response were observed when sleep restricted but not when rested. McEwen and Seeman (1999) hypothesised that allostatic load may manifest as dysregulated patterns of stress response *across* systems, rather than alterations in individual system activity. According to this model, one of the putative causes of allostatic load, is an inadequate response of some allostatic systems leading to compensatory upregualtion of associated mediators. The authors give the example of inadequate secretion of glucocorticoids (i.e., cortisol), resulting in increased levels of inflammatory cytokines, that are normally counter-regulated by glucocorticoids.

The pathogenesis of inflammation as a contributory factor in cardiovascular disease is complex and most likely multifactorial. However, neuroendocrine functions are known to contribute to the inflammatory process (McEwen et al., 1997). The HPA axis is involved in the containment of immune-mediated inflammatory reactions. Activation of the axis has profound inhibitory effects on the inflammatory/immune response because virtually all the components of the immune response are inhibited by cortisol (Tsigos & Chrousos, 2002). Further, the physiological effect of cortisol is immunomodulatory rather than solely immunosuppressive, causing a shift of cytokine production from a primarily pro-inflammatory to an anti-inflammatory pattern. A dysfunctional (i.e., reduced) HPA axis

function may thus involve a failure to resolve inflammation (Nijm et al., 2007). Accordingly, impaired HPA response has been associated with increased susceptibility to inflammatory diseases (Sternberg, 2001) and impaired cortisol responses have been found to be associated with increased circulating levels of serum CRP (Nijm et al., 2007).

As such, the present trends, particularly under conditions of acute sleep restriction, suggest an association between increases in a measure of salivary CRP and increased BMI, a known correlate of systemic inflammation. Further, together with results of reduced cortisol response identified in Study 4, the results identify this association as occurring contemporaneously with a significantly reduced cortisol response, suggesting a possible reciprocal physiological relationship of impaired cortisol response and upregulated CRP inflammatory activity, associated with acute sleep restriction and increased BMI. While such effects were observed under acute conditions (partial sleep loss over one night), the observed pattern of effects are also consistent with effects observed under more chronic stressor conditions. Chronic stress (as characterised by parents of children undergoing cancer treatments; Miller, Cohen, & Ritchey, 2002) was also found to alter the inflammatory (pro-inflammatory cytokine IL-6) response, which occurred in the presence of a more flattened (as opposed to increased) diurnal cortisol secretion, primarily due to a reduced output at 1 hr post-awakening (similar to awakening results identified in Study 4). Such results are suggestive of a state whereby, rather than increases in glucocorticoid (i.e., cortisol) secretion, inhibition of cortisol response related to both acute and chronic stress experience, may co-occur with an increased inflammatory response, particularly in relation to pre-existing risk for cardiovascular health.

Data suggest positive correlations between saliva and serum CRP concentrations, even when taken longitudinally, 2 years apart; while higher BMI was consistently associated with higher salivary and serum CRP across time (Out, Hall, Granger, Page, & Woods, 2012).

However, given the novelty of the hs-CRP salivary assay, the degree to which levels of immune and inflammatory markers in oral fluids represent systemic immune activity is a key issue. In the current sample, 28 rested and 30 sleep restricted samples were outside of the expected range for salivary CRP reported by the manufacturer, representing 25 per cent and 27 per cent of the total samples collected at each sleep phase. Ouellet-Morin et al. (2011) also reported relatively large (17 per cent) out of range values in their sample (with no correspondence seen in serum samples). Testing for the effects of calibration of the salivary assays, salivary flow rate, blood contamination, saliva pH and health conditions could not explain these out of range values. They speculate that local inflammatory processes could underlie these out of range values. Megson et al. (2010) demonstrated that CRP in gingival crevicular fluid is likely to be reflective of systemic inflammation, and as whole saliva contains secretions from the salivary glands as well as from the GCF, it is thought this may account for the significant associations between serum and saliva CRP levels. However, local production of CRP in the oral cavity must also be considered (Out et al., 2012). As such, poor oral hygiene and local inflammatory processes may also be an important consideration. In the current study, while physical health and intake of medications was subjectively reported, and controlled for using manufacturer expected CRP ranges, further studies may wish to include a more comprehensive specific assessment of oral health status, or inclusion of oral health examination as part of participants' health assessment. This may help identify issues related to oral health/hygiene or underlying inflammatory processes and reduce unexplained out of range values in unknown samples.

In sum, the present data indicate positive trends between BMI and CRP as a function of sleep duration, in a sample of young healthy adults. Consistent with previous examinations of combined HPA and inflammatory response, the data also suggest the possibility that the observed effects may be physiologically linked with impaired cortisol response, previously

shown to be associated with sleep restriction. Taken together, these findings raise the possibility that insufficient sleep duration, on a chronic basis, may contribute to the development of sustained inflammatory response, in particular amongst those with higher BMIs.

While the current study was exploratory in nature, aiming to contribute to the limited literature on this novel marker of inflammation and present the first study of salivary CRP examined in relation to experimental sleep duration, the trends observed were in line with previous findings of increased salivary inflammatory activity associated with BMI, providing initial support for the utility of salivary CRP as a novel marker of this correlate of systemic inflammation, assessed substantially less invasively in saliva. Although these findings are far from complete and must be viewed with caution, such data suggest even short-term, partial reductions of sleep restriction, may be associated with systemic inflammatory activity.

# Chapter 7.

# OVERALL DISCUSSION

Reduced sleep duration has been associated with adverse health outcomes, in particular negative cardiovascular health. The mechanisms by which sleep loss may influence cardiovascular health are unclear but may be related to alterations in cardiovascular stress responding. The current project sought to assess the association between sleep duration and cardiovascular response to laboratory social stress in addition to examining ambulant cardiovascular functioning, using experimental partial restriction of sleep duration in a sample of healthy young adults. To objectively monitor adherence to the sleep manipulation, participants were provided with a wrist activity monitor. Associated physiological processes, including salivary markers of neuroendocrine stress functioning and inflammation were additionally monitored to further explore the effects of acute sleep restriction on cardiovascular related functioning across both laboratory and ambulatory contexts. Resulting from pooled data collected from 128 college students, five empirical studies were reported, incorporating methodological refinements to help advance understanding of the effects of sleep loss on cardiovascular health.

## 7.1. General Conclusions

Despite epidemiological evidence identifying sleep loss as an important risk factor for cardiovascular health, including increased hypertension risk, the relations between sleep loss and cardiovascular activity remain equivocal. A key imperative of this research was to consider the direct cardiovascular effects attributed to sleep restriction. This was pursued in both laboratory and ambulatory contexts. Findings were largely consistent throughout, with results appearing to differ by category of outcome variable. That the effects were vascular, rather than myocardial (Study 1), and indicated increased (SBP and DBP) blood pressure response to naturally occurring ambulatory interactions (Study3), support the apparent

adverse effects of restricted sleep on cardiovascular health proposed by population based studies.

Study 1 confirmed previous laboratory findings, revealing overall blood pressure response (SBP, DBP, and HR) remained unchanged between rested and sleep restricted participants, perhaps suggesting that altered patterns of HP may underlie non-significant overall blood pressure responses to sleep loss as reported in some previous laboratory investigations. While the underlying hemodynamic pattern of blood pressure response was delineated by the findings of Study 1 (in the absence of overall cardiovascular alterations), in keeping with reports that stress responses obtained in real life are commonly larger than those obtained in the laboratory (Phillips & Hughes, 2011; Zanstra & Johnston, 2011), underscoring the ecological validity of field based stressors, Study 3 revealed augmented responses associated with social contact observed in overall ABP activity. Study 3 further suggested the potential role that sleep restriction may have in negatively affecting resting nocturnal (i.e., lower nighttime DBP) blood pressure in ways previously linked to the etiology of heightened risk for cardiovascular disease amongst at risk individuals.

Examining the effect of sleep restriction on salivary biomarkers of neuroendocrine function, the present research identifies alterations to biomarkers of both SAM and HPA systems following one night of reduced sleep duration. The influence of sleep restriction on both sAA and CAR activity, representing immediate and more prolonged neuroendocrine activation respectively, appeared to implicate sleep loss in both temporal patterns of stress responding. Marked increases in basal sAA following to one night of partial sleep restriction were identified (Study 2). The data additionally contributed new insight regarding the sensitivity of α-amylase to laboratory social stress exposure, demonstrating increased sAA response following participant exposure to negative social evaluation, presented over video relay.

Similarly, the pattern of awakening response evident in cortisol identified in Study 4 offers new evidence implicating sleep restriction in etiological mechanisms pertaining to cardiovascular disease risk, particularly concurrent with the preliminary findings from Study 5. Extending previous findings of CAR responses following reduced sleep duration, the results indicated partial restriction of sleep duration may alter the morning stimulation of the HPA-axis, dampening morning cortisol levels. These studies indicate that sleep restriction tends to be associated with patterns of sAA and CAR response markedly different than when at rest. As chronic dysfunction of these systems has been implicated with health risk, over time, such neuroendocrine response patterns appear to be a marker for, if not a causal factor in, increased risk to cardiovascular health. Further, as a biomarker of physiological stress, the data collected also suggest that saliva offers distinct advantages over more invasive sampling techniques, a particularly important consideration in stress research.

Contributing to the burgeoning literature on salivary CRP, the present research additionally suggests that short sleep duration may be related to this novel marker of systemic inflammation. Relative to when rested, one night of sleep restriction was not associated significantly increased salivary CRP level. However, the results further indicated positive trends between BMI and salivary CRP level as a function of sleep duration, in this sample of young healthy adults. While the findings of Study 5 are preliminary, these results suggest that sleep restriction may be associated with increased CRP level detectable in saliva, in conjunction with pre-existing cardiovascular risk factors.

## 7.2. Theoretical Implications

A central objective of the present research was to consider the effects of sleep restriction on physiological function from a stress reactivity perspective. The consistency in the findings across the investigations demonstrated value for the reactivity construct in terms of examining potentially maladaptive response patterns following reduced sleep duration. In

keeping with the empirical support for blood pressure level as a key predictor of cardiovascular disease, and that blood pressure-lowering regimens are important to cardiovascular disease protection (Czernichow et al., 2011), the reactivity hypothesis is most commonly used in reference to blood pressure and HR reactivity. While in line with previous reports of little or no effects of sleep loss on overall blood pressure levels (Meney et al., 1998; Smith & Maben, 1993), results from Study 1 revealed that relative to rest, acute sleep restriction was associated with a markedly vascular response to social stress in the sleep restricted group. Such data are significant as they corroborate similar effects reported by James and Gregg (James & Gregg, 2004b), who also identified a vascular HP following sleep restriction, while further extending the observance of such effects in response to laboratory social stress. This is an important addition given the superior predictive power of social stress reactivity tasks for ambulatory blood pressure level (Ewart & Kolodner, 1993; Linden & Con, 1994).

As discussed in Chapter 1, as a general proposition, the reactivity hypothesis is not specific with regard to the particular physiological processes which may be implicated in health risk (James et al., 2012). Addressing the call for a multivariate systems approach to help establish links between behavioural and psychosocial pathways to health outcomes with greater certainty (Singer & Ryff, 2001), non-invasive salivary sampling was utilised to examine neuroendocrine stress system functioning. With confirmation (provided in Study 2) of the effects of sleep restriction on the first phase neuroendocrine stress response, as evidenced by upregulated SAM related sAA activity in response to laboratory social stress, examination of the longer-term neuroendocrine stress response (while additionally observing ambulatory salivary physiological responsivity in relation to sleep restriction), was examined in Study 4, which demonstrated altered HPA axis activity by means of reduced CAR profile following sleep loss, assessed in the home setting. These data follow a distinct pattern of markedly altered neuroendocrine stress system activation post-sleep restriction when

compared to rested states, and may be further evidence (alongside the laboratory and ambulatory blood pressure investigations) of the health-compromising characteristics of sleep restriction, while further demonstrating the value of a multi-systems approach to stress reactivity research.

### 7.3. Clinical Implications

As reviewed in Chapter 1, present prevalence data suggest that an increasing number of individuals are chronically sleep restricted, while the present epidemiological record presents a compelling picture of the effects of such chronic sleep debt on incidence of cardiovascular morbidity and mortality. Highlighting inconsistencies in previous data investigating both clinical and non-clinical samples (i.e., confirmed hypertensive/CVD status versus healthy cohorts, respectively) the findings of Study 1 highlight the potential for previous characterisations of null changes in blood pressure response subsequent to sleep loss to be characterised by robust differences in underlying hemodynamic functioning. Further, as the observed effects were found to be vascular, associated with relatively larger increases in TPR compared to CO, this may suggest that vascular hemodynamic responses, and their inducement by increased peripheral resistance, (considered to be maladaptive, in particular in relation to development of established [i.e., chronic] hypertension;Mayet & Hughes, 2003), may, over continued periods sleep loss, contribute to increased risk to cardiovascular health, particularly hypertensive states.

Extending the traditional CVR laboratory protocol, Study 3 complemented the laboratory cardiovascular examination in Study 1, affording increased ecological validity in an examination of sleep restriction and associated cardiovascular function in an ambulatory context. Such dual-assessment methods for blood pressure monitoring (clinic and ambulatory) are routinely employed in clinical practice, providing a representation of blood pressure response in non-artificial settings and involving presented assessment (though both

daytime and nighttime periods), thereby increasing reliability of observed effects. The observed changes in the present research, whereby occurrences of high (compared to low) levels of naturally occurring interpersonal contact throughout the day, were associated with increased SBP and DBP blood pressure responses, when sleep restricted but not when rested, are of particular note. These findings implicate such naturally occurring interaction as a possible mechanism in the sleep loss-health association, given that the observed increase (i.e., 2 to 3 mmHg) in SBP/DBP ABP reactivity has been implicated in cardiovascular risk at the population level. In addition to highlighting CVR to interpersonal contact as a possible mediating mechanism to cardiovascular risk post-sleep loss, Study 3 confirmed that nocturnal blood pressure (considered to be an important representation of basal cardiovascular function due to low physical and/or psychological concomitants), was lower when sleep restricted compared to rested, consistent with previous findings (Lusardi et al., 1996). Together with trends toward increases in nocturnal DBP dipping (both independently and in combination with high levels of contact) while sleep restricted**,** the observed reduced nocturnal blood pressure may be indicative of a pattern of sleep loss related lowering of nocturnal DBP, to levels attributable to "extreme dippers", which have previously been reported to be associated with negative cardiovascular outcome amongst at risk individuals.

Following participant provision of four waking saliva samples, in their own homes, over the first 45 mins post-awakening, acute sleep restriction was found to be related to morning stimulation of the HPA-axis and, in particular, dampening of morning cortisol levels, relative to when rested. These findings corroborated the recent findings by Wu et al. (2008) and Gribbin et al. (2011), both observing a reduced CAR after short-term sleep restriction. Moreover, present results confirm these earlier findings in a sizeable sample of young adults, including methodological refinement over previous investigations, using a more comprehensive (four sample) waking CAR profile, in addition to actigraph verification of sleep duration. Further, relative to SAM axis activation (Study 2), representing more

immediate neurohormonal stress response, the CAR profile suggested alteration to normal HPA response persisting up to 45 mins post-stimulus (awakening) when sleep restricted.

While such alteration to neuroendocrine response patterning demonstrates a noticeable adjustment to stress hormone signalling observed under rested states, after as little as one night of partial sleep restriction, the degree to which these effects may be independently physiologically related to health states remains to be determined. However, as proposed by McEwen and Seeman (1999) cortisol secretion failing to increase in response to stress may permit an upregulation of inflammatory processes (normally counter regulated by cortisol). Inflammatory activity, and associated measureable increases in levels of CRP, may be useful in predicting CHD as well as other vascular events, for example stroke and peripheral vascular disease. Further, elevated serum CRP levels are associated with traditional cardiovascular risk factors (i.e., obesity; Buckley et al., 2009). For example, overweight and obese persons are more likely to have elevated serum CRP levels than their normal-weight counterparts (Visser et al., 1999). Importantly from a stress research perspective, using non-invasive techniques, peripheral (i.e., salivary) CRP measures could offer an estimate of increases in such systemic inflammation, resulting from stress-related acute phase activation.

Extending previous findings reported by Ouellet-Morin et al. (2011) and Out et al. (2012), the findings of Study 5 similarly indicated a positive trend between BMI and salivary CRP level when rested. Together with previous findings, such effects add to the literature which suggests an association between salivary CRP and a known correlate of systemic inflammation. Moreover, the data revealed a moderate strengthening of this positive association following one night of acute sleep restriction. Such findings suggests a mutual relationship between BMI and conditions of reduced sleep (known to increase serum inflammatory markers) influencing CRP detectable in saliva, although as this marker has only

recently been introduced in saliva, this is an emerging area of investigation, requiring further exploration.

## 7.2. Methodological Issues

The present research is strengthened by a number of methodological advancements. Highlighted as a key area of scientific opportunity to advance understanding of how biological and behavioural processes interact to determine health risk, it has been suggested that multiple measurements of stress-related biological processes be attained (Bauer, Quas, & Boyce, 2002; Granger et al., 2006; Singer & Ryff, 2001). A conspicuous methodological strength is the examination of concurrent activity (i.e., reduced sleep duration) across stress systems, permitting a more thorough observation of the physiological correlates of sleep loss on stress system functioning, when compared to examination of activity exclusively in a single system. Moreover, such strengths were extended to the experimental manipulation of sleep duration. Advancing on the methodological limitations of a number of previous cross sectional and prospective epidemiological studies, in addition to a number of experimental studies, reliance on a measure of sleep duration using wrist actigraphy provided objective verification regarding participant implementation of the sleep restriction protocol and bolstered the validity of the observed findings. Additionally, where called for, the empirical studies included methodological refinement specific to the dependent variable being examined, including clarifying the effect of verified sleep loss on sAA as a purported non-invasive (relative to venepuncture) biomarker of SAM stress reactivity, in addition to more rigorous CAR profiling in a larger, and older, sample than previously examined.

The experimental manipulation of sleep duration in the present study offers an advantage over previous (i.e., epidemiological) examinations of the cardiovascular effects of short sleep. However, while there was objective data to identify adherence to the sleep restriction protocol, those individuals in the rested group may not have received a night of

complete rest as directed. This lack of objective measurement of sleep quality in the rested group prior to the laboratory session is a potential weakness. Nevertheless, self-reported fatigue data, across multiple domains of functioning, confirmed that those in the sleep restricted group were significantly more fatigued than the rested group. Issues around expectation bias aside, this offers some evidence supporting the validity of the rested protocol.

As with any study that draws its sample from a convenience population, in this case university students, the generalisability of findings must be considered. However, given recent findings conducted on both older and younger cohorts indicating sizable proportions of short sleepers at the population level of both cohorts, the findings of the present research are useful beyond younger populations. Having demonstrated specific patterns of physiological responding relevant to cardiovascular health in this sub-sample of healthy young adults who were sleep restricted, without additional (e.g., clinical-disease status and age) confounds, future research can build on this model using more diverse populations.

Men constituted a relatively small proportion of the participants (approximately 24 per cent), which is a reflection of the psychology student population from which the sample was drawn. However, sample size and generalisability issues are fairly ubiquitous problems for research, and moreover, gender was not uniformly highlighted as a lightly confound across the outcome variables assessed (e.g., Almeida et al., 2009; Rohleder & Nater, 2009; Steptoe, 2000), the inclusion of males therefore was useful in terms of overall external validity.

The laboratory social stress task employed in Study 1 and 2 to elicit cardiovascular and sAA stress reactions used a protocol successfully utilised in prior research (O'Leary et al.; Thorsteinsson et al., 1998). The stressor manipulation, using video-relayed presentation, further advocated the use of such a methodology in the observance of laboratory physiological stress effects with a greater degree of precision and standardisation. Since the

literature reporting sAA findings is continually growing, methodological guidelines are also evolving over time. While both empirical data and assay supplier recommendations at the time of sAA data collection for the present study indicated that sAA concentrations are not affected by salivary flow rates (see Rohleder et al., 2006), current recommendations advise examination of the secretion rate for sAA (i.e., the output as u/min) in addition to sAA concentration (u/mL) when using sAA as a marker of nervous activity (Bosch et al., 2011). Increased reporting of both parameters will help to establish if secretion/flow rate of sAA (which is largely reflective of parasympathetic as opposed to sympathetic activity) is a factor that necessitates adjustment.

While some reports have suggested that morning exposure to bright light results in an increase in cortisol levels compared to remaining in dim light (Figueiro & Rea, 2012; Leproult, Colecchia, L'Hermite-Balériaux, & Van Cauter, 2001), others have reported the opposite effect, indicating a suppressive effect on cortisol levels following bright light exposure (Jung et al., 2010). As exposure to light was not controlled for in the present study, it may be possible that participants received differing levels of initial light exposure across sleep phases. While the previous data is unclear as to what effect this may have on CAR response, consideration of light levels in the home setting (i.e., same bedroom and same light source) across sleep phases may be an advantage.

The monitoring afforded by the Finometer facilitated a more detailed examination of cardiovascular variables than has been achieved in many previous studies of sleep loss and cardiovascular response (e.g., Franzen et al., 2011; Meney et al., 1998). Further, few studies have measured how sleep loss affects cardiovascular response to naturalistic stressors, while periods of interpersonal contact have been suggested as a useful task for investigating CVR in the field. ABP offered a profile of repeated assessments of cardiovascular activity to ambulant stimuli away from laboratory settings. Examination of nocturnal blood pressure dipping was also conducted to explore if the habitual nocturnal reduction in blood pressure

was associated with reduced sleep duration. However, while mean levels of self-reported stress were significantly higher when sleep restricted compared to rested, ABP was unaffected by ratings of self-reported stress, when either rested or sleep restricted. This finding contrasts with some previous findings which observed increased cardiovascular responses to instances of high stress and interpersonal interactions (Lehman & Conley, 2010). Underlying such an effect, specific characteristics of the reported stress (i.e., anxiety over others' evaluative reactions during the social interaction) appeared to have a bearing on response strength. It is therefore possible that such variables may have influenced the specific interaction of ambulatory stress and cardiovascular response similarly in the current sample. Previous findings (Holt-Lunstad et al., 2003) have also found interactions with ambivalent others to be associated with higher ABP compared to interactions with family members and spouses, which were associated with lower responses. As such, further ambulatory physiological examinations incoroprating examination of aspects of social relationships and characteristics of the interpersonal contact across daily interactions, when both rested and sleep restricted, could be revealing.

The use of a novel marker of systemic inflammation, salivary CRP, expanded on the emerging literature of this biomarker, while additionally identifying an association between one night of acute sleep restriction, CRP response, and a known correlate of systemic inflammation (BMI). Given the novelty of the hs-CRP salivary assay, and the high proportion of CRP samples outside the expected range for salivary CRP reported by the assay manufacturer found in the present research (and similarly reported by others; Ouellet-Morin et al., 2011), suggests the need for further examination. In particular, overall health/inflammatory processes in the oral cavity may require closer scrutiny and help to account for the number of out of range values.

Collectively, findings from the current study show that altered cardiovascular, neuroendocrine, and inflammatory activity are correlates of reduced sleep duration in young

adults. Studies aiming to track sleep duration over time and designs aimed to enhance sleep duration in young adults would be beneficial. For example, a sleep extension intervention, in short sleepers, would aid demonstration of causality in the opposite direction, supporting the claim that short sleep is associated with health problems (Grandner, Patel, et al., 2010).

Testing such effects, a recent study failed to find a blood pressure lowering effect in sleep extension of 2 hrs per weekend day over a period of 1 week in workers with habitual sleep durations of less than 6 hrs (Kubo et al., 2011). However, blood pressure was assessed by means of a single daytime measurement. Using repeated measurements, utilising ABP monitoring over two 24-hr periods, mirroring the protocol used in the current research, a recent pilot study found that a 6-week behavioural intervention, which extended actigraphy verified daily sleep by 35 mins in a sample of 22 hypertensive and pre-hypertensive individuals, with habitual sleep duration of < 7 hrs, demonstrated a reduction in average 24-hr SBP and DBP. By comparison, the control group who maintained their habitual sleep duration did not show a significant blood pressure decrease (Haack et al., in press). The decreases observed after 6 weeks in the sleep extension group, corresponding to a drop in 24-hr drop in SBP and DBP of 14 and 8 mmHg, respectively, are of comparable magnitude to standard blood pressure lowering behavioural intervention strategies, such as exercise and diet. For example, Blumenthal et al. (2010) reported that a 9-week behavioural intervention, including dietary and exercise improvements, reduced 24-hr ambulatory SBP and DBP in the region of 10 and 5 mmHg respectively. Such findings highlight sleep loss as a modifiable cardiovascular risk factor, and the potential for the reversibility of such effects through interventions such as sleep extension. Interventions designed to reduce hypertension and cardiovascular disease risk (e.g., Blumenthal et al., 2010) may therefore benefit from additionally targeting sleep duration to provide maximal benefits.

The blood pressure reductions reported by Haack et al. (in press) were not paralleled by pre- to post-intervention changes in inflammatory (IL-6 and CRP) or sympatho-adrenal

(NE) markers, which were assessed in single time-point measurement in blood and urine samples. However, given previous findings indicating alterations in these markers with experimental sleep loss, and demonstrated in the present research using salivary markers of sAA and CRP assessed at rest and again following sleep restriction, while the findings of Haack et al. did not show a change in these markers within 6 weeks of sleep extension, it is, as they suggest, an open question whether a single measurement 6 weeks apart is sufficient to detect a meaningful change in these systems. A more accurate reflection of parallel neuroendocrine and inflammatory activity may therefore be achieved using a more comprehensive (i.e., repeated assessment) sampling schedule in future sleep extension research methodologies.

## 7.3. Overall Conclusions

The overarching aim of this research was to investigate the association, as suggested by epidemiological evidence, relating self-reported short sleep duration to adverse cardiovascular health on a population level. Throughout the research, there was an emphasis on the experimental examination of the stress response, and the activation of the physiological stress systems, as an area which suggested clinically significant endpoints which aligned with the cardiovascular risks associated with short sleep. At a primary level, influences of sleep restriction on laboratory and ambulatory cardiovascular levels were assessed, with subsequent studies extending to associated stress processes, examining biomarkers of HPA and SAM system activation, followed by examination of stress responsivity in a novel marker of systemic inflammation, as measured using salivary CRP. In the first instance, effects of actigraphy-assessed short sleep duration were found on hemodynamic determinants of blood pressure in response to laboratory social stress and ambulatory cardiovascular indices of reactivity to naturally occurring interpersonal contact and nocturnal blood pressure, in ways that have been implicated in the etiology of heightened cardiovascular disease risk. Alterations to

associative physiological stress processes, as evidenced in both sAA and CAR activity, representing immediate and more prolonged neuroendocrine stress response, respectively, were further observed following sleep restriction, implicating sleep loss in change to both temporal patterns of stress responding, assessed in laboratory and ambulatory conditions. Examining inflammatory activity, preliminary correlational evidence for increased salivary CRP associated with sleep restriction was observed in the final study, when such sleep restriction occurred in the context of other cardiovascular risk factors.

Overall, the present data have (i) confirmed that relative to a rested group, acute sleep restriction was associated with alterations in the hemodynamic determinants of blood pressure, indicative of vascular responding, in response to social stress, while overall blood pressure response remained unchanged between rested and sleep restricted groups (ii) indicated marked increases in basal sAA activity following sleep restriction, additionally corroborating previous findings regarding the sensitivity of sAA to laboratory social stress exposure, while further demonstrating a significant increase in sAA in response to an acute social stress protocol where the primary social evaluative element was presented by virtual (i.e., video-relayed) observers (iii) suggested the potential role that sleep restriction may have in negatively influencing both nocturnal blood pressure and reactivity to naturally occurring interpersonal contact, observed in ambulatory cardiovascular indices (iv) consolidated the involvement of acute sleep loss in morning stimulation of the HPA-axis and, specifically, the dampening of morning cortisol levels, and (v) contributed to the growing literature on salivary CRP, demonstrating for the first time that short sleep duration may influence this novel marker of systemic inflammation in conjunction with a known cardiovascular risk factor. This research adds substantially to investigations of short sleep duration and effects on health, highlighting potential physiological pathways though which reduced sleep duration may, over time, influence cardiovascular pathogenesis.

## References

Adam, E. K. (2006). Transactions among adolescent trait and state emotion and diurnal and momentary cortisol activity in naturalistic settings. *Psychoneuroendocrinology, 31*, 664-679.

Åkerstedt, T. (2006). Psychosocial stress and impaired sleep. *Scandinavian Journal of Work, Environment & Health, 32*, 493-501.

Allan, R., & Scheidt, S. (1996). Empirical basis for cardiac psychology. In R. Allan & S. Scheidt (Eds.), *Heart and mind: The practice of cardiac psychology* (pp. 63–123). Washington, DC: American Psychological Association.

Allen, M. T., Obrist, P. A., Sherwood, A., & Growell, M. D. (2007). Evaluation of myocardial and peripheral vascular responses during reaction time, mental arithmetic, and cold pressor tasks. *Psychophysiology, 24*, 648-656.

Almeida, D. M., Piazza, J. R., & Stawski, R. S. (2009). Interindividual differences and intraindividual variability in the cortisol awakening response: An examination of age and gender. *Psychology and Aging, 24*, 819–827.

Altman, D. G., & Royston, P. (2006). Statistics notes: the cost of dichotomising continuous variables. *BMJ: British Medical Journal, 332*, 1080.

Am-Meijer, H., & Enober, G. (2000). Time in bed, quality of sleep and school functioning of children. *Journal of Sleep Research, 9*, 145-153.

American Psychiatric Association. (2000). *Diagnostic and statistical manual of mental disorders*. *4*. Washington, DC: American Psychiatric Association.

Anderson, L., Garrett, J., Johnson, D., Kauffman, D., Keller, P., & Thulin, A. (1984). Influence of circulating catecholamines on protein secretion into rat parotid saliva during parasympathetic stimulation. *The Journal of Physiology, 352*, 163-171.

Aranda, P., De La Cruz, J., Fernandez-Garcia, J., Ribo-Crusat, F., De Miguel, A., Perez-Vidal, S., . . . De La Sierra, A. (2010). Measuring cardiovascular risk in normotensive people: Impact of a non-dipper pattern of blood pressure. *Journal of Hypertension, 28*, e84.

Axelrod, J., & Reisine, T. D. (1984). Stress hormones - Their interaction and regulation. *Science, 224*(4648), 452-459.

Ayas, N. T., White, D. P., Al-Delaimy, W. K., Manson, J. A. E., Stampfer, M. J., Speizer, F. E., . . . Hu, F. B. (2003). A prospective study of self-reported sleep duration and incident diabetes in women. *Diabetes Care, 26*, 380-384.

Ayas, N. T., White, D. P., Manson, J. A. E., Stampfer, M. J., Speizer, F. E., Malhotra, A., & Hu, F. B. (2003). A prospective study of sleep duration and coronary heart disease in women. *Archives of Internal Medicine, 163*, 205-209.

Backhaus, J., Junghanns, K., & Hohagen, F. (2004). Sleep disturbances are correlated with decreased morning awakening salivary cortisol. *Psychoneuroendocrinology, 29*, 1184-1191.

Balbo, M., Leproult, R., & Van Cauter, E. (2010). Impact of sleep and its disturbances on hypothalamo-pituitary-adrenal axis activity. *International Journal of Endocrinology, 2010*, 1-16.

Banks, J., Breeze, E., Crawford, R., Demakakos, P., Oliveira Cd, G. E., Green, R., . . . Marmot, M. (2010). Financial circumstances, health and well-being of the older population in England. *The 2008 English Longitudinal Study of Ageing (Wave 4)*.

Bastard, J. P., Jardel, C., Delattre, J., Hainque, B., Bruckert, E., & Oberlin, F. (1999). Evidence for a link between adipose tissue interleukin-6 content and serum C-reactive protein concentrations in obese subjects. *Circulation, 99*, 2219-2222.

Bauer, A. M., Quas, J. A., & Boyce, W. T. (2002). Associations between physiological reactivity and children's behavior: Advantages of a multisystem approach. *Journal of Developmental & Behavioral Pediatrics, 23*, 102-113.

Baum, A., & Grunberg, N. (1995). Measurement of stress hormones. In S. Cohen, R. C. Kessler & L. Underwood Gordon (Eds.), *Measuring stress: A guide for health and social scientists* (pp. 175-192). Oxford: Oxford University Press.

Bayer, O., Neuhauser, H., & von Kries, R. (2009). Sleep duration and blood pressure in children: A cross-sectional study. *Journal of Hypertension, 27,* 1789-1793.

Bhagwagar, Z., Hafizi, S., & Cowen, P. J. (2005). Increased salivary cortisol after waking in depression. *Psychopharmacology, 182*, 54-57.

Bin, Y. S., Marshall, N. S., & Glozier, N. (2012). Secular trends in adult sleep duration: A systematic review. *Sleep Medicine Reviews, 16,* 223-230.

Bishop, G. D., Pek, J., & Ngau, F. (2006). Blunted cardiovascular responses to daytime activities as related to reduced nocturnal blood pressure decline. *Annals of Behavioral Medicine, 31*, 248-253.

Bixler, E. (2009). Sleep and society: An epidemiological perspective. *Sleep Medicine, 10,* S3-S6.

Blankenberg, S., & Yusuf, S. (2006). The Inflammatory Hypothesis. *Circulation, 114*, 1557-1560.

Bliwise, D. L., & Young, T. B. (2007). The parable of parabola: What the U-shaped curve can and cannot tell us about sleep. *Sleep, 30*, 1614-1615.

Blumenthal, J. A., Babyak, M. A., Hinderliter, A., Watkins, L. L., Craighead, L., Lin, P.-H., . . . Sherwood, A. (2010). Effects of the DASH diet alone and in combination with exercise and weight loss on blood pressure and cardiovascular biomarkers in men and

women with high blood pressure: The ENCORE study. *Archives of Internal Medicine, 170*, 126-135.

Bonnafoux, P. (1996). Auscultatory and oscillometric methods of ambulatory blood pressure monitoring, advantages and limits: A technical point of view. *Blood Pressure Monitoring, 1*, 181-185.

Bonnet, M. H., & Arand, D. L. (1995). We are chronically sleep deprived. *Sleep, 18*, 908-911.

Born, J., & Fehm, H. (1998). Hypothalamus-pituitary-adrenal activity during human sleep: A coordinating role for the limbic hippocampal system. *Experimental and Clinical Endocrinology and Diabetes, 106*, 153-163.

Bosch, J., Brand, H., Ligtenberg, A., Bermond, B., Hoogstraten, J., & Nieuw-Amgerongen, A. (1998). The response of salivary protein levels and S-IgA to an academic examination are associated with daily stress. *Journal of Psychophysiology, 12*, 384-391.

Bosch, J. A., Brand, H. S., Ligtenberg, T., Bermond, B., Hoogstraten, J., & Amerongen, A. V. N. (1996). Psychological stress as a determinant of protein levels and salivary-induced aggregation of Streptococcus gordonii in human whole saliva. *Psychosomatic Medicine, 58*, 374-382.

Bosch, J. A., de Geus, E. J. C., Veerman, E. C. I., Hoogstraten, J., & Amerongen, A. V. N. (2003). Innate secretory immunity in response to laboratory stressors that evoke distinct patterns of cardiac autonomic activity. *Psychosomatic Medicine, 65*, 245-258.

Bosch, J. A., Veerman, E. C. I., de Geus, E. J., & Proctor, G. B. (2011). α-Amylase as a reliable and convenient measure of sympathetic activity: Don't start salivating just yet! *Psychoneuroendocrinology, 36*, 449-453.

Brondolo, E., Grantham, K. I., Karlin, W., Taravella, J., Mencía-Ripley, A., Schwartz, J. E., . . . Contrada, R. J. (2009). Trait hostility and ambulatory blood pressure among traffic enforcement agents: the effects of stressful social interactions. *Journal of Occupational Health Psychology, 14*, 110-121.

Brondolo, E., Karlin, W., Alexander, K., Bobrow, A., & Schwartz, J. (1999). Workday communication and ambulatory blood pressure: Implications for the reactivity hypothesis. *Psychophysiology, 36*, 86-94.

Buckley, D. I., Fu, R., Freeman, M., Rogers, K., & Helfand, M. (2009). C-reactive protein as a risk factor for coronary heart disease: A systematic review and meta-analyses for the US Preventive Services Task Force. *Annals of Internal Medicine, 151*, 483-495.

Burt, V. L., Whelton, P., Roccella, E. J., Brown, C., Cutler, J. A., Higgins, M., . . . Labarthe, D. (1995). Prevalence of hypertension in the US adult population results from the Third National Health and Nutrition Examination Survey, 1988-1991. *Hypertension, 25*, 305-313.

Buske-Kirschbaum, A., Geiben, A., Höllig, H., Morschhäuser, E., & Hellhammer, D. (2002). Altered responsiveness of the hypothalamus-pituitary-adrenal axis and the sympathetic adrenomedullary system to stress in patients with atopic dermatitis. *Journal of Clinical Endocrinology & Metabolism, 87*, 4245-4251.

Calabro, P., Chang, D. W., Willerson, J. T., & Yeh, E. T. H. (2005). Release of C-reactive protein in response to inflammatory cytokines by human adipocytes: Linking obesity to vascular inflammation. *Journal of the American College of Cardiology, 46*, 1112-1113.

Cannon, W. B. (1929a). Bodily changes in pain, hunger, fear, and rage. *Southern Medical Journal, 22*, 870.

Cannon, W. B. (1929b). Organization for physiological homeostasis. *Physiological Reviews, 9*, 399-431.

Capaldi, I., Vincent F, Handwerger, K., Richardson, E., & Stroud, L. R. (2005). Associations between sleep and cortisol responses to stress in children and adolescents: A pilot study. *Behavioral Sleep Medicine, 3*, 177-192.

Cappuccio, F. P., Cooper, D., D'Elia, L., Strazzullo, P., & Miller, M. A. (2011). Sleep duration predicts cardiovascular outcomes: A systematic review and meta-analysis of prospective studies. *European Heart Journal, 32*, 1484-1492.

Cappuccio, F. P., Stranges, S., Kandala, N. B., Miller, M. A., Taggart, F. M., Kumari, M., . . . Marmot, M. G. (2007). Gender-specific associations of short sleep duration with prevalent and incident hypertension: The Whitehall II Study. *Hypertension, 50*, 693-700.

Carroll, D. (1992). *Health psychology: Stress, behaviour and disease*. London: Falmer Press

Carroll, D., Phillips, A. C., & Der, G. (2008). Body mass index, abdominal adiposity, obesity, and cardiovascular reactions to psychological stress in a large community sample. *Psychosomatic Medicine, 70*, 653-660.

Carroll, D., Smith, G. D., Shipley, M. J., Steptoe, A., Brunner, E. J., & Marmot, M. G. (2001). Blood pressure reactions to acute psychological stress and future blood pressure status: A 10-year follow-up of men in the Whitehall II study. *Psychosomatic Medicine, 63*, 737-743.

Castell, J. V., Gómez-lechón, M. J., David, M., Fabra, R., Trullenque, R., & Heinrich, P. C. (2005). Acute-phase response of human hepatocytes: Regulation of acute-phase protein synthesis by interleukin-6. *Hepatology, 12*, 1179-1186.

Chae, C. U., Lee, R. T., Rifai, N., & Ridker, P. M. (2001). Blood pressure and inflammation in apparently healthy men. *Hypertension, 38*, 399-403.

Chatterton, R. T., Vogelsong, K. M., Lu, Y.-C., Ellman, A. B., & Hudgens, G. A. (1996). Salivary α-amylase as a measure of endogenous adrenergic activity. *Clinical Physiology, 16,* 433-448.

Chatterton, R. T., Vogelsong, K. M., Lu, Y., & Hudgens, G. A. (1997). Hormonal responses to psychological stress in men preparing for skydiving. *Journal of Clinical Endocrinology & Metabolism, 82,* 2503-2509.

Chatzitheochari, S., & Arber, S. (2009). Lack of sleep, work, and the long hours culture: Evidence from the UK Time Use Survey. *Work, Employment & Society, 23,* 30-48.

Chatzizisis, Y. S., Coskun, A. U., Jonas, M., Edelman, E. R., Stone, P. H., & Feldman, C. L. (2007). Risk stratification of individual coronary lesions using local endothelial shear stress: A new paradigm for managing coronary artery disease. *Current Opinion in Cardiology, 22,* 552-564.

Chen, H. (1991). Effects of 30-h sleep loss on cardiorespiratory functions at rest and in exercise. *Medicine and Science in Sports and Exercise, 23,* 193-198.

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: A systematic review and meta-analysis. *Biological Psychology, 80,* 265-278.

Chida, Y., & Steptoe, A. (2010). Greater Cardiovascular Responses to Laboratory Mental Stress Are Associated With Poor Subsequent Cardiovascular Risk Status A Meta-Analysis of Prospective Evidence. *Hypertension, 55,* 1026-1032.

Chikanza, I., Petrou, P., Kingsley, G., Chrousos, G., & Panayi, G. (1992). Defective hypothalamic response to immune and inflammatory stimuli in patients with rheumatoid arthritis. *Arthritis and Rheumatism, 35,* 1281-1288.

Chobanian, A. V., Bakris, G. L., Black, H. R., Cushman, W. C., Green, L. A., Izzo, J. L., . . . Wright, J. T. (2003). Seventh report of the joint national committee on prevention,

detection, evaluation, and treatment of high blood pressure. *Hypertension, 42*, 1206-1252.

Choi, K., Lee, J., Park, H., Baik, S., Choi, D., & Kim, S. (2008). Relationship between sleep duration and the metabolic syndrome: Korean National Health and Nutrition Survey 2001. *International Journal of Obesity, 32*, 1091-1097.

Christensen, N. J., Vestergaard, P., Sørensen, T., & Rafaelsen, O. (2007). Cerebrospinal fluid adrenaline and noradrenaline in depressed patients. *Acta Psychiatrica Scandinavica, 61*, 178-182.

Clow, A., Hucklebridge, F., Stalder, T., Evans, P., & Thorn, L. (2010). The cortisol awakening response: more than a measure of HPA axis function. *Neuroscience & Biobehavioral Reviews, 35*, 97-103.

Coffield, T. G., & Tryon, W. W. (2004). Construct validation of actigraphic sleep measures in hospitalized depressed patients. *Behavioral Sleep Medicine, 2*, 24-40.

Cohen, H., Zohar, J., Gidron, Y., Matar, M. A., Belkind, D., Loewenthal, U., . . . Kaplan, Z. (2006). Blunted HPA axis response to stress influences susceptibility to posttraumatic stress response in rats. *Biological Psychiatry, 59*, 1208-1218.

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. NJ: Erlbaum: Hillsdale.

Cohen, J. (1992). A power primer. *Psychological Bulletin, 112*, 155-159.

Colten, H. R., & Altevogt, B. M. (2006). *Sleep disorders and sleep deprivation: An unmet public health problem*. Washington DC: National Academies Press.

Czernichow, S., Zanchetti, A., Turnbull, F., Barzi, F., Ninomiya, T., Kengne, A. P., . . . Arima, H. (2011). The effects of blood pressure reduction and of different blood pressure-lowering regimens on major cardiovascular events according to baseline blood pressure: Meta-analysis of randomized trials. *Journal of hypertension, 29*, 4.

Danesh, J., Wheeler, J. G., Hirschfield, G. M., Eda, S., Eiriksdottir, G., Rumley, A., . . . Gudnason, V. (2004). C-reactive protein and other circulating markers of inflammation in the prediction of coronary heart disease. *New England Journal of Medicine, 350*, 1387-1397.

de Boer, O. J., van der Wal, A. C., & Becker, A. E. (2000). Atherosclerosis, inflammation, and infection. *The Journal of Pathology, 190*, 237-243.

de Rooij, S. R. (in press). Blunted cardiovascular and cortisol reactivity to acute psychological stress: A summary of results from the Dutch Famine Birth Cohort Study. *International Journal of Psychophysiology*.

Deak, T. (2007). From classic aspects of the stress response to neuroinflammation and sickness: Implications for individuals and offspring. *International Journal of Comparative Psychology, 20*, 96-110.

Dement, W., Richardson, G., Prinz, P., Carskadon, M., Kripke, O., & Czeisler, C. (1985). Changes of sleep and wakefulness with age. *Handbook of the Biology of Aging*, 692-717.

DeSantis, A. S., Adam, E. K., Mendelsohn, K. A., & Doane, L. D. (2010). Concordance between self-reported and objective wakeup times in ambulatory salivary cortisol research. *International Journal of Behavioral Medicine, 17*, 74-78.

Dickerson, S. S., & Kemeny, M. E. (2004). Acute stressors and cortisol responses: A theoretical integration and synthesis of laboratory research. *Psychological Bulletin, 130*, 355-391.

Dillon, M. C., Opris, D. C., Kopanczyk, R., Lickliter, J., Cornwell, H. N., Bridges, E. G., . . . Bridges, K. G. (2010). Detection of Homocysteine and C-Reactive Protein in the Saliva of Healthy Adults: Comparison with Blood Levels. *Biomarker Insights*, *5*(2010), 57-61.

Dimsdale, J. E. (2008). Psychological stress and cardiovascular disease. *Journal of the American College of Cardiology, 51*, 1237-1246.

Dinges, D., Douglas, S., Zaugg, L., Campbell, D., McMann, J., Whitehouse, W., . . . Orne, M. (1994). Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64 hours of sleep deprivation. *Journal of Clinical Investigation, 93*, 1930-1939.

Dinges, D. F. (2009). An overview of sleepiness and accidents. *Journal of sleep research, 4*, 4-14.

Ditzen, B., Neumann, I. D., Bodenmann, G., von Dawans, B., Turner, R. A., Ehlert, U., & Heinrichs, M. (2007). Effects of different kinds of couple interaction on cortisol and heart rate responses to stress in women. *Psychoneuroendocrinology, 32*, 565-574.

Dolan, E., Stanton, A., Thijs, L., Hinedi, K., Atkins, N., McClory, S., . . . O'Brien, E. (2005). Superiority of ambulatory over clinic blood pressure measurement in predicting mortality the Dublin outcome study. *Hypertension, 46*, 156-161.

Edwards, S., Clow, A., Evans, P., & Hucklebridge, F. (2001). Exploration of the awakening cortisol response in relation to diurnal cortisol secretory activity. *Life Sciences, 68*, 2093-2103.

Edwards, S., Evans, P., Hucklebridge, F., & Clow, A. (2001). Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology, 26*, 613-622.

Ekstedt, M., Söderström, M., Åkerstedt, T., Nilsson, J., Søndergaard, H. P., & Aleksander, P. (2006). Disturbed sleep and fatigue in occupational burnout. *Scandinavian Journal of Work, Environment, & Health, 32*, 121-131.

Ellenbogen, M. A., Hodgins, S., Walker, C. D., Couture, S., & Adam, S. (2006). Daytime cortisol and stress reactivity in the offspring of parents with bipolar disorder. *Psychoneuroendocrinology, 31*, 1164-1180.

Epstein, F. H., Gabay, C., & Kushner, I. (1999). Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine, 340*, 448-454.

Epstein, F. H., & Ross, R. (1999). Atherosclerosis—an inflammatory disease. *New England Journal of Medicine, 340*, 115-126.

Esch, T., Stefano, G. B., Fricchione, G. L., & Benson, H. (2002). Stress in cardiovascular diseases. *Medical Science Monitor, 8*(5), 93-101.

Ewart, C. K., & Kolodner, K. B. (1993). Predicting ambulatory blood pressure during school: Effectiveness of social and nonsocial reactivity tasks in black and white adolescents. *Psychophysiology, 30*, 30-38.

Faraut, B., Boudjeltia, K. Z., Dyzma, M., Rousseau, A., David, E., Stenuit, P., . . . Kerkhofs, M. (2011). Benefits of napping and an extended duration of recovery sleep on alertness and immune cells after acute sleep restriction. *Brain, Behavior, and Immunity, 25*, 16-24.

Faraut, B., Boudjeltia, K. Z., Vanhamme, L., & Kerkhofs, M. (2012). Immune, inflammatory, and cardiovascular consequences of sleep restriction and recovery. *Sleep Medicine Reviews, 16*, 137-149.

Federenko, I., Wüst, S., Hellhammer, D. H., Dechoux, R., Kumsta, R., & Kirschbaum, C. (2004). Free cortisol awakening responses are influenced by awakening time. *Psychoneuroendocrinology, 29*, 174-184.

Feinsilver, S. H. (2003). Sleep in the elderly: What is normal? *Clinics in Geriatric Medicine, 19*, 177-188.

Fekedulegn, D. B., Andrew, M. E., Burchfiel, C. M., Violanti, J. M., Hartley, T. A., Charles, L. E., & Miller, D. B. (2007). Area under the curve and other summary indicators of repeated waking cortisol measurements. *Psychosomatic Medicine, 69*, 651-659.

Felsten, G., & Wilcox, K. (1992). Influences of stress and situation-specific mastery beliefs and satisfaction with social support on well-being and academic performance. *Psychological Reports, 70*, 291-303.

Ferrie, J. E., Shipley, M. J., Cappuccio, F. P., Brunner, E., Miller, M. A., Kumari, M., & Marmot, M. G. (2007). A prospective study of change in sleep duration: Associations with mortality in the Whitehall II cohort. *Sleep, 30*, 1659–1666.

Figueiro, M., & Rea, M. (2011). Sleep opportunities and periodic light exposures: Impact on biomarkers, performance and sleepiness. *Lighting Research and Technology, 43*, 349-369.

Figueiro, M. G., & Rea, M. S. (2012). Short-wavelength light enhances cortisol awakening response in sleep-restricted adolescents. *International Journal of Endocrinology, 2012*, 1-7.

Foster, R., & Roenneberg, T. (2012). Times Cheltenham Science Festival. Retrieved from http://www.ox.ac.uk/media/news_stories/2012/120612_1.html

Franzen, P. L., Gianaros, P. J., Marsland, A. L., Hall, M. H., Siegle, G. J., Dahl, R. E., & Buysse, D. J. (2011). Cardiovascular reactivity to acute psychological stress following sleep deprivation. *Psychosomatic Medicine, 73*, 679-682.

Freeman, D. J., Norrie, J., Caslake, M. J., Gaw, A., Ford, I., Lowe, G. D., . . . Sattar, N. (2002). C-reactive protein is an independent predictor of risk for the development of diabetes in the West of Scotland Coronary Prevention Study. *Diabetes, 51*, 1596-1600.

Freitag, M. H., & Vasan, R. S. (2003). What is normal blood pressure? *Current Opinion in Nephrology and Hypertension, 12*, 285-292.

Frey, D. J., Fleshner, M., & Wright, K. P. (2007). The effects of 40 hours of total sleep deprivation on inflammatory markers in healthy young adults. *Brain, Behavior, and Immunity, 21*, 1050-1057.

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology, 72*, 67-73.

Gallicchio, L., & Kalesan, B. (2009). Sleep duration and mortality: A systematic review and meta-analysis. *Journal of Sleep Research, 18*, 148-158.

Gangwisch, J. E., Heymsfield, S. B., Boden-Albala, B., Buijs, R. M., Kreier, F., Pickering, T. G., . . . Malaspina, D. (2006). Short sleep duration as a risk factor for hypertension: Analyses of the First National Health and Nutrition Examination Survey. *Hypertension, 47*, 833-839.

Garrett, J. (1987). The proper role of nerves in salivary secretion: A review. *Journal of Dental Research, 66*, 387-397.

Geiss, A., Varadi, E., Steinbach, K., Bauer, H. W., & Anton, F. (1997). Psychoneuroimmunological correlates of persisting sciatic pain in patients who underwent discectomy. *Neuroscience Letters, 237*(2), 65-68.

Germano, G., Hoes, A., Karadeniz, S., Mezzani, A., Prescott, E., Ryden, L., . . . Vrints, C. (2012). European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). *European Heart Journal, 33*, 1635-1701.

Gilman, S., Fischer, G., Biersner, R., Thornton, R., & Miller, D. (1979). Human parotid alpha-amylase secretion as a function of chronic hyperbaric exposure. *Undersea Biomedical Research, 6*, 303-307.

Girdler, S. S., Jamner, L. D., Jarvik, M., Soles, J. R., & Shapiro, D. (1997). Smoking status and nicotine administration differentially modify hemodynamic stress reactivity in men and women. *Psychosomatic Medicine, 59*, 294-306.

Girdler, S. S., Turner, J. R., Sherwood, A., & Light, K. C. (1990). Gender differences in blood pressure control during a variety of behavioral stressors. *Psychosomatic Medicine, 52*, 571-591.

Gleick, J. (2000). *Faster: The acceleration of just about everything*. New York: Pantheon Books.

Golden, J., Conroy, R. M., Bruce, I., Denihan, A., Greene, E., Kirby, M., & Lawlor, B. A. (2009). Loneliness, social support networks, mood and wellbeing in community-dwelling elderly. *International Journal of Geriatric Psychiatry, 24*, 694-700.

Goodwin, J., Bilous, M., Winship, S., Finn, P., & Jones, S. C. (2007). Validation of the Oscar 2 oscillometric 24-h ambulatory blood pressure monitor according to the British Hypertension Society protocol. *Blood Pressure Monitoring, 12*, 113-117.

Gordis, E. B., Granger, D. A., Susman, E. J., & Trickett, P. K. (2006). Asymmetry between salivary cortisol and α-amylase reactivity to stress: Relation to aggressive behavior in adolescents. *Psychoneuroendocrinology, 31*, 976-987.

Gottlieb, D. J., Redline, S., Nieto, F. J., Baldwin, C. M., Newman, A. B., Resnick, H. E., & Punjabi, N. M. (2006). Association of usual sleep duration with hypertension: The Sleep Heart Health Study. *Sleep, 29*, 1009-1014.

Gramer, M. (2006). Social anxiety and cardiovascular responses to active coping conditions. *Psychology Science, 48*, 39-52.

Grandner, M. A. (2012). Sleep duration across the lifespan: Implications for health. *Sleep Medicine Reviews, 16*, 199-201.

Grandner, M. A., & Drummond, S. (2007). Who are the long sleepers? Towards an understanding of the mortality relationship. *Sleep Medicine Reviews, 11,* 341-360.

Grandner, M. A., Hale, L., Moore, M., & Patel, N. P. (2010). Mortality associated with short sleep duration: The evidence, the possible mechanisms, and the future. *Sleep Medicine Reviews, 14,* 191-203.

Grandner, M. A., Patel, N. P., Gehrman, P. R., Perlis, M. L., & Pack, A. I. (2010). Problems associated with short sleep: Bridging the gap between laboratory and epidemiological studies. *Sleep Medicine Reviews, 14,* 239-247.

Granger, D. A., Kivlighan, K. T., Blair, C., El-Sheikh, M., Mize, J., Lisonbee, J. A., . . . Schwartz, E. B. (2006). Integrating the measurement of salivary α-amylase into studies of child health, development, and social relationships. *Journal of Social and Personal Relationships, 23,* 267-290.

Gregg, M. E., James, J. E., Matyas, T. A., & Thorsteinsson, E. B. (1999). Hemodynamic profile of stress-induced anticipation and recovery. *International Journal of Psychophysiology, 34,* 147-162.

Gregg, M. E., Matyas, T. A., & James, J. E. (2002). A new model of individual differences in hemodynamic profile and blood pressure reactivity. *Psychophysiology, 39,* 64-72.

Gribbin, C. E., Watamura, S. E., Cairns, A., Harsh, J. R., & LeBourgeois, M. K. (2011). The cortisol awakening response (CAR) in 2- to 4- year-old children: Effects of acute nighttime sleep restriction, wake time, and daytime napping. *Developmental Psychobiology, 54,* 412-422.

Gröschl, M. (2008). Current status of salivary hormone analysis. *Clinical Chemistry, 54,* 1759-1769.

Gruenewald, T. L., Kemeny, M. E., Aziz, N., & Fahey, J. L. (2004). Acute threat to the social self: shame, social self-esteem, and cortisol activity. *Psychosomatic Medicine, 66*, 915-924.

Guelen, I., Westerhof, B. E., van der Sar, G. L., van Montfrans, G. A., Kiemeneij, F., Wesseling, K. H., & Bos, W. J. W. (2003). Finometer, finger pressure measurements with the possibility to reconstruct brachial pressure. *Blood Pressure Monitoring, 8*, 27-30.

Guo, X., Zheng, L., Wang, J., Zhang, X., Zhang, X., Li, J., & Sun, Y. (2013). Epidemiological evidence for the link between sleep duration and high blood pressure: A systematic review and meta-analysis. *Sleep Medicine, 14*, 324-332.

Guyton, A. C., & Hall, J. E. (1997). *Human physiology and mechanisms of disease* (6th ed.). Philadelphia: Saunders

Haack, M., Sanchez, E., & Mullington, J. M. (2007). Elevated inflammatory markers in response to prolonged sleep restriction are associated with increased pain experience in healthy volunteers. *Sleep, 30*, 1145-1452.

Haack, M., Serrador, J., Cohen, D., Simpson, N., Meier-Ewert, H., & Mullington, J. M. (in press). Increasing sleep duration to lower beat-to-beat blood pressure: A pilot study. *Journal of Sleep Research*.

Hamrahian, A. H., Oseni, T. S., & Arafah, B. M. (2004). Measurements of serum free cortisol in critically ill patients. *New England Journal of Medicine, 350*, 1629-1638.

Hansson, G. K. (2005). Inflammation, atherosclerosis, and coronary artery disease. *New England Journal of Medicine, 352*, 1685-1695.

Heaney, J. L. J., Ginty, A. T., Carroll, D., & Phillips, A. C. (2011). Preliminary evidence that exercise dependence is associated with blunted cardiac and cortisol reactions to acute psychological stress. *International Journal of Psychophysiology, 79*, 323-329.

Heaney, J. L. J., Phillips, A. C., & Carroll, D. (2012). Aging, health behaviors, and the diurnal rhythm and awakening response of salivary cortisol. *Experimental Aging Research, 38,* 295-314.

Herity, N. A. (2000). Interleukin 6: A message from the heart. *Heart, 84*, 9-10.

Hofman, M. A. (2000). The human circadian clock and aging. *Chronobiology international, 17*, 245-259.

Holt-Lunstad, J., Jones, B. Q., & Birmingham, W. (2009). The influence of close relationships on nocturnal blood pressure dipping. *International Journal of Psychophysiology, 71,* 211-217.

Holt-Lunstad, J., Uchino, B. N., Smith, T. W., Olson-Cerny, C., & Nealey-Moore, J. B. (2003). Social relationships and ambulatory blood pressure: Structural and qualitative predictors of cardiovascular function during everyday social interactions. *Health Psychology, 22*, 388-397.

Hossain, J. L., & Shapiro, C. M. (2002). The prevalence, cost implications, and management of sleep disorders: An overview. *Sleep and Breathing, 6*, 85-102.

House, J. S. (2001). Social isolation kills, but how and why? *Psychosomatic Medicine, 63*, 273-274.

Howard, S., Hughes, B. M., & James, J. E. (2011). Type D personality and hemodynamic reactivity to laboratory stress in women. *International Journal of Psychophysiology, 80*, 96-102.

Huber, T. J., Issa, K., Schik, G., & Wolf, O. T. (2006). The cortisol awakening response is blunted in psychotherapy inpatients suffering from depression. *Psychoneuroendocrinology, 31*, 900-904.

Hughes, B. M. (2001). Memory and arithmetic as laboratory stressors for analyses of cardiovascular reactivity: A cursory assessment. *Studia Psychologica, 43,* 3-12.

Hurwitz Eller, N., Netterstrom, B., & Marie Hansen, A. (2001). Cortisol in urine and saliva: Relations to the intima media thickness, IMT. *Atherosclerosis, 159*, 175-185.

Imai, Y., Ohkubo, T., Tsuji, I., Satoh, H., & Hisamichi, S. (1999). Clinical significance of nocturnal blood pressure monitoring. *Clinical and Experimental Hypertension, 21*, 717-727.

Irwin, M., Thompson, J., Miller, C., Gillin, J. C., & Ziegler, M. (1999). Effects of sleep and sleep deprivation on catecholamine and interleukin-2 levels in humans: Clinical implications. *Journal of Clinical Endocrinology & Metabolism, 84*, 1979-1985.

Irwin, M. R., Wang, M., Campomayor, C. O., Collado-Hidalgo, A., & Cole, S. (2006). Sleep deprivation and activation of morning levels of cellular and genomic markers of inflammation. *Archives of Internal Medicine, 166*, 1756-1762.

Ivanenko, A., McLaughlin Crabtree, V., & Gozal, D. (2005). Sleep and depression in children and adolescents. *Sleep Medicine Reviews, 9*, 115-129.

James, J. E. (2004). Critical review of dietary caffeine and blood pressure: A relationship that should be taken more seriously. *Psychosomatic Medicine, 66*, 63-71.

James, J. E., Gregg, M., Matyas, T. A., Hughes, B. M., & Howard, S. (2012). Stress reactivity and the Hemodynamic Profile-Compensation Deficit (HP-CD) Model of blood pressure regulation. *Biological Psychology, 90*, 161-170.

James, J. E., & Gregg, M. E. (2004a). Effects of dietary caffeine on mood when rested and sleep restricted. *Human Psychopharmacology: Clinical and Experimental, 19*, 333-341.

James, J. E., & Gregg, M. E. (2004b). Hemodynamic effects of dietary caffeine, sleep restriction, and laboratory stress. *Psychophysiology, 41*, 914-923.

James, J. E., Gregg, M. E., Kane, M., & Harte, F. (2005). Dietary caffeine, performance, and mood: Enhancing and restorative effects after controlling for withdrawal reversal. *Neuropsychobiology, 52*, 1-10.

Jennings, J. R., Kamarck, T., Stewart, C., Eddy, M., & Johnson, P. (1992). Alternate cardiovascular baseline assessment techniques: Vanilla or resting baseline. *Psychophysiology, 29*, 742-750.

Julius, S. (1988). The blood pressure seeking properties of the central nervous system. *Journal of Hypertension, 6*, 177-185.

Jung, C. M., Khalsa, S. B. S., Scheer, F. A., Cajochen, C., Lockley, S. W., Czeisler, C. A., & Wright, K. P. (2010). Acute effects of bright light exposure on cortisol levels. *Journal of Biological Rhythms, 25*, 208-216.

Juster, R.-P., McEwen, B. S., & Lupien, S. J. (2010). Allostatic load biomarkers of chronic stress and impact on health and cognition. *Neuroscience and Biobehavioral Reviews, 35*, 2-16.

Kahn-Greene, E. T., Lipizzi, E. L., Conrad, A. K., Kamimori, G. H., & Killgore, W. D. S. (2006). Sleep deprivation adversely affects interpersonal responses to frustration. *Personality and Individual Differences, 41*, 1433-1443.

Kamarck, T. W., Debski, T. T., & Manuck, S. B. (2000). Enhancing the laboratory-to-life generalizability of cardiovascular reactivity using multiple occasions of measurement. *Psychophysiology, 37*, 533-542.

Kamarck, T. W., & Lovallo, W. R. (2003). Cardiovascular reactivity to psychological challenge: Conceptual and measurement considerations. *Psychosomatic Medicine, 65*, 9-21.

Kamarck, T. W., Schwartz, J. E., Janicki, D. L., Shiffman, S., & Raynor, D. A. (2003). Correspondence between laboratory and ambulatory measures of cardiovascular reactivity: A multilevel modeling approach. *Psychophysiology, 40*, 675-683.

Kamarck, T. W., Schwartz, J. E., Shiffman, S., Muldoon, M. F., Sutton-Tyrrell, K., & Janicki, D. L. (2005). Psychosocial stress and cardiovascular risk: What is the role of daily experience? *Journal of Personality, 73*, 1749-1774.

Kamarck, T. W., Shiffman, S. M., Smithline, L., Goodie, J. L., Paty, J. A., Gnys, M., & Jong, J. Y. K. (1998). Effects of task strain, social conflict, and emotional activation on ambulatory cardiovascular activity: Daily life consequences of recurring stress in a multiethnic adult sample. *Health Psychology, 17*, 17-29.

Kamdar, B. B., Kaplan, K. A., Kezirian, E. J., & Dement, W. C. (2004). The impact of extended sleep on daytime alertness, vigilance, and mood. *Sleep Medicine, 5*, 441.

Kamphuis, J., Meerlo, P., Koolhaas, J. M., & Lancel, M. (2012). Poor sleep as a potential causal factor in aggression and violence. *Sleep Medicine, 13*, 327–333.

Kao, T. W., Lu, I. S., Liao, K. C., Lai, H. Y., Loh, C. H., & Kuo, H. K. (2009). Associations between body mass index and serum levels of C-reactive protein. *South African Medical Journal, 99*, 326-330.

Kario, K., Pickering, T. G., Matsuo, T., Hoshide, S., Schwartz, J. E., & Shimada, K. (2001). Stroke prognosis and abnormal nocturnal blood pressure falls in older hypertensives. *Hypertension, 38*, 852-857.

Kario, K., Schwartz, J. E., & Pickering, T. G. (2000). Changes of nocturnal blood pressure dipping status in hypertensives by nighttime dosing of α-adrenergic blocker, doxazosin: Results from the HALT Study. *Hypertension, 35*, 787-794.

Kario, K., & Shimada, K. (2004). Risers and extreme-dippers of nocturnal blood pressure in hypertension: Antihypertensive strategy for nocturnal blood pressure. *Clinical and Experimental Hypertension, 26,* 177-189.

Kasprowicz, A. L., Manuck, S. B., Malkoff, S. B., & Krantz, D. S. (1990). Individual differences in behaviorally evoked cardiovascular response: Temporal stability and hemodynamic patterning. *Psychophysiology, 27,* 605-619.

Kato, M., Phillips, B. G., Sigurdsson, G., Narkiewicz, K., Pesek, C. A., & Somers, V. K. (2000). Effects of sleep deprivation on neural circulatory control. *Hypertension, 35,* 1173-1175.

Kawabe, H., & Saito, I. (2008). Does short sleep duration in daily life affect morning home blood pressure? Evaluation in Japanese people. *Clinical and Experimental Hypertension, 30,* 183-190.

Kelsey, R. M., Ornduff, S. R., & Alpert, B. S. (2007). Reliability of cardiovascular reactivity to stress: Internal consistency. *Psychophysiology, 44,* 216-225.

Kenny, N. D., & Ríain, N. (2009). Cardiovascular disease in women. *Irish College of General Practitioners (ICGP).* Retrieved from http://hdl.handle.net/10147/135421

Kershaw, E. E., & Flier, J. S. (2004). Adipose tissue as an endocrine organ. *Journal of Clinical Endocrinology & Metabolism, 89,* 2548-2556.

King, C. R., Knutson, K. L., Rathouz, P. J., Sidney, S., Liu, K., & Lauderdale, D. S. (2008). Short sleep duration and incident coronary artery calcification. *The Journal of the American Medical Association, 300,* 2859-2866.

Kirschbaum, C., Pirke, K., & Hellhammer, D. (1993). The'Trier Social Stress Test'-A tool for investigating psychobiological stress responses in a laboratory setting. *Neuropsychobiology, 28,* 76-81.

Klein, L. C., & Corwin, E. J. (2002). Seeing the unexpected: How sex differences in stress responses may provide a new perspective on the manifestation of psychiatric disorders. *Current Psychiatry Reports, 4*, 441-448.

Knutson, K. L. (2010). Sleep duration and cardiometabolic risk: A review of the epidemiologic evidence. *Best Practice & Research Clinical Endocrinology & Metabolism, 24*, 731-743.

Knutson, K. L. (2013). Sociodemographic and cultural determinants of sleep deficiency: Implications for cardiometabolic disease risk. *Social Science & Medicine, 79*, 7-15.

Knutson, K. L., Van Cauter, E., Rathouz, P. J., DeLeire, T., & Lauderdale, D. S. (2010). Trends in the prevalence of short sleepers in the USA: 1975–2006. *Sleep, 33*, 37-45.

Knutson, K. L., Van Cauter, E., Rathouz, P. J., Yan, L. L., Hulley, S. B., Liu, K., & Lauderdale, D. S. (2009). Association between sleep and blood pressure in midlife: The CARDIA sleep study. *Archives of Internal Medicine, 169*, 1055-1061.

Koenig, W., Löwel, H., Baumert, J., & Meisinger, C. (2004). C-reactive protein modulates risk prediction based on the Framingham score. *Circulation, 109*, 1349-1353.

Kopanczyk, R., Opris, D. C., Lickliter, J., Bridges, E. D., Nazar, A. M., & Bridges, K. G. (2010). C-reactive protein levels in blood and saliva show no correlation in young, healthy adults. *The Federation of American Societies for Experimental Biology, 24 (Meeting Abstract Suppl.)*, lb409.

Kotani, K., Saiga, K., Sakane, N., Mu, H., & Kurozawa, Y. (2008). Sleep status and blood pressure in a healthy normotensive female population. *International Journal of Cardiology, 125*, 425-427.

Kripke, D., Garfinkel, L., Wingard, D., Klauber, M., & Marler, M. (2002). Mortality associated with sleep duration and insomnia. *Archives of General Psychiatry, 59*, 131-136.

Kubo, T., Takahashi, M., Sato, T., Sasaki, T., Oka, T., & Iwasaki, K. (2011). Weekend sleep intervention for workers with habitually short sleep periods. *Scandinavian Journal of Work Environment andHealth, 37,* 418-426.

Kucukler, N., Yalçin, F., Abraham, T., & Garcia, M. (2011). Stress induced hypertensive response: Should it be evaluated more carefully? *Cardiovascular Ultrasound, 9,* 1-8.

Kudielka, B. M., Broderick, J. E., & Kirschbaum, C. (2003). Compliance with saliva sampling protocols: Electronic monitoring reveals invalid cortisol daytime profiles in noncompliant subjects. *Psychosomatic Medicine, 65,* 313-319.

Kudielka, B. M., & Kirschbaum, C. (2003). Awakening cortisol responses are influenced by health status and awakening time but not by menstrual cycle phase. *Psychoneuroendocrinology, 28,* 35-48.

Kudielka, B. M., & Kirschbaum, C. (2005). Sex differences in HPA axis responses to stress: A review. *Biological Psychology, 69,* 113-132.

Kunz-Ebrecht, S. R., Kirschbaum, C., Marmot, M., & Steptoe, A. (2004). Differences in cortisol awakening response on work days and weekends in women and men from the Whitehall II cohort. *Psychoneuroendocrinology, 29,* 516-528.

Kvetnansky, R., Sabban, E. L., & Palkovits, M. (2009). Catecholaminergic systems in stress: Structural and molecular genetic approaches. *Physiological Reviews, 89,* 535-606.

Lauderdale, D. S., Knutson, K. L., Yan, L. L., Liu, K., & Rathouz, P. J. (2008). Self-reported and measured sleep duration: How similar are they? *Epidemiology, 19,* 838-845.

Lawler, K. A., Kline, K. A., Adlin, R. F., Wilcox, Z. C., Craig, F. W., Krishnamoorthy, J. S., & Piferi, R. L. (2001). Psychophysiological correlates of individual differences in patterns of hemodynamic reactivity. *International Journal of Psychophysiology, 40,* 93-107.

Lazarus, R. S., & Folkman, S. (1984). *Stress, appraisal, and coping*. New York: Springer Publishing Company.

Leary, A. C., Donnan, P. T., MacDonald, T. M., & Murphy, M. B. (2000). Physical activity level is an independent predictor of the diurnal variation in blood pressure. *Journal of Hypertension, 18*, 405-410.

Lechner, O., Hu, Y., Jafarian-Tehrani, M., Dietrich, H., Schwarz, S., Herold, M., . . . Wick, G. (1996). Disturbed immunoendocrine communication via the hypothalamo–pituitary–adrenal axis in murine lupus. *Brain, Behavior, and Immunity, 10*, 337-350.

Leger, D. (1994). The cost of sleep-related accidents: A report for the National Commission on Sleep Disorders Research. *Sleep, 17*, 84-93.

Lehman, B. J., & Conley, K. M. (2010). Momentary reports of social-evaluative threat predict ambulatory blood pressure. *Social Psychological and Personality Science, 1*, 51-56.

Leproult, R., Colecchia, E. F., L'Hermite-Balériaux, M., & Van Cauter, E. (2001). Transition from dim to bright light in the morning induces an immediate elevation of cortisol levels. *Journal of Clinical Endocrinology & Metabolism, 86*, 151-157.

Leproult, R., Copinschi, G., Buxton, O., & VanCauter, E. (1997). Sleep loss results in an elevation of cortisol levels the next evening. *Sleep, 20*, 865-870.

Libby, P. (2002). Inflammation in atherosclerosis. *Nature, 420*, 868-874.

Linden, W., & Con, A. (1994). Laboratory reactivity models as predictors of ambulatory blood pressure and heart rate. *Journal of psychosomatic research, 38*, 217-228.

Linden, W., Rutledge, T., & Con, A. (1998). A case for the usefulness of laboratory social stressors. *Annals of Behavioral Medicine, 20*, 310-316.

Lorendo, J., Nelesen, R., Ancoli-lsrael, S., & Dimsdale, J. (2004). Sleep quality and blood pressure dipping in normal adults. *Sleep, 27*, 1097-1103.

Lovallo, W. R. (2005). Cardiovascular reactivity: Mechanisms and pathways to cardiovascular disease. *International Journal of Psychophysiology, 58,* 119-132.

Lovallo, W. R., Dickensheets, S. L., Myers, D. A., Thomas, T. L., & Nixon, S. J. (2000). Blunted stress cortisol response in abstinent alcoholic and polysubstance-abusing men. *Alcoholism: Clinical and Experimental Research, 24,* 651-658.

Lovallo, W. R., & Gerin, W. (2003). Psychophysiological reactivity: Mechanisms and pathways to cardiovascular disease. *Psychosomatic Medicine, 65,* 36-45.

Luecken, L., Kraft, A., Appelhans, B., & Enders, C. (2009). Emotional and cardiovascular sensitization to daily stress following childhood parental loss. *Developmental Psychology, 45,* 296-302.

Lund-Johansen, P. (1991). Twenty-year follow-up of hemodynamics in essential hypertension during rest and exercise. *Hypertension, 18,* III54.

Lusardi, P., Mugellini, A., Preti, P., Zoppi, A., Derosa, G., & Fogari, R. (1996). Effects of a restricted sleep regimen on ambulatory blood pressure monitoring in normotensive subjects. *American Journal of Hypertension, 9,* 503-505.

Lusardi, P., Zoppi, A., Preti, P., Pesce, R. M., Piazza, E., & Fogari, R. (1999). Effects of insufficient sleep on blood pressure in hypertensive patients: A 24-h study. *American Journal of Hypertension, 12,* 63-68.

MacCallum, R. C., Zhang, S., Preacher, K. J., & Rucker, D. D. (2002). On the practice of dichotomization of quantitative variables. *Psychological Methods, 7,* 19-40.

Manuck, S. B. (1994). Cardiovascular reactivity in cardiovascular disease: "Once more unto the breach". *International Journal of Behavioral Medicine, 1,* 4-31.

Marler, M. R., Jacob, R. G., Lehoczky, J. P., & Shapiro, A. P. (2006). The statistical analysis of treatment effects in 24-hour ambulatory blood pressure recordings. *Statistics in Medicine, 7,* 697-716.

Martin, P. R. (2003). *Counting sheep: The science and pleasures of sleep and dreams*. Great Britain: Harper Perennial.

Maruyama, Y., Kawano, A., Okamoto, S., Ando, T., Ishitobi, Y., Tanaka, Y., . . . Higuma, H. (2012). Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the Trier Social Stress Test. *PLoS One, 7,* e39375.

Matthews, K. A., Kamarck, T. W., H. Hall, M., Strollo, P. J., Owens, J. F., Buysse, D. J., . . . Reis, S. E. (2008). Blood pressure dipping and sleep disturbance in African-American and Caucasian men and women. *American Journal of Hypertension, 21,* 826-831.

Matthews, K. A., Katholi, C. R., McCreath, H., Whooley, M. A., Williams, D. R., Zhu, S., & Markovitz, J. H. (2004). Blood pressure reactivity to psychological stress predicts hypertension in the CARDIA study. *Circulation, 110,* 74-78.

Matthews, K. A., Woodall, K. L., & Allen, M. T. (1993). Cardiovascular reactivity to stress predicts future blood pressure status. *Hypertension, 22,* 479-485.

Mayet, J., & Hughes, A. (2003). Cardiac and vascular pathophysiology in hypertension. *Heart, 89,* 1104–1109.

McEwen, B. S. (2000). The neurobiology of stress: From serendipity to clinical relevance. *Brain Research, 886,* 172-189.

McEwen, B. S. (2006). Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism, 55,* S20-S23.

McEwen, B. S., Biron, C. A., Brunson, K. W., Bulloch, K., Chambers, W. H., Dhabhar, F. S., . . . Weiss, J. M. (1997). The role of adrenocorticoids as modulators of immune function in health and disease: Neural, endocrine, and immune interactions. *Brain Research Reviews, 23,* 79-133.

McEwen, B. S., & Seeman, T. (1999). Protective and damaging effects of mediators of stress: Elaborating and testing the concepts of allostasis and allostatic load. *Annals of the New York Academy of Sciences, 896*, 30-47.

McEwen, B. S., & Stellar, E. (1993). Stress and the individual: Mechanisms leading to disease. *Archives of Internal Medicine, 153*, 2093-2091.

Meeran, K., Hattersley, A., Mould, G., & Bloom, S. (1993). Venepuncture causes rapid rise in plasma ACTH. *The British Journal of Clinical Practice, 47*, 246-247.

Meerlo, P., Sgoifo, A., & Suchecki, D. (2008). Restricted and disrupted sleep: Effects on autonomic function, neuroendocrine stress systems, and stress responsivity. *Sleep Medicine Reviews, 12*, 197-210.

Megson, E., Fitzsimmons, T., Dharmapatni, K., & Mark Bartold, P. (2010). C-reactive protein in gingival crevicular fluid may be indicative of systemic inflammation. *Journal of Clinical Periodontology, 37*, 797-804.

Meier-Ewert, H. K., Ridker, P. M., Rifai, N., Price, N., Dinges, D. F., & Mullington, J. M. (2001). Absence of diurnal variation of C-reactive protein concentrations in healthy human subjects. *Clinical Chemistry, 47*, 426-430.

Meier-Ewert, H. K., Ridker, P. M., Rifai, N., Regan, M. M., Price, N. J., Dinges, D. F., & Mullington, J. M. (2004). Effect of sleep loss on C-Reactive protein, an inflammatory marker of cardiovascular risk. *Journal of the American College of Cardiology, 43*, 678-683.

Meney, I., Waterhouse, J., Atkinson, G., Reilly, T., & Davenne, D. (1998). The effect of one night's sleep deprivation on temperature, mood, and physical performance in subjects with different amounts of habitual physical activity. *Chronobiology International, 15*, 349-363.

Miller, G. E., Cohen, S., & Ritchey, A. K. (2002). Chronic psychological stress and the regulation of pro-inflammatory cytokines: A glucocorticoid-resistance model. *Health Psychology, 21,* 531-541.

Minkel, J. D., Banks, S., Htaik, O., Moreta, M. C., Jones, C. W., McGlinchey, E. L., . . . Dinges, D. F. (2012). Sleep deprivation and stressors: Evidence for elevated negative affect in response to mild stressors when sleep deprived. *Emotion, 12,* 1015-1020.

Miró, E., Cano-Lozano, M., & Buela-Casal, G. (2002). Electrodermal activity during total sleep deprivation and its relationship with other activation and performance measures. *Journal of Sleep Research, 11,* 105-112.

Miura, K., Dyer, A. R., Greenland, P., Daviglus, M. L., Hill, M., Liu, K., . . . Stamler, J. (2001). Pulse pressure compared with other blood pressure indexes in the prediction of 25-year cardiovascular and all-cause mortality rates: The Chicago Heart Association Detection Project in Industry study. *Hypertension, 38*, 232-237.

Mottram, P. M., Haluska, B., Yuda, S., Leano, R., & Marwick, T. H. (2004). Patients with a hypertensive response to exercise have impaired systolic function without diastolic dysfunction or left ventricular hypertrophy. *Journal of the American College of Cardiology, 43,* 848-853.

Muenter, N., Watenpaugh, D., Wasmund, W., Wasmund, S., Maxwell, S., & Smith, M. (2000). Effect of sleep restriction on orthostatic cardiovascular control in humans. *Journal of Applied Physiology, 88*, 966-972.

Müller, H. U., Riemann, D., Berger, M., & Müller, W. (1993). The influence of total sleep deprivation on urinary excretion of catecholamine metabolites in major depression. *Acta Psychiatrica Scandinavica, 88,* 16-20.

Mullington, J. M., Haack, M., Toth, M., Serrador, J. M., & Meier-Ewert, H. K. (2009). Cardiovascular, inflammatory, and metabolic consequences of sleep deprivation. *Progress in Cardiovascular Diseases, 51*, 294-302.

Munakata, M., Saito, Y., Nunokawa, T., Ito, N., Fukudo, S., & Yoshinaga, K. (2002). Clinical significance of blood pressure response triggered by a doctor's visit in patients with essential hypertension. *Hypertension Research, 25*, 343-349.

Murphy, J. K., Alpert, B. S., Walker, S. S., & Willey, E. S. (2007). Children's cardiovascular reactivity: Stability of racial differences and relation to subsequent blood pressure over a one-year period. *Psychophysiology, 28*, 447-457.

Nater, U., & Rohleder, N. (2009a). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology, 34*, 486-496.

Nater, U. M., Ditzen, B., & Ehlert, U. (2012). *Associations between salivary alpha-amylase and catecholamines - a multilevel modelling approach*. Paper presented at the 70th annual meeting of the American Psychosomatic Society, Athens, Greece.

Nater, U. M., La Marca, R., Florin, L., Moses, A., Langhans, W., Koller, M. M., & Ehlert, U. (2006). Stress-induced changes in human salivary alpha-amylase activity-associations with adrenergic activity. *Psychoneuroendocrinology, 31*, 49-58.

Nater, U. M., & Rohleder, N. (2009b). Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology, 34*, 486-496.

Nater, U. M., Rohleder, N., Gaab, J., Berger, S., Jud, A., Kirschbaum, C., & Ehlert, U. (2005). Human salivary alpha-amylase reactivity in a psychosocial stress paradigm. *International Journal of Psychophysiology, 55*, 333-342.

Nater, U. M., Rohleder, N., Schlotz, W., Ehlert, U., & Kirschbaum, C. (2007). Determinants of the diurnal course of salivary alpha-amylase. *Psychoneuroendocrinology, 32,* 392-401.

National Sleep Foundation. (2011). The 2011 Sleep in America® poll Retrieved from http://www.sleepfoundation.org/sites/default/files/sleepinamericapoll/SIAP_2011_Summary_of_Findings.pdf

Nederfors, T., & Dahlöf, C. (1992). Effects of the β-adrenoceptor antagonists atenolol and propranolol on human whole saliva flow rate and composition. *Archives of Oral Biology, 37,* 579-584.

Nguyen, V., & McLaughlin, M. A. (2002). Coronary artery disease in women: A review of emerging cardiovascular risk factors. *The Mount Sinai Journal of Medicine, New York, 69,* 338-349.

Nijm, J., Kristenson, M., Olsson, A. G., & Jonasson, L. (2007). Impaired cortisol response to acute stressors in patients with coronary disease. Implications for inflammatory activity. *Journal of Internal Medicine, 262,* 375-384.

O'Brien, E., Coats, A., Owens, P., Petrie, J., Padfield, P., Littler, W., . . . Mee, F. (2000). Use and interpretation of ambulatory blood pressure monitoring: Recommendations of the British Hypertension Society. *BMJ, 320,* 1128-1134.

O'Brien, E., Sheridan, J., & O'Malley, K. (1988). Dippers and non-dippers. *The Lancet, 332,* 397.

O'Connell, C. (2012, April 5th). A lack of sleep can make you ill, *The Irish Times*.

O'Connor, T., O'Halloran, D., & Shanahan, F. (2000). The stress response and the hypothalamic-pituitary-adrenal axis: From molecule to melancholia. *QJM, 93,* 323-333.

O'Leary, É. D., Howard, S., Hughes, B. M., & James, J. E. (2013). An experimental test of blunting using sleep-restriction as an acute stressor in Type D and non-Type D women. *International Journal of Psychophysiology, 90*, 37-43.

O'Brien, E. (2010). Ambulatory blood pressure monitoring: 24-hour blood pressure control as a therapeutic goal for improving cardiovascular prognosis. *Medicographia, 32*, 241-249.

Obrist, P. A. (1981). *Cardiovascular psychophysiology: A perspective*. New York: Plenum Press.

Obrist, P. A. (1982). Beta-adrenergic hyperresponsivity to behavioral challenges: A possible hypertensive risk factor. In J. F. Orlebke, G. Mulder & L. J. P. van Dooren (Eds.), *Psychophysiology of cardiovascular control: Methods, models, and data.* (Vol. 26). New York: Plenum Press.

Ohayon, M., Priest, R., Zulley, J., Smirne, S., & Paiva, T. (2002). Prevalence of narcolepsy symptomatology and diagnosis in the European general population. *Neurology, 58*, 1826-1833.

Ohkubo, T., Hozawa, A., Yamaguchi, J., Kikuya, M., Ohmori, K., Michimata, M., . . . Araki, T. (2002). Prognostic significance of the nocturnal decline in blood pressure in individuals with and without high 24-h blood pressure: The Ohasama study. *Journal of Hypertension, 20*, 2183-2189.

Okun, M. L., Krafty, R. T., Buysse, D. J., Monk, T. H., Reynolds, C. F., Begley, A., & Hall, M. (2010). What constitutes too long of a delay? Determining the cortisol awakening response (CAR) using self-report and PSG-assessed wake time. *Psychoneuroendocrinology, 35*, 460-468.

Omisade, A., Buxton, O. M., & Rusak, B. (2010). Impact of acute sleep restriction on cortisol and leptin levels in young women. *Physiology & Behavior, 99*, 651-656.

Ottaviani, C., Shapiro, D., Goldstein, I. B., James, J. E., & Weiss, R. (2006). Hemodynamic profile, compensation deficit, and ambulatory blood pressure. *Psychophysiology, 43,* 46-56.

Ouchi, N., Kihara, S., Funahashi, T., Nakamura, T., Nishida, M., Kumada, M., . . . Kishida, K. (2003). Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation, 107,* 671-674.

Ouellet-Morin, I., Danese, A., Williams, B., & Arseneault, L. (2011). Validation of a high-sensitivity assay for C-reactive protein in human saliva. *Brain, Behavior, and Immunity, 25,* 640-646.

Out, D. e., Hall, R. J., Granger, D. A., Page, G. G., & Woods, S. J. (2012). Assessing salivary C-reactive protein: Longitudinal associations with systemic inflammation and cardiovascular disease risk in women exposed to intimate partner violence. *Brain, Behavior, and Immunity, 26,* 543-551.

Owens, J. A. (2004). Sleep in children: Cross-cultural perspectives. *Sleep and Biological Rhythms, 2,* 165-173.

Pai, J. K., Pischon, T., Ma, J., Manson, J. E., Hankinson, S. E., Joshipura, K., . . . Stampfer, M. J. (2004). Inflammatory markers and the risk of coronary heart disease in men and women. *New England Journal of Medicine, 351,* 2599-2610.

Pearson, T., Mensah, G., Alexander, R., Anderson, J., Cannon 3rd, R., Criqui, M., . . . Myers, G. (2003). Markers of inflammation and cardiovascular disease: Application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation, 107,* 499-511.

Penaz, J. (1973). *Photoelectric measurement of blood pressure, volume and flow in the finger.* Paper presented at the Digest of the 10th International Conference on Medical and Biological Engineering, Dresden.

Pfaffe, T., Cooper-White, J., Beyerlein, P., Kostner, K., & Punyadeera, C. (2011). Diagnostic potential of saliva: Current state and future applications. *Clinical Chemistry, 57,* 675-687.

Phillips, A. C., Der, G., & Carroll, D. (2009). Self-reported health and cardiovascular reactions to psychological stress in a large community sample: Cross-sectional and prospective associations. *Psychophysiology, 46,* 1020-1027.

Phillips, A. C., Ginty, A. T., & Hughes, B. M. (2013). The other side of the coin: Blunted cardiovascular and cortisol reactivity is also associated with negative health outcomes. *International Journal of Psychophysiology, 90,* 1-7.

Phillips, A. C., & Hughes, B. M. (2011). Introductory paper: Cardiovascular reactivity at a crossroads: Where are we now? *Biological Psychology, 86,* 95-97.

Piazza, J. R., Almeida, D. M., Dmitrieva, N. O., & Klein, L. C. (2010). Frontiers in the use of biomarkers of health in research on stress and aging. *The Journals of Gerontology Series B: Psychological Sciences and Social Sciences, 65,* 513-525.

Pickering, T. (1996). Recommendations for the use of home (self) and ambulatory blood pressure monitoring. *American Journal of Hypertension, 9,* 1-11.

Pickering, T. G., Hall, J. E., Appel, L. J., Falkner, B. E., Graves, J., Hill, M. N., . . . Roccella, E. J. (2005). Recommendations for blood pressure measurement in humans and experimental animals. *Circulation, 111,* 697-716.

Poirier, P., & Eckel, R. H. (2002). Obesity and cardiovascular disease. *Current Atherosclerosis Reports, 4,* 448-453.

Poole, C. D., Conway, P., & Currie, C. J. (2007). An evaluation of the association between systemic inflammation – as measured by C-reactive protein – and hospital resource use. *Current Medical Research and Opinion, 23,* 2785-2792.

Pottier, P., Hardouin, J. B., Dejoie, T., Bonnaud, A., Le Loupp, A. G., Planchon, B., & LeBlanc, V. (2011). Stress responses in medical students in ambulatory and in-hospital patient consultations. *Medical Education, 45,* 678-687.

Pruessner, J. C., Kirschbaum, C., Meinlschmid, G., & Hellhammer, D. H. (2003). Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology, 28,* 916-931.

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., Buske-Kirschbaum, A., von Auer, K., Jobst, S., . . . Kirschbaum, C. (1997). Free cortisol levels after awakening: A reliable biological marker for the assessment of adrenocortical activity. *Life Sciences, 61,* 2539-2549.

Pruessner, M., Hellhammer, D. H., Pruessner, J. C., & Lupien, S. J. (2003). Self-reported depressive symptoms and stress levels in healthy young men: Associations with the cortisol response to awakening. *Psychosomatic Medicine, 65,* 92-99.

Raber, J. (1998). Detrimental effects of chronic hypothalamic—pituitary—adrenal axis activation. *Molecular Neurobiology, 18,* 1-22.

Rafey, M. (2009). Beyond office sphygmomanometry: Ways to better assess blood pressure. *Cleveland Clinic Journal of Medicine, 76,* 657-662.

Räikkönen, K., Matthews, K. A., Pesonen, A.-K., Pyhälä, R., Paavonen, E. J., Feldt, K., . . . Kajantie, E. (2010). Poor sleep and altered hypothalamic-pituitary-adrenocortical and sympatho-adrenal-medullary system activity in children. *Journal of Clinical Endocrinology & Metabolism, 95,* 2254-2261.

Rajaratnam, S. M. W., & Arendt, J. (2001). Health in a 24-h society. *The Lancet, 358*, 999-1005.

Reynolds, C. F., Kupfer, D. J., Hoch, C. C., & Stack, J. A. (1986). Sleep deprivation in healthy elderly men and women: Effects on mood and on sleep during recovery. *Sleep, 9*, 492-501.

Ridker, P. M. (2001). High-sensitivity C-reactive protein: potential adjunct for global risk assessment in the primary prevention of cardiovascular disease. *Circulation, 103*, 1813-1818.

Ridker, P. M., Cushman, M., Stampfer, M. J., Tracy, R. P., & Hennekens, C. H. (1997). Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. *New England Journal of Medicine, 336*, 973-979.

Ridker, P. M., Hennekens, C. H., Buring, J. E., & Rifai, N. (2000). C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. *New England Journal of Medicine, 342*, 836-843.

Rifai, N., & Ridker, P. M. (2001). High-sensitivity C-reactive protein: A novel and promising marker of coronary heart disease. *Clinical Chemistry, 47*, 403-411.

Rifai, N., Tracy, R. P., & Ridker, P. M. (1999). Clinical efficacy of an automated high-sensitivity C-reactive protein assay. *Clinical Chemistry, 45*, 2136-2141.

Roberts, A. D. L., Wessely, S., Chalder, T., Papadopoulos, A., & Cleare, A. J. (2004). Salivary cortisol response to awakening in chronic fatigue syndrome. *The British Journal of Psychiatry, 184*, 136-141.

Rodenbeck, A., Huether, G., Rüther, E., & Hajak, G. (2002). Interactions between evening and nocturnal cortisol secretion and sleep parameters in patients with severe chronic primary insomnia. *Neuroscience Letters, 324*(2), 159-163.

Rohleder, N., & Nater, U. M. (2009). Determinants of salivary alpha-amylase in humans and methodological considerations. *Psychoneuroendocrinology, 34*, 469-485.

Rohleder, N., Nater, U. M., Wolf, J. M., Ehlert, U., & Kirschbaum, C. (2004). Psychosocial stress-induced activation of salivary alpha-amylase: An indicator of sympathetic activity? *Annals of the New York Academy of Sciences, 1032*(1), 258-263.

Rohleder, N., Wolf, J. M., Maldonado, E. F., & Kirschbaum, C. (2006). The psychosocial stress-induced increase in salivary alpha-amylase is independent of saliva flow rate. *Psychophysiology, 43*, 645-652.

Rosansky, S. J., Menachery, S. J., Wagner, C. M., & Jackson, K. (1995). The effect of sleep intervals on analysis of 24-h ambulatory blood pressure data. *American Journal of Hypertension, 8*, 672-675.

Royston, P., Altman, D. G., & Sauerbrei, W. (2006). Dichotomizing continuous predictors in multiple regression: a bad idea. *Statistics in medicine, 25*, 127-141.

Sadeh, A., Hauri, P. J., Kripke, D. F., & Lavie, P. (1995). The role of actigraphy in the evaluation of sleep disorders. *Sleep, 18*, 288-302.

Salimetrics. (12/09/2010). Salivary C-reactive Protein ELISA Kit, from http://www.salimetrics.com/documents/CRP_Kit_Insert.pdf

Sapolsky, R. M. (2000). Glucocorticoids and hippocampal atrophy in neuropsychiatric disorders. *Archives of General Psychiatry, 57*, 925-935.

Schlotz, W., Hellhammer, J., Schulz, P., & Stone, A. A. (2004). Perceived work overload and chronic worrying predict weekend/weekday differences in the cortisol awakening response. *Psychosomatic Medicine, 66*, 207-214.

Schmidt-Reinwald, A., Pruessner, J. C., Hellhammer, D. H., Federenko, I., Rohleder, N., Schürmeyer, T. H., & Kirschbaum, C. (1999). The cortisol response to awakening in

relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sciences, 64*, 1653-1660.

Schumacher, S., Kirschbaum, C., Fydrich, T., & Ströhle, A. (2013). Is salivary alpha-amylase an indicator of autonomic nervous system dysregulations in mental disorders?—A review of preliminary findings and the interactions with cortisol. *Psychoneuroendocrinology, 38*, 729-743.

Schutte, A. E., Huisman, H. W., van Rooyen, J. M., Oosthuizen, W., & Jerling, J. C. (2003). Sensitivity of the Finometer device in detecting acute and medium-term changes in cardiovascular function. *Blood Pressure Monitoring, 8*, 195-201.

Selye, H. (1956). *The stress of life*. New York: McGraw-Hill.

Seugnet, L., Boero, J., Gottschalk, L., Duntley, S. P., & Shaw, P. J. (2006). Identification of a biomarker for sleep drive in flies and humans. *Proceedings of the National Academy of Sciences of the United States of America, 103*, 19913-19918.

Shahid, A., Shen, J., & Shapiro, C. M. (2010). Measurements of sleepiness and fatigue. *Journal of Psychosomatic Research, 69*, 81-89.

Sherwood, A., Dolan, C. A., & Light, K. C. (1990). Hemodynamics of blood pressure responses during active and passive coping. *Psychophysiology, 27*, 656-668.

Sigurdson, K. S. K., & Ayas, N. T. (2007). The public health and safety consequences of sleep disorders. *Canadian Journal of Physiology and Pharmacology, 85*, 179-183.

Singer, B., & Ryff, C. D. (2001). *New horizons in health: An integrative approach*. Washington DC: National Academies Press.

Smets, E., Garssen, B., Bonke, B., & De Haes, J. (1995). The Multidimensional Fatigue Inventory (MFI) psychometric qualities of an instrument to assess fatigue. *Journal of Psychosomatic Research, 39*, 315-325.

Smith, A., & Maben, A. (1993). Effects of sleep deprivation, lunch, and personality on performance, mood, and cardiovascular function. *Physiology & Behavior, 54*, 967-972.

Späth-Schwalbe, E., Schöller, T., Kern, W., Fehm, H., & Born, J. (1992). Nocturnal adrenocorticotropin and cortisol secretion depends on sleep duration and decreases in association with spontaneous awakening in the morning. *Journal of Clinical Endocrinology & Metabolism, 75*, 1431-1435.

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *The Lancet, 354*, 1435-1439.

Staessen, J. A., Thijs, L., Fagard, R., O'Brien, E. T., Clement, D., de Leeuw, P. W., . . . Parati, G. (1999). Predicting cardiovascular risk using conventional vs ambulatory blood pressure in older patients with systolic hypertension. *The Journal of the American Medical Association, 282*, 539-546.

Stang, A., Moebus, S., Möhlenkamp, S., Erbel, R., & Jöckel, K. H. (2008). Gender-specific associations of short sleep duration with prevalent hypertension. *Hypertension, 51*, e15-e16.

Stanley, N. (2005). The physiology of sleep and the impact of ageing. *European Urology Supplements, 3*, 17-23.

Steptoe, A. (2000). Stress, social support, and cardiovascular activity over the working day. *International Journal of Psychophysiology, 37*, 299-308.

Steptoe, A., Hamer, M., & Chida, Y. (2007). The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. *Brain, Behavior, and Immunity, 21*, 901-912.

Steptoe, A., Peacey, V., & Wardle, J. (2006). Sleep duration and health in young adults. *Archives of Internal Medicine, 166*, 1689-1692.

Sterling, P., & Eyer, J. (1988). Allostasis: A new paradigm to explain arousal pathology. In S. Fisher & J. Reason (Eds.), *Handbook of Life Stress, Cognition, and Health* (pp. 629-650). Chichester: Wiley.

Sternberg, E. (2001). Neuroendocrine regulation of autoimmune/inflammatory disease. *Journal of Endocrinology, 169*, 429-435.

Stetler, C., & Miller, G. E. (2005). Blunted cortisol response to awakening in mild to moderate depression: Regulatory influences of sleep patterns and social contacts. *Journal of Abnormal Psychology, 114*, 697-705.

Stores, G., & Crawford, C. (1998). Medical student education in sleep and its disorders. *Journal of the Royal College of Physicians of London, 32*, 149-153.

Strahler, J., Mueller, A., Rosenloecher, F., Kirschbaum, C., & Rohleder, N. (2010). Salivary α-amylase stress reactivity across different age groups. *Psychophysiology, 47*, 587-595.

Stranges, S., Dorn, J. M., Shipley, M. J., Kandala, N.-B., Trevisan, M., Miller, M. A., . . . Marmot, M. G. (2008). Correlates of short and long sleep duration: A cross-cultural comparison between the United Kingdom and the United States the Whitehall II study and the Western New York health study. *American journal of epidemiology, 168*, 1353-1364.

Tabachnick, B. G., & Fidell, L. S. (1989). *Using multivariate statistics*. New York: Harper Collins.

Takai, N., Yamaguchi, M., Aragaki, T., Eto, K., Uchihashi, K., & Nishikawa, Y. (2004). Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Archives of Oral Biology, 49*, 963-968.

Taylor, A. B., West, S. G., & Aiken, L. S. (2006). Loss of power in logistic, ordinal logistic, and probit regression when an outcome variable is coarsely categorized. *Educational and Psychological Measurement, 66*, 228-239.

Taylor, W. R. (1999). Hypertensive vascular disease and inflammation: Mechanical and humoral mechanisms. *Current Hypertension Reports, 1*, 96-101.

Thoma, M. V., Kirschbaum, C., Wolf, J. M., & Rohleder, N. (2012). Acute stress responses in salivary alpha-amylase predict increases of plasma norepinephrine. *Biological Psychology, 91*, 342-348.

Thorn, L., Hucklebridge, F., Esgate, A., Evans, P., & Clow, A. (2004). The effect of dawn simulation on the cortisol response to awakening in healthy participants. *Psychoneuroendocrinology, 29*, 925-930.

Thorsteinsson, E. B., James, J. E., & Gregg, M. E. (1998). Effects of video-relayed social support on hemodynamic reactivity and salivary cortisol during laboratory-based behavioral challenge. *Health Psychology, 17*, 436-444.

Tochikubo, O., Ikeda, A., Miyajima, E., & Ishii, M. (1996). Effects of Insufficient Sleep on Blood Pressure Monitored by a New Multibiomedical Recorder. *Hypertension, 27*, 1318-1324.

Treiber, F. A., Kamarck, T., Schneiderman, N., Sheffield, D., Kapuku, G., & Taylor, T. (2003). Cardiovascular reactivity and development of preclinical and clinical disease states. *Psychosomatic Medicine, 65*, 46-62.

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research, 53*, 865-871.

Turner, J. R. (1994). *Cardiovascular reactivity and stress: Patterns of physiological response*. New York: Plenum Press.

Turner, J. R., Girdler, S. S., Sherwood, A., & Light, K. C. (1990). Cardiovascular responses to behavioral stressors: Laboratory-field generalization and inter-task consistency. *Journal of Psychosomatic Research, 34*, 581-589.

Turner, J. R., Sherwood, A., & Light, K. C. (1994). Intertask consistency of hemodynamic responses to laboratory stressors in a biracial sample of men and women. *International Journal of Psychophysiology, 17*, 159-164.

Uchino, B. N. (2006). Social support and health: A review of physiological processes potentially underlying links to disease outcomes. *Journal of Behavioral Medicine, 29*, 377-387.

Van Cauter, E., & Spiegel, K. (1999). Sleep as a mediator of the relationship between socioeconomic status and health: a hypothesis. *Annals of the New York Academy of Sciences, 896*, 254-261.

Van Cauter, E., Spiegel, K., Tasali, E., & Leproult, R. (2008). Metabolic consequences of sleep and sleep loss. *Sleep Medicine, 9*, S23-S28.

van den Berg, J. F., Tulen, J. H. M., Neven, A. K., Hofman, A., Miedema, H. M. E., Witteman, J. C. M., & Tiemeier, H. (2007). Sleep duration and hypertension are not associated in the elderly. *Hypertension, 50*, 585-589.

Van Leeuwen, W. M. A., Lehto, M., Karisola, P., Lindholm, H., Luukkonen, R., Sallinen, M., . . . Alenius, H. (2009). Sleep restriction increases the risk of developing cardiovascular diseases by augmenting proinflammatory responses through IL-17 and CRP. *PLoS One, 4*, e4589.

Van Reeth, O., Weibel, L., Spiegel, K., Leproult, R., Dugovic, C., & Maccari, S. (2000). Physiology of sleep (review)-interactions between stress and sleep: From basic research to clinical situations. *Sleep Medicine Reviews, 4*, 201-219.

van Stegeren, A., Rohleder, N., Everaerd, W., & Wolf, O. T. (2006). Salivary alpha amylase as marker for adrenergic activity during stress: Effect of betablockade. *Psychoneuroendocrinology, 31*, 137-141.

VanItallie, T. B. (2002). Stress: A risk factor for serious illness. *Metabolism-Clinical and Experimental, 51*, 40-45.

Vedhara, K., Brant, H., Alexiou, A., Petrie, K., Miles, J., & Lightman, S. (2010). An investigation into the effects of social evaluation on cardiovascular and endocrine responses to the CO2 stress test in humans. *Stress: The International Journal on the Biology of Stress, 13*, 195-202.

Verdecchia, P. (2000). Prognostic value of ambulatory blood pressure current evidence and clinical implications. *Hypertension, 35*, 844-851.

Verdecchia, P., Angeli, F., Borgioni, C., Gattobigio, R., & Reboldi, G. (2007). Ambulatory blood pressure and cardiovascular outcome in relation to perceived sleep deprivation. *Hypertension, 49*, 777-783.

Verdecchia, P., Porcellati, C., Schillaci, G., Borgioni, C., Ciucci, A., Battistelli, M., . . . Santucci, A. (1994). Ambulatory blood pressure. An independent predictor of prognosis in essential hypertension. *Hypertension, 24*, 793-801.

Vgontzas, A. N., Zoumakis, M., Bixler, E. O., Lin, H. M., Prolo, P., Vela-Bueno, A., . . . Chrousos, G. P. (2003). Impaired nighttime sleep in healthy old versus young adults is associated with elevated plasma interleukin-6 and cortisol levels: Physiologic and therapeutic implications. *Journal of Clinical Endocrinology & Metabolism, 88*, 2087-2095.

Vigushin, D. M., Pepys, M. B., & Hawkins, P. N. (1993). Metabolic and scintigraphic studies of radioiodinated human C-reactive protein in health and disease. *Journal of Clinical Investigation, 91*, 1351–1357.

Visser, M., Bouter, L. M., McQuillan, G. M., Wener, M. H., & Harris, T. B. (1999). Elevated C-reactive protein levels in overweight and obese adults. *The Journal of the American Medical Association, 282*, 2131-2135.

Voderholzer, U., Piosczyk, H., Holz, J., Feige, B., Loessl, B., Kopasz, M., . . . Nissen, C. (2012). The impact of increasing sleep restriction on cortisol and daytime sleepiness in adolescents. *Neuroscience Letters, 507*(2), 161-166.

Walker, S., O'Connor, D. B., Schaefer, A., Talbot, D., & Hendrickx, H. (2011). The cortisol awakening response: Associations with trait anxiety and stress reactivity. *Personality and Individual Differences, 51*, 123-127.

Wallace, J. P., Park, S., Zakutansky, D. W., Lehmkuhl, L. A. A., & Jastremski, C. A. (2005). Time of day to monitor ambulatory blood pressure affects the outcome. *Blood Pressure Monitoring, 10*, 43-50.

Wang, T. J., Gona, P., Larson, M. G., Tofler, G. H., Levy, D., Newton-Cheh, C., . . . Robins, S. J. (2006). Multiple biomarkers for the prediction of first major cardiovascular events and death. *New England Journal of Medicine, 355*, 2631-2639.

Waye, K. P., Clow, A., Edwards, S., Hucklebridge, F., & Rylander, R. (2003). Effects of nighttime low frequency noise on the cortisol response to awakening and subjective sleep quality. *Life Sciences, 72*, 863-875.

Wessa, M., Rohleder, N., Kirschbaum, C., & Flor, H. (2006). Altered cortisol awakening response in posttraumatic stress disorder. *Psychoneuroendocrinology, 31*, 209-215.

Westermann, J., Hubl, W., Kaiser, N., & Salewski, L. (2002). Simple, rapid, and sensitive determination of epinephrine and norepinephrine in urine and plasma by non-competitive enzyme immunoassay, compared with HPLC method. *Clinical Laboratory, 48*, 61-72.

Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W., & Wüst, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology, 32*, 358-366.

Wingenfeld, K., Schulz, M., Damkroeger, A., Philippsen, C., Rose, M., & Driessen, M. (2010). The diurnal course of salivary alpha-amylase in nurses: An investigation of potential confounders and associations with stress. *Biological Psychology, 85*, 179-181.

Wirtz, P. H., von Känelb, R., Emini, L., Ruedisueli, K., Groessbauer, S., Maercker, A., & Ehlert, U. (2007). Evidence for altered hypothalamus-pituitary-adrenal axis functioning in systemic hypertension: Blunted cortisol response to awakening and lower negative feedback sensitivity. *Psychoneuroendocrinology, 32*, 430–436.

Wolfram, M., Bellingrath, S., & Kudielka, B. M. (2011). The cortisol awakening response (CAR) across the female menstrual cycle. *Psychoneuroendocrinology, 36*, 905-912.

Wright, C. E., Valdimarsdottir, H. B., Erblich, J., & Bovbjerg, D. H. (2007). Poor sleep the night before an experimental stress task is associated with reduced cortisol reactivity in healthy women. *Biological Psychology, 74*, 319-327.

Wu, H., Zhao, Z., Stone, W. S., Huang, L., Zhuang, J., He, B., . . . Li, Y. (2008). Effects of sleep restriction periods on serum cortisol levels in healthy men. *Brain Research Bulletin, 77*, 241-245.

Wust, S., Wolf, J., Hellhammer, D. H., Federenko, I., Schommer, N., & Kirschbaum, C. (2000). The cortisol awakening response-normal values and confounds. *Noise and Health, 2*, 79-88.

Yang, H., Durocher, J. J., Larson, R. A., DellaValla, J. P., & Carter, J. R. (2012). Total sleep deprivation alters cardiovascular reactivity to acute stressors in humans. *Journal of Applied Physiology, 113*, 903-908.

York, K. M., Hassan, M., Li, Q., Li, H., Fillingim, R. B., & Sheps, D. S. (2007). Coronary artery disease and depression: Patients with more depressive symptoms have lower cardiovascular reactivity during laboratory-induced mental stress. *Psychosomatic Medicine, 69*, 521-528.

Zanstra, Y. J., & Johnston, D. W. (2011). Cardiovascular reactivity in real life settings: Measurement, mechanisms, and meaning. *Biological Psychology, 86*, 98-105.

Zethelius, B., Berglund, L., Sundstr m, J., Ingelsson, E., Basu, S., Larsson, A., . . . Ärnlöv, J. (2008). Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *New England Journal of Medicine, 358*, 2107-2116.

## APPENDIX A

## Research Consent Form

crols
CENTRE FOR RESEARCH ON OCCUPATIONAL AND LIFE STRESS

**SLEEP, HEALTH, AND WELLNESS**

**2009-2011**

HRB
Health Research Board

# PARTICIPANT CONSENT FORM

**SLEEP, HEALTH, AND WELLNESS**
**2009-2011**

**Project Manager:** Dr Siobhán Howard
**Doctoral Candidate:** Mr Éanna O'Leary

| | | Please Initial |
|---|---|---|
| 1 | I confirm that I have read the information sheet for the above study and have had the opportunity to ask questions | |
| | | |
| 2 | I am satisfied that I understand the information provided | |
| | | |
| 3 | I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason | |
| | | |
| 4 | I confirm that the day after I am sleep restricted, I will not drive a car or operate any heavy machinery | |
| | | |
| 5 | I agree to take part in the above study | |
| | | |

| Name of Participant | Signature | Date |
|---|---|---|
| Name of Researcher | Signature | Date |

## APPENDIX B

### Participant Information Sheet


**SLEEP, HEALTH, AND WELLNESS**

**2009-2011**


**PARTICIPANT INFORMATION SHEET**

**SLEEP, HEALTH, AND WELLNESS**
**2009-2011**

You are invited to take part in a research study. Before you decide, it is important that you understand why the research is being done and what it will involve. This *Participant Information Sheet* tells you about the purpose, risks and benefits of this research study. If you agree to take part, we will ask you to sign a *Consent Form*. If there is anything that you are not clear about, we will be happy to explain it to you. Please take as much time as you need to read this information. You should only consent to participate in this research study when you feel you understand what is being asked of you, and you have had enough time to think about your decision. Thank you for reading this.

**Purpose of the Study:** This study is concerned with the effect that amount of night-time sleep has on daytime mood and blood pressure. You have been asked to take part because significant insights can be gained by examining healthy people. We will ask you to report your mood using standard forms for that purpose, as well as a measure of sleep quantity and quality. We also wish to measure your blood pressure while you perform some computer tasks, as well as while you go about your usual business at work. For one night in this study, you will be required to restrict your sleep to about 40% of your normal sleeping time.

**Taking Part – What it Involves**

**Do I have to take part?** It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect your rights in any way.

**What will happen to me if I take part?** We will ask you to answer some standard questionnaires that ask you about your general health and lifestyle (e.g., whether you are a smoker, drink alcohol, your sleep patterns, etc.). We also wish to measure your blood pressure at rest and while you perform some computer tasks. In addition, we wish to measure your blood pressure throughout the day, using a small portable device that you carry in a pouch on your hip. This device measures your blood pressure as you go about your daily activities. We also wish to take a few saliva samples during the day. Relating these measurements to one another will allow us to

see how mood and sleep patterns affect blood pressure overall. For one night, you will only receive 40% of your usual sleep.

**How long will my part in the study last?** In total, your participation will last approximately four hours in the laboratory and you will wear a blood pressure monitor for a total of 48 hours as you go about your daily activities.

**What are the possible benefits in taking part?** You will learn something about your general level of cardiovascular fitness and the factors that affect blood pressure level. We will also give you a report on your ambulatory blood pressure. You can take this report with you and discuss it further with your family doctor if you wish.

In return for your participation, we will also provide you with your personal scores returned from the psychometric tests. In addition, you will receive €20 and will be granted with full research credit in exchange for taking part in this project.

**What are the possible disadvantages and risks of taking part?** The blood pressure monitor you will wear during the day may be an inconvenience (you cannot shower or do vigorous activity that day), though the device is entirely harmless. However, we are interested in monitoring your blood pressure while you engage in your usual activities. Also, this study includes questionnaires that measure your feelings and well-being, now and in the recent past. You might find while you are answering them that you would like to talk to someone about some of the issues raised. We will be happy to recommend someone to you. The day after you are sleep restricted, you will be extremely tired as you go about your normal daily activities. For this reason, you will be asked not to drive or operate any heavy machinery.

**What happens at the end of the study?** When all participants have been tested (this should be within 12 months of your participation), you will receive a summary (no more than 2 pages) of our main findings. While it could be up to 2 years before final results are published, we would be pleased to include you on an address list to receive publications arising from the study. Only general findings will be reported, without reference to identifiable individual results.

Your individual blood pressure report will be provided to you at the end of **your** participation. The individual report that outlines your blood pressure levels and scores on the psychometric measures will be provided to you at the end of the study.

**What happens if I change my mind during the study?** Your participation is voluntary and you are free to withdraw at any time without giving any reason and without your rights being affected in any way.

**What if I have a complaint during my participation in the study?** If at anytime during the study, or after, you have any concerns or complaints arising from your participation you should contact the Researcher. If your concerns or complaints are not dealt with to your satisfaction you should take the matter to the **Chairperson of the NUI Galway Research Ethics Committee, c/o Office of the Vice President for Research, NUI Galway, ethics@nuigalway.ie.**

**Whom do I contact for more information or if I have further concerns?**
If you would like further information or have concerns, you should contact the Researcher:

Dr. Siobhán Howard
Centre for Research on Occupational and Life Stress
NUI Galway
Extension: 4069
siobhan.howard@nuigalway.ie

**Confidentiality:** All information that is collected about you during the course of the research will be kept strictly confidential and will not be shared with anyone else. The information collected in this research study will be stored in a way that protects your identity. Results from the study will be reported as group data and will not identify you in any way.

**Summary:** If you would like further information or have concerns, do not hesitate to contact the Project Manager Dr. Siobhán Howard, CROLS and School of Psychology, NUI Galway, Phone (091) 494069.

For further information on this project, please see http://studentstudy.wordpress.com

**THANK YOU**

## APPENDIX C

### Sleep Diary

**Sleep Diary**

**Please complete the following sleep diary for the next seven nights. Please complete each morning, answering each question as honestly as possible.**

| | Night 1 | Night 2 | Night 3 | Night 4 | Night 5 | Night 6 | Night 7 |
|---|---|---|---|---|---|---|---|
| **Date** | ____/____/2009 | ____/____/2009 | ____/____/2009 | ____/____/2009 | ____/____/2009 | ____/____/2009 | ____/____/2009 |
| **What time did you go to bed?** | ..... ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm |
| **How long did it take to fall asleep?** | .....min | .....min | .....min | .....min | .....min | .....min | .....min |
| **How many times did you wake up?** | .....times | .....times | .....times | .....times | .....times | .....times | .....times |
| **How long did it take you to fall asleep again?** *(If you woke more than 4 times, use the other side of this sheet.)* | 1st time:..….min<br>2nd time:.....min<br>3rd time.....min<br>4th time.....min | 1st time:..….min<br>2nd time:.....min<br>3rd time.....min<br>4th time.....min | 1st time:..….min<br>2nd time:.....min<br>3rd time.....min<br>4th time.....min | 1st time:..….min<br>2nd time:.....min<br>3rd time.....min<br>4th time.....min | 1st time:..….min<br>2nd time:.....min<br>3rd time.....min<br>4th time.....min | 1st time:..….min<br>2nd time:.....min<br>3rd time.....min<br>4th time.....min | 1st time:..….min<br>2nd time:.....min<br>3rd time.....min<br>4th time.....min |
| **What time did you wake up?** | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm | …….. ☐ am ☐ pm |

**APPENDIX D**

**Sleep Restricted Night Leaflet**

---

crols
CENTRE FOR RESEARCH ON OCCUPATIONAL AND LIFE STRESS

NUI Galway
OÉ Gaillimh

HRB
Health Research Board

# Sleep, Health, and Wellness 2009-2011

## Sleep Restriction Protocol

Tonight, please retire to bed at _____

Please set your alarm for _____

When you wake at this time, please stay awake for the rest of the night

When you wake, please activate the Actiwatch monitor. Instructions for use of the Actiwatch monitor are on page 2

Please take a saliva sample every 15 minutes from when you wake up. This means, you will provide 4 saliva samples in the first 45 minutes after you wake. Saliva sample instructions are provided on page 3

Please bring the saliva samples along with the Actiwatch and this completed booklet to your laboratory appointment tomorrow

Your laboratory appointment will take place in Room 205/222 Cois Abhann at _____ **sharp**. Please ensure you are not late for this appointment. Thank you.

**Successful completion of the sleep restriction protocol is an essential part of this project**

**It is important that you avoid eating, smoking, and drinking any fluids (other than water) for 60 minutes prior to your laboratory session tomorrow morning**

**Study ID:**

## Actiwatch Monitor

The Activity Monitor you are wearing is called an Actiwatch.

This Actiwatch will monitor every small movement you make. It will gather data to indicate whether you were successful in adhering to the sleep restriction protocol. More importantly, however, this monitor will **help** you to adhere to the protocol. It is important that you wear this watch at all times during the sleep deprivation period.

The Actiwatch is an expensive piece of equipment. Please ensure it does not get wet.

Please put this monitor on the wrist of your **dominant** hand. Please activate the Actiwatch at 5pm this evening (if you have a 5pm lecture, activate it at 6pm).

**To activate the Actiwatch:**

- Press the red button and hold it down until you hear **4 beeps**
- When activated, the little red LED will start to flash. You will know from this if you have activated the watch properly
- If you are inactive during the time we want you to stay awake, it will sound an alarm. **Briefly** press the red button to stop the alarm and start an activity that will keep you awake.

**To deactivate the Actiwatch:**

- Press the red button until you hear **one** single tone

**Instructions**

**Today**

- Activate the Actiwatch at 5pm

**Tonight**

- Deactivate the watch before you go to bed at ____
- Set your alarm for_____
- Activate the Actiwatch as soon as you wake
- Remain awake for the rest of the night

**Note**:
The Actiwatch is extremely sensitive to your movement (or failure to move). The alarm may sound when you are engaging in activities such as reading a book or watching TV. Try to ensure you move your arm frequently during this time, to minimize this annoyance. Please note that this is not an error. Please ensure you wear the watch for the entire night.



## Saliva Sample Schedule

When you wake tonight, we would like you to take a number of saliva samples in the first 45 minutes after you wake. By examining the levels of cortisol in these four samples, we can study the "Cortisol Awakening Response".

The Cortisol Awakening Response (CAR) is an increase in the secretion of cortisol in the first 30-45 minutes after wakening. This is thought to be a valuable biological marker of psychosocial and health status.

So, to enable us to examine your individual CAR, please take a saliva sample

- Immediately after wakening
- 15 minutes after wakening
- 30 minutes after wakening
- 45 minutes after wakening

**Please do not eat, smoke, brush your teeth, or drink anything other than water until you have taken your final sample.**

**Taking a saliva sample:**

1. Swallow once
2. Tilting your head slightly forward, allow saliva to pool in your mouth for 2 minutes
3. Using the straw, deposit saliva in tube
4. Replace cap and secure into place
5. Please note the time you took each sample in the table below

| TUBE | Sample | Please take….. | Note time |
|---|---|---|---|
| Red cap | S1 | On Awakening | |
| Yellow cap | S2 | 15 minutes after wakening | |
| Blue cap | S3 | 30 minutes after wakening | |
| Green cap | S4 | 45 minutes after wakening | |

**Please bring all your saliva samples with you to your laboratory session**

**APPENDIX E**

**Rested Night Leaflet**

---


## Instructions

Tonight, please retire to bed at _____

Please set your alarm for _____

Please take a saliva sample every 15 minutes from when you wake up. This means, you will provide 4 saliva samples in the first 45 minutes after you wake. Saliva sample instructions are provided on page 2

Please bring the saliva samples along with this completed booklet to your laboratory appointment tomorrow

Your laboratory appointment will take place in Room 205/222 Cois Abhann at _____ **sharp**. Please ensure you are not late for this appointment. Thank you.

**It is important that you avoid eating, smoking, and drinking any fluids (other than water) for 60 minutes prior to your laboratory session tomorrow morning**

**Study ID:**

## Saliva Sample Schedule

When you wake tomorrow morning, we would like you to take a number of saliva samples in the first 45 minutes after you wake. By examining the levels of cortisol in these four samples, we can study the "Cortisol Awakening Response".

The Cortisol Awakening Response (CAR) is an increase in the secretion of cortisol in the first 30-45 minutes after wakening. This is thought to be a valuable biological marker of psychosocial and health status.

So, to enable us to examine your individual CAR, please take a saliva sample

- Immediately after wakening
- 15 minutes after wakening
- 30 minutes after wakening
- 45 minutes after wakening

**Please do not eat, smoke, brush your teeth, or drink anything other than water until you have taken your final sample.**

**Taking a saliva sample:**

1. Swallow once
2. Tilting your head slightly forward, allow saliva to pool in your mouth for 2 minutes
3. Using the straw, deposit saliva in tube
4. Replace cap and secure into place
5. Please note the time you took each sample in the table below

| TUBE | Sample | Please take….. | Note time |
|---|---|---|---|
| Red cap | S1 | On Awakening | |
| Yellow cap | S2 | 15 minutes after wakening | |
| Blue cap | S3 | 30 minutes after wakening | |
| Green cap | S4 | 45 minutes after wakening | |

**Please bring all your saliva samples with you to your laboratory session**



## Tomorrow Morning

**When you wake:**

- Provide 1st Saliva sample (S1)
- Note time of Saliva sample on Saliva Sample Schedule (previous page)

**15 minutes after you wake:**

- Provide 2nd Saliva sample (S2)
- Note time of Saliva sample on Saliva Sample Schedule

**30 minutes after you wake:**

- Provide 3rd Saliva sample (S3)
- Note time of Saliva sample on Saliva Sample Schedule

**45 minutes after you wake:**

- Provide 4th Saliva sample (S4)
- Note time of Saliva sample on Saliva Sample Schedule

**Showering/Bathing**

As you will be wearing the ambulatory blood pressure monitor for 24 hours after you laboratory session; and as you cannot shower during this time, if you wish, please bath/shower before your laboratory appointment.

**Breakfast**

As we will be collecting saliva samples from you in the laboratory, please ensure you have your breakfast at least 90 minutes before your laboratory appointment.

**It is important that you avoid eating, smoking, and drinking any fluids (other than water) for 60 minutes prior to your laboratory session**

## APPENDIX F

### Multidimensional Fatigue Inventory

Questionnaire

Study ID#:

crols
CENTRE FOR RESEARCH ON OCCUPATIONAL AND LIFE STRESS

**CONFIDENTIAL**

HRB
Health Research Board

**SECTION A**

Please indicate by marking the appropriate box, how accurately each statement describes your present feelings; that is, **how you feel right now**

| | | True | > | > | > | False |
|---|---|---|---|---|---|---|
| **A1** | I feel fit | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A2** | Physically, I feel only able to do a little | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A3** | I feel very active | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A4** | I feel like doing all sorts of nice things | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A5** | I feel tired | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A6** | I think I do a lot in a day | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A7** | When I am doing something, I can keep my thoughts on it | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A8** | Physically I can take on a lot | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A9** | I dread having to do things | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A10** | I think I do very little in a day | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A11** | I can concentrate well | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A12** | I am rested | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A13** | It takes a lot of effort to concentrate on things | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A14** | Physically I feel I am in a bad condition | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A15** | I have a lot of plans | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A16** | I tire easily | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A17** | I get little done | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A18** | I don't feel like doing anything | ☐ | ☐ | ☐ | ☐ | ☐ |

| | | | | | | |
|---|---|---|---|---|---|---|
| **A19** | My thoughts easily wander | ☐ | ☐ | ☐ | ☐ | ☐ |
| **A20** | Physically I feel I am in an excellent condition | ☐ | ☐ | ☐ | ☐ | ☐ |

**SECTION B**

| **Please answer the following questions by marking the appropriate box** | | ***Not at all*** → → → → ***Extremely*** | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **1** | **2** | **3** | **4** | **5** | **6** | **7** | **8** | **9** | **10** |
| **B1** | How difficult did you find the previous tasks? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **B2** | How calm do you feel right now? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **B3** | How enjoyable did you find the previous tasks? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **B4** | How frustrated do you feel right now? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **B5** | How stressful did you find the previous tasks? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |
| **B6** | How angry do you feel right now? | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ | ☐ |

**Thank You**

# APPENDIX G

## Ambulatory Diary

crols

Sleep, Health, and Wellness
2009-2011

HRB

### Information

Thank you for agreeing to take part in the *Sleep, Health, and Wellness Project, 2009-2011*. By now, you have concluded the *Sleep, Health, and Wellness* ***Laboratory*** *Study*. Welcome to the second part of this research project; *The Sleep, Health, and Wellness* ***Ambulatory*** *Study*.

For this part of the project, you will be wearing an ambulatory blood pressure monitor. This monitor will automatically take your blood pressure at various times throughout the day and night. You will feel the arm cuff inflate when it is taking a measurement. This unit is entirely harmless. The arm cuff will inflate three times every hour during the day, and once every 45 minutes while you are asleep.

Monitoring your blood pressure over a 24-hour period allows us to view in detail the rise and fall in your blood pressure over your day. The activity diary, which we will ask you to complete each time a measurement is taken, allows us to see what sort of factors affect our blood pressure throughout the day. This information gives us important insights into the psychological and social factors that affect our health, and how these factors might protect us from disease. This activity diary does not need to be completed when you are asleep.

We hope you enjoyed taking part in the Sleep, Health, and Wellness Laboratory Study and we hope you will enjoy the Sleep, Health, and Wellness Ambulatory Study. If you have any queries over the next few hours, please contact the Project Manager, Dr. Siobhán Howard, at 091-494069 or 086-1223963.

Your voluntary contribution has helped to advance what we know about how the body works and the factors that may influence our bodily processes. Thank you for taking part in this study. I look forward to seeing you at your next laboratory appointment.



crols

**Ambulatory Study**

HRB

### Information and Instructions

The ambulatory blood pressure monitor you are attached to is called an Oscar 2 unit. It consists of a small electronic unit connected to an arm cuff that is wrapped around your non-dominant arm.

When you feel the arm cuff inflate, please relax your arm. You can continue with the activity you are involved in; however, it is best to try not to bend your arm during a measurement. When the unit has finished taking the measurement, you will feel the arm cuff deflate. As soon as possible after the measurement has finished, **please complete the activity diary.**

The monitor is entirely harmless; however, if it gets wet, the monitor will be damaged. Please avoid any activity that would result in the monitor getting wet.

To stop a measurement, please the stop/play button just once. To turn the unit off, press the stop/play button for six seconds until you hear 5 beeps in quick succession.

| Practice Measure | | | |
|---|---|---|---|
| **Time:** | | | |
| *At time of measurement:* | | | |
| **Social Contact** | | | |
| Low | | | High |
| ☐ | ☐ | ☐ | ☐ |
| **Stressed** | | | |
| Not at all | | | High |
| ☐ | ☐ | ☐ | ☐ |
| **Angry** | | | |
| Not at all | | | High |
| ☐ | ☐ | ☐ | ☐ |
| **Positive Mood** | | | |
| Not at all | | | High |
| ☐ | ☐ | ☐ | ☐ |

# APPENDIX H

## Instructions and Dialogue Provided to Video Confederates

Research participant will be instructed that a co-researcher will be helping on the project and they will provide them with video-relayed feedback as they complete the task. They will be informed that the co-researcher can view them through the camera.

**Dialogue to induce a sense of negative social evaluation:**

Confederate is seen entering a room containing a desk, chair, computer and microphone. Confederate arranges notes for 1 min, sits, and introduces themselves, addressing the camera and speaking into the provided microphone.

"Hello, my name is ___ and I'm here today to watch you as you complete your task"

"It's important to remember as you complete the task that both your speed and accuracy is important and I'll be rating you on both throughout"

"It's therefore important for you to concentrate throughout the task and do as well as you can"

"When I tell you to begin, please start the task according to the instructions you were given"

"Begin now please" (6 min stressor begins)

1 min – "Try to keep concentrating, compared to other participants your pace has begun to slow.."

2 min – "Okay…Looking at your progress here, most participants have performed slightly better than you at this point"

3 min – "You're now halfway through the task"

"Try to pick up your pace while still concentrating….most participants have still performed slightly better than you at this point".

5 min – "You're almost finished…keep your accuracy and rate of answers high"

6 min – "Time is up… Thanks". " Please wait for the experimenter to re-enter the room".

Confederate gathers notes, leaves room.

# APPENDIX I

## Laboratory Debriefing Report

crols
CENTRE FOR RESEARCH ON OCCUPATIONAL AND LIFE STRESS

**SLEEP, HEALTH, AND WELLNESS**
**2009-2011**

HRB
Health Research Board

**THE LABORATORY STUDY**

**PARTICIPANT FEEDBACK INFORMATION PACK**

The information presented here relates to your participation in the *Sleep, Health, and Wellness Laboratory Study*.

**RESEARCH TEAM**

**The people you met:**

| | |
|---|---|
| *Project Manager:* | Dr Siobhán Howard |
| *Doctoral Candidate:* | Mr Éanna O'Leary |

**The people behind the scenes:**

| | |
|---|---|
| *Principle Investigator:* | Prof. Jack James |
| *Co-Investigator:* | Dr Brian Hughes |

**Project funded by the Health Research Board**

***Sleep, Health, and Wellness Laboratory Study***

***Laboratory Study:***

**Acclimatization (20 minutes)**
*What happened?* For this session, you were seated in one of our cardiovascular reactivity laboratories and seated at a desk. You were asked to rest for approximately 20 minutes while completing some questionnaires. Once you had completed the questionnaires, you were attached to the blood pressure monitor. This monitor is called a Finometer and it assesses cardiovascular function at every heart-beat. This monitor recorded a range of measurements, such as how fast your heart was beating, your blood pressure, as well as the volume of blood your heart was pumping. This allowed us to monitor changes in your cardiovascular functioning while you engaged in different activities.

*Purpose:* The main purpose of the acclimatization period is to ensure all participants are beginning from a similar starting point. So, if you had ran to your laboratory session, your heart rate would be significantly higher than a person who took a taxi. In order to ensure all participants are beginning from a similar point, we set aside a 20-30 minute period during which participants sit quietly while completing questionnaires or reading magazines. Likewise, the Finometer is attached to you during this time to allow you to become acclimatized to the monitor.

**Baseline (10 minutes)**
*What happened?* The Finometer was connected to your middle finger, on your non-dominant hand. You were asked to sit quietly and read some magazines during this time. Your blood pressure was monitored.

*Purpose* : The purpose of the baseline period (also referred to as the resting period) is to obtain measurements of your cardiovascular functioning while at rest. This allows comparisons to be made between your blood pressure when you are resting, and your blood pressure when you are engaging in cognitive tasks.

**StressTask (6 minutes)**
*What happened?* You were asked to complete a mental arithmetic task, receiving feedback from either Áine or Eoin through a video-relayed connection. Áine and Eoin provided false feedback to you and gave identical feedback to every person. You were watching a pre-recorded video of Áine and Eoin. The feedback had no reflection on your actual performance

*Purpose:* Our aim was to examine how your blood pressure increased or decreased as a result of receiving evaluative feedback on your performance.

***Sleep Deprivation***
As you know, for one night prior to one of your laboratory sessions, you were asked to restrict your sleep. On that night, you received 40% of your usual night's sleep. The purpose of this was to examine your blood pressure in response to tasks when you were fully rested and when you were sleep deprived. This is one of the main aims of this project.

# APPENDIX J

## Ambulatory Debriefing Report

**SLEEP, HEALTH, AND WELLNESS**
**2009-2011**

### THE AMBULATORY STUDY

### PARTICIPANT FEEDBACK INFORMATION PACK

The information presented here relates to your participation in the *Sleep, Health, and Wellness Ambulatory Study*.

Please note that although this information pack contains data on your blood pressure as well as some generic advice for enhancing cardiovascular health, the information contained herein does not constitute professional medical advice.

### RESEARCH TEAM

**The people you met:**

| | |
|---|---|
| *Project Manager:* | Dr Siobhán Howard |
| *Doctoral Candidate:* | Mr Éanna O Leary |
| *Researcher:* | Dr Diarmuid Verrier |

**The people behind the scenes:**

| | |
|---|---|
| *Principle Investigator:* | Prof. Jack James |
| *Co-Investigator:* | Dr Brian Hughes |

**Project funded by the Health Research Board**

***Sleep, Health, and Wellness Ambulatory Study***

As part of your participant information feedback pack, you are provided with your ambulatory blood pressure report. For two separate 24-hour periods throughout one working day, you wore an Oscar 2 unit. The Oscar 2 unit is an ambulatory blood pressure monitor that is clinically validated to all three internationally recognized standards; the British Hypertension Society, the European Society of Hypertension International Protocol, and the AAMI SP10. This monitor measured your blood pressure, approximately once every **20 minutes**. These 20 minute intervals were set to vary by 5 minutes. At night, the monitor measured your blood pressure, once every **45 minutes**. You will see on your report, the fluctuation in your blood pressure throughout the day and night. Systolic blood pressure readings above 140mmHg and diastolic blood pressure readings above 85mmHg during the day are displayed in red. Systolic blood pressure readings above 120mmHg and diastolic blood pressure readings above 80mmHg during the night are displayed in red. We recommend that you bring this ambulatory blood pressure report to your doctor on your next visit, if you wish to discuss it in more detail. However, in the meantime, the information provided below will aid you in interpreting your report.

Much of the following information is adapted from the booklet, *Blood Pressure: Heart Information Series Number 4*, published by the British Heart Foundation (2009).

**What is blood pressure?**
Blood pressure is the pressure of the blood in your arteries – the tubes that take the blood away from your heart to the rest of your body. Obviously, you need a certain amount of pressure to keep the blood flowing. Your heart is a pump that beats by contracting and then relaxing. The pressure of blood flowing through the arteries varies at different times in the heartbeat cycle. The highest pressure, known as systolic blood pressure, is the pressure when the beat or contraction of your heart forces blood round your body. The lowest pressure, diastolic blood pressure is the pressure between heartbeats when the heart is resting. Blood pressure is measured in millimetres of mercury (shortened to 'mmHg'). A blood-pressure reading usually gives two numbers. The first number is the systolic pressure and the second is the diastolic pressure.

To maintain hear health, your target is to have a blood pressure below 140/85mmHg (140 systolic and 85 diastolic) If you have diabetes, kidney disease, or disease of the heart and circulation, your target is below 130/80mmHg. There is no fixed dividing line between normal blood pressure and slightly raised blood pressure. Doctors differ in how they interpret 'borderline' blood-pressure levels.

For medical purposes, before you have your blood pressure taken, you should have rested for at least five minutes. You should be sitting down when you have the measurement taken. In this study, it is possible (perhaps likely) that many of your measurements were recorded when you were not in a suitably rested condition. As such, some of your measurements in this study will be higher than measurements that would be taken for purely medical purpose. **Compare your average resting blood pressure with the target of 140/85mmHg cited by the British Heart Foundation**. Remember that many of your measurements were recorded when you were not in a rested state. Therefore focus your attention on your lowest measurements. These are likely to be most representative of your resting blood pressure.

**What if you appear to have low blood pressure?**
People with low blood pressure tend to live longer than people with high or even 'normal' blood pressure. Low blood pressure is sometimes discovered during routine medical examination. Most people with low blood pressure don't have any noticeable 'symptoms'. However, in some people who have blood pressure below 90/60mmHg, it can cause dizziness or even fainting when they get up after bending over or lying down, especially in older people.

Sometimes low blood pressure can be the result of another illness or condition. So if you are having symptoms of dizziness, it is important that you see your doctor. If your blood-pressure reading is strikingly low, your doctor should check to make sure there is not a medical cause.

There is usually no need to treat low blood pressure. Only a very small number of people need to take medication for it.

**What if you appear to have high blood pressure?**
To put it very plainly, the higher your blood pressure, the shorter your life expectancy. People with high blood pressure run a higher risk of having a stroke or a heart attack. If left untreated for a long time, high blood pressure can lead to kidney failure and even damage your sigh. It can also make the heart abnormally large

and less efficient (a condition called 'left ventricular hypertrophy'). This can lead to heart failure, which is when the pumping action of the heart becomes less effective.

People with high blood pressure are not alone. About four in every ten people in the UK either have high blood pressure or are being treated for high blood pressure, and there is no reason to expect the pattern to be significantly different in Ireland. People who know they have high blood pressure are lucky. Nearly a third of people with high blood pressure are not being treated. If you have high blood pressure, reducing your blood pressure can lower your risk of having a heart attack.

**What can you do to help control blood pressure that is high?**
Heart disease is by far the largest cause of premature death in the industrialised world, killing more than twice the number of people who die from cancer. Apart from genetic factors, gender, and ageing, most of the major risk factors for heart disease are lifestyle factors. Therefore, unlike many other diseases, risk for heart disease can be very effectively addressed through behaviour change. This is why psychologists have for many years been involved in cardiac medicine. The following information describes a number of measures people with high blood pressure can take to help reduce their blood pressure.

1. **Be more physically active.** The type of activity recommended for the heart is moderate, rhythmic, activity such as brisk walking, cycling or dancing. If you do not have heart disease (or angina) then your target is to build up to 30 minutes of moderate activity on at least five days of the week. (Moderate activity means any activity that makes you feel warm and slightly out of breath). If you do have angina, you should consult your doctor to advise you as to what you can easily manage without chest pain.

2. **Become less overweight.** If you are overweight, shedding the pounds will help control your blood pressure. For some people, losing weight is all they need to do to get their blood pressure down. Health eating can also help you to lower your blood cholesterol level. Of course, being physically active plays an important part in losing weight.

3. **Avoid smoking.** Smoking is a major risk factor for coronary heart disease. If you smoke and you also have high blood pressure, your arteries will become narrowed more quickly. If you stop smoking, your risk of a heart attack falls to about half that of a smoker within one year.

4. **Cut down on salt.** There is a link between having too much salt in your diet and high blood pressure. Most of us eat at least double the amount of salt we need. Try cooking without adding any salt, and not adding salt to your food at the table. Most of the salt you eat is 'hidden' in processed foods, so avoid these high-salt foods as much as possible.

5. **Eat more fruit and vegetables.** Fruit and vegetables contain potassium, which can help keep your blood pressure down. They're also low in salt. Aim to eat at least five portions of a variety of fruit and vegetables each day. One portion can be in the form of fruit juice.

6. **Drink alcohol within the sensible limits.** Moderate drinking-between 1 and 2 units of alcohol a day-may have a protective effect on the heart, *but only in men aged over 40 and in women who have been through the menopause.* Heavier drinking can contribute to disorders of the heart and circulation, including high blood pressure and stroke. Men should drink no more than 4 units a day and a total of no more than 21 units of alcohol a week. Women should drink no more than 3 units a day and a total of no more than 14 units of alcohol a week. If you drink over this limit, your systolic blood pressure is likely to increase.

7. **Avoid stress.** Stressful situations can cause your blood pressure to rise, but the blood pressure usually returns to normal once the stress has gone away. However, sometimes stress does not go away quickly or at all (for example, severe work stress of the stress of a stressful personal relationship). Relaxation may help you to avoid short-stress. For long-term stress, the solution may lie in changing your view on life such that you become solution-focused rather than emotion-focused when faced with problems. Strategies that aid planning, such as time management strategies, may help prevent stress arising in the future.