The impact of night shift and sleep deprivation on cortisol

by

Linda Rachel Grosser

Bachelor of Psychology (Honours)

Cognitive Neuroscience

A thesis submitted for the degree of

Doctor of Philosophy in Psychology

Behaviour-Brian-Body Research Centre

Sleep and Chronobiology Laboratory

Justice and Society



University of South Australia

April 2023

Contents

Contents ..... **i**
List of Figures ..... **v**
List of Tables ..... **vi**
Glossary ..... **vii**
Contextual statement ..... **viii**
Publication context ..... **x**
Summary ..... **xiv**
Declaration ..... **xvi**
Acknowledgments ..... **xvii**
Funding and scholarship statement ..... **xix**
Chapter 1 Cortisol and shiftwork: A scoping review **1**
Abstract **2**
1.1 Introduction **3**
1.2 Method **6**
1.3 Findings **10**
1.4 Cortisol levels **24**
1.4.1 Night shift 24
1.4.2 Shift schedules 25
1.4.3 Sleep restriction 27
1.5 Cortisol rhythm **29**
1.5.1 Night shift 29
1.5.2 Shift schedules 30
1.5.3 Recovery 32
1.6 The cortisol awakening response **33**
1.7 Discussion **36**
1.8 Conclusion **39**
Chapter 2 Narrative review: Sleep deprivation and cortisol **41**
Abstract **41**
2.1 Introduction **43**
2.1.2 Sleep deprivation 43
2.1.3 Cortisol – the stress hormone 44
2.1.4 The cortisol awakening response 45
2.1.5 Cortisol awakening response measurement 48

2.2 Sleep deprivation and cortisol 48
2.3 Sleep deprivation and the cortisol awakening response 55
2.4 Conclusion 57
Chapter 3: Thesis Aims 59
Chapter 4: Methods 62
4.1 Ethics and informed consent 63
4.1.1 Ethics approval 63
4.1.2 Informed consent 63
4.2 Recruitment 64
4.2.1 Screening session one 66
4.2.2 Screening session two 69
4.3 Participants 70
4.4 Study design 70
4.4.1 Laboratory setting 70
4.2.4 Laboratory protocol 73
4.4.3 Food intake 73
4.5 Materials 74
4.5.1 Biological outcome measures 74
4.5.2 Assay procedure 75
Chapter 5: Circadian and behavioural impacts on cortisol during simulated night shifts 76
Abstract 77
5.1 Introduction 79
5.2 Ethical considerations 82
5.3 Participants 82
5.4 Study protocol design 84
5.4.1 Food intake 86
5.4.2 Saliva collection 87
5.4.3 Saliva assay analysis 88
5.5 Statistical analyses 89
5.6 Results 90
5.6.1 Participants 90
5.6.2 The effects of simulated night shifts on the cortisol rhythm 93
5.6.3 The impact of eating at night and meal size on cortisol response 94

5.6.4 The impact of eating at night and meal size on the circadian morning cortisol rise ... 96
5.7 Discussion ... 99
5.8 Conclusion ... 103
Chapter 6: The impact of 62h sleep deprivation on cortisol ... 106
Abstract ... 106
6.1 Introduction ... 108
6.2 Ethical considerations ... 111
6.3 Participants ... 111
6.4 Study protocol ... 112
6.5 Materials ... 113
6.5.1 Saliva sampling ... 113
6.5.2 Assay procedure ... 114
6.6 Statistical analyses ... 115
6.7 Results ... 116
6.7.1 Participants ... 116
6.7.2 Area under the curve ... 118
6.7.3 The effects of 62h total sleep deprivation on the cortisol rhythm ... 118
6.8 Discussion ... 121
6.9 Conclusion ... 123
Chapter 7: The impact of 62h sleep deprivation on the cortisol awakening response ... 124
Abstract ... 125
7.1 Introduction ... 127
7.2 Ethical considerations ... 131
7.3 Participants ... 131
7.4 Study protocol ... 132
7.4.1 At home pre and post study saliva sampling protocol ... 132
7.4.2 In-laboratory study protocol ... 133
7.4.3 Saliva sampling ... 134
7.4.4 Assay procedure ... 135
7.5 Statistical analyses ... 136
7.5.1 Missing data ... 137
7.6 Results ... 137
7.6.1 Participants ... 137

7.6.2 The effects of 62h total sleep deprivation on the cortisol awakening response 139
7.6.2.1 Analyses by timepoint 139
7.6.2.2 Area under the curve analyses 139
7.7 Discussion 142
7.8 Conclusion 146
Chapter 8 Discussion and Conclusion 148
Summary of research aims 147
8.1 Cortisol displays a strong circadian rhythm and does not increase when awake during the night: Impact of night shift and total sleep deprivation 150
8.2 Behavioural changes with night work and sleep deprivation: Impact of eating at night on cortisol 153
8.3 Sleep and wake are essential elements for the cortisol awakening response ... 155
8.4 Conclusion 157
References 159
Appendix 1: Permission to reproduce article 193
Appendix 2: Written permission of co-authors 194
Appendix 3: Participant information sheet − Study 1 195
Appendix 4: Participant information Sheet – Study 2 200
Appendix 5: Particpant consent form – Study 1 205
Appendix 6: Participant consent form – Study 2 208
Appendix 7: Advertising flyer – Study 1 209
Appendix 8: Advertising flyer – Study 2 210

List of Figures

Figure 1. PRISMA flow diagram for article selection 9
Figure 2. Floor plan of sleep and chronobiology laboratory (City East campus) 70
Figure 3. Floor plan of sleep and chronobiology laboratory (Magill campus) 72
Figure 4. Protocol schematic displaying sleep periods, general mealtimes, test meal/snack time and saliva collection times across time of day 86
Figure 5. Eating behaviour varied between conditions 87
Figure 6. Consort diagram 91
Figure 7. Overnight cortisol across the four simulated night shifts in the no meal at night group 93
Figure 8. Cortisol following meal, snack, or no meal across four simulated night shifts 96
Figure 9. Cortisol outcomes per condition between 03:00h and 05:30h across the four night shifts 98
Figure 10. Protocol schematic 113
Figure 11. Consort diagram of participants 117
Figure 12. Predicted salivary cortisol levels vs. actual cortisol values across 62h total sleep deprivation 119
Figure 13. Protocol schematic 134
Figure 14. Consort diagram of participants who were screened, enrolled and completed the laboratory protocol and data analysed 138
Figure 15. Mean cortisol levels at 15min intervals across the salivary cortisol sampling period 0700h–0745h 141

List of Tables

Table 1. Research studies in review 11
Table 2. Shift schedules 28
Table 3 Studies exploring total sleep deprivation and cortisol 53
Table 4. Studies exploring the cortisol awakening response and total sleep deprivation 57
Table 5. Inclusion criteria 65
Table 6. Screened urinalysis criteria 67
Table 7 Screened bloods criteria 68
Table 8 Participant demographics for each study 69
Table 9. Exclusion criteria 84
Table 10. Descriptive statistics for sleep variables prior to commencing in-laboratory study 90
Table 11. Reactivity changes in cortisol across four consecutive simulated night shifts 94
Table 12. Mean cortisol concentrations per condition 00:30h–02:30h across each night shift 95
Table 13. Mean AUC_g and AUC_i for cortisol across each condition and night shift 98
Table 14. Models for the effect of 62h total sleep deprivation on cortisol 120
Table 15. Salivary cortisol values (ng/ml) at 0 minutes across the study days for waking cortisol AUC_i and AUC_g 140
Table 16. Overarching aims of the thesis 149
Table 17. Summary of thesis study results 149

Glossary

ACTH	Adrenocorticotropic hormone
AIC	Akaike's information criterion
ANOVA	Analysis of variance
AUC	Area under the curve
AUC_g	Area under the curve in relation to ground
AUC_i	Area under the curve in relation to increase
BDI	Beck depression inventory
BMI	Body mass index
CAR	Cortisol awakening response
CAR_i	Identifies changes in the CAR overtime but is sensitive to the first awakening sample
CR	Constant routine
CRH	Corticotrophin releasing hormone
ELISA	Enzyme Linked Immunosorbent Assay
HPA	Hypothalamic-pituitary-adrenal
HRP	Horse radish peroxidase
LLoQ	Lower limit of quantitation
MEMS	Medication Event Monitoring System
MnInc	Mean increase
PFC	Prefrontal cortices
PSQI	Pittsburgh sleep quality index
SCBL	Sleep and Chronobiology Laboratory
SCN	Suprachiasmatic nucleus
SPSS	Statistical package for social sciences
TIB	Time in bed
TMB	tetramethylbenzidine
TSD	Total sleep deprivation

Contextual statement

This thesis is submitted as a 'Thesis Containing Published Research' in accordance with the University of South Australia's Academic Regulations for Higher Degrees by Research Clause 16.1.1.b. This presentation method allows students to submit a combination of conventional chapters and publications. It is required that publications be produced, published, or accepted for publication in peer-reviewed journals or conference proceedings during the student's candidature. The requirements of a 'Thesis Containing Published Research' are as follows:

- Each publication must be peer-reviewed and recognised for the University's publication collection.
- Each publication must be published or accepted for publication pending revisions for publication in significant journals, books and/or conference proceedings relevant to the discipline.
- Conference papers published in conference proceedings and book chapters can only be included where there is evidence of peer-review of the full work.
- For publications that have been accepted for publication pending revisions, the principal supervisor must provide written confirmation that revisions required by referees and/or the editor have been made. This confirmation must be incorporated in an appendix to the thesis.
- Each co-authored publication included in the thesis must be accompanied by a statement by the candidate describing the contribution of each author to each publication. The statements must be signed by each author and incorporated in an appendix to the thesis.
- The candidate must be the greatest or equal greatest contributor to any co-authored publications included in the thesis.

- The subject matter of the publications must be closely related and form a cohesive narrative.
- The publications must be compliant with Copyright law and intellectual property requirements.
- Where copyright has been assigned to the publisher, permission will need to be sought to reproduce the work in the print and digital versions of the thesis. It is the candidate's responsibility to obtain permission to reproduce the work in the thesis. Permission statements from publishers must be included as an appendix to the thesis.
- Publications published, accepted, or submitted for publication prior to commencing the degree, either at University of South Australia or at another university, must not be included in the thesis.

Publication context

This thesis contains one manuscript that has been published in a peer-reviewed international journal within the field of sleep and health. Permission to reproduce this article in this thesis was obtained from the relevant journal (Appendix 1). The signed co-authors' contribution statement to this publication is presented in Appendix 2.

Chapter 1

Grosser L, Knayfati S, Yates C, Dorrian J, & Banks S. (2022). Cortisol and shiftwork: A scoping review. Sleep Medicine Reviews, 64, 101581.

The above paper was submitted for publication in *Sleep Medicine Reviews*, a peer-reviewed medical journal covering research on the diagnosis and therapy of sleep disturbances and disorders. Revisions were completed as part of the publication process. The manuscript was accepted for publication in November 2021.

Overview of author's contributions

The following section describes the author's contribution to the conception/design, data collection and data analysis/interpretation for the studies included in this thesis.

Chapter 5: Study 1

Study data collection and data analysis

This study had already begun to collect data prior to commencement of my PhD candidature. I developed the research questions addressed in Chapter 4. I was involved in collecting data during the last four of 15 study runs. During each study run I was responsible for various tasks involved in data collection. I was responsible for monitoring participants at all times while on shift, setting up and conducting performance test batteries, preparing meals and conducting collection of saliva samples.

This study required participants to remain in the laboratory for the duration of the 7-day study. At the conclusion of each study run I was responsible for entering the data relating to my research questions. Once all runs were complete, I was responsible for preparing and delivering saliva samples, so they could be analysed off site. Once all raw data were available, I was responsible for identifying the most appropriate statistical tests, collating the spreadsheets, running the tests and interpreting the results.

Chapters 6 and 7: Study 2

Study design, data collection and data analysis

For this study, I actively participated in the study design and developed the research questions addressed in Chapters 6 and 7. My data were collected over 12 months, six 5-day study runs, with up to four participants per run. I was responsible for designing the minute-by-minute protocol, recruitment, screening, creating and modifying in-laboratory standard operation procedures to accommodate COVID-19, design menus, calculating individual dietary intake and ordering food. Additionally, I helped train numerous vacation scholars and undergraduate students who worked on this study.

During each study run I was responsible for various tasks involved in data collection. I was responsible for monitoring participants at all times while on shift, setting up and conducting performance test batteries, preparing meals, and conducting numerous saliva samples. This study required participants to remain in the laboratory for the duration of the 5-day study, with a team of staff and students working in the laboratory for five consecutive 24h days. I was available on-call 24h a day for enquiries that could not be answered by the staff on duty. At the conclusion of each study run, I was responsible for entering the study data. Once all runs were complete, I was responsible for preparing and delivering saliva samples, so they could be analysed off site. Once all raw data were

available, I was responsible for identifying the most appropriate statistical tests, collating the spreadsheets, running the tests and interpreting the results.

Summary of publications submitted during candidature

Peer-reviewed publications within the thesis – Chapter 1

Grosser, L., Knayfati, S., Yates, C., Dorrian, J., & Banks, S. (2022). Cortisol and shiftwork: A scoping review. Sleep Medicine Reviews, 64, 101581. https://doi.org/10.1016/j.smrv.2021.101581

Additional publications during candidature

Peer-reviewed journal articles

Marando, I., Matthews, R. W., Grosser, L., Yates, C., & Banks, S. (2022). The effect of time on task, sleep deprivation, and time of day on simulated driving performance. Sleep, 45(9), zsac167.

Volmer, B., Baumeister, J., Matthews, R., Grosser, L., Von Itzstein, S., Banks, S., & Thomas, B. H. (2022). A comparison of spatial augmented reality predictive cues and their effects on sleep deprived users. In *2022 IEEE Conference on Virtual Reality and 3D User Interfaces (VR)*, 589–598. IEEE.

Commissioned industry reports

Matthews, R.W., Yates, C., Grosser, L., Volmer, B., Marando, I., Hanley, B., & Banks, S. (2021) Optimising human performance with AI: Developing a database of performance metrics that dynamically reflect a range of human responses under conditions of fatigue and varying time of day.

Grosser, L., Yates, C., Ngo, M., Wilkin, B., Banks, S. (2022) Integrating physiological and tactical data to improve command decision making: A scoping literature review.

Marando, I., Owen, M., Grosser, L., Matthews, R.W., Yates, C., & Banks, S. (2022) Maximising the habitability proposition for a future maritime platform: Circadian watch routine options.

Summary

Contemporary work and lifestyle factors have contributed to a modern 24h society with disrupted sleep–wake and meal schedules. Cortisol is an important hormone that is impacted by such disruptions. The purpose of this thesis is twofold. First, examine the cumulative effects of simulated night shifts and the concomitant behavioural changes that occur with altering sleep–wake patterns such as eating during the night on cortisol rhythm. Second, explore the impact of two nights of total sleep deprivation (TSD) on the cortisol rhythm and the cortisol awakening response. This thesis contains eight chapters comprised of a systematic review, narrative review, literature summary and thesis aims, study methods, three results chapters from two studies and final discussion/conclusion.

The scoping review (Chapter 1) explored the extent and nature of evidence investigating shiftwork and disruptions to cortisol. It found shiftwork, especially night shift, significantly disrupts the cortisol rhythm and the CAR. Also, irregular shift schedules produce greater disruptions to cortisol than regular shift schedules. It further identified a lack of consistency between studies on variables used to determine reversal of the cortisol rhythm, and the majority of the literature focused on acute rather than chronic disruptions. Furthermore, it revealed limited research examining the impact of eating at night on cortisol. The narrative review (Chapter 2) explored the implications of TSD on cortisol and the CAR. It found that TSD produces significant disruptions to cortisol. However, results are inconsistent with papers reporting change at different times of day, increase only, increase and decrease, decrease only or no effect of TSD on cortisol. It also found literature exploring the CAR during and post TSD is minimal.

Study 1 (Chapter 5) investigated the impact of simulated night shifts with varied eating patterns at night on salivary cortisol. Participants completed four in-laboratory consecutive night shifts and were assigned to either a meal or snack at night, or a no eating at night condition. Results showed night shift induces a temporal shift in the cortisol rhythm rather than a reversal. Furthermore, eating during the night increased cortisol levels ~30min post food consumption with levels remaining high for ~1h.

Study 2 (Chapter 6) investigated the impact of 62h of TSD on cortisol. Results showed a small increase in cortisol in the early evening suggesting that sleep deprivation is a mild physiological stressor . Study 2 (Chapter 7) also investigated the CAR prior to, during and post 62h TSD. Results showed the CAR is absent when waking does not occur but recovers to baseline levels following a recovery sleep opportunity.

The results from this thesis advance understanding of the possible mechanisms associated with cortisol and poor health outcomes in night workers.

Declaration

This thesis presents work carried out by myself and does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; to the best of my knowledge it does not contain any materials previously published or written by another person except where due reference is made in the text; and all substantive contributions by others to the work presented, including jointly authored publications, are clearly acknowledged.



Linda Rachel Grosser 20th April, 2023

Acknowledgments

The PhD journey stretched me more than I anticipated, developed my skills in so many areas, gave me an opportunity to work alongside amazing people who have all counselled, encouraged and inspired me, and some who have become great friends.

This journey and thesis would not have been possible without three amazing women. Firstly my principal supervisor, Professor Siobhan Banks. If not for you I would not have experienced or remained on this journey. I am so very thankful for your invaluable advice, continuous support, and patience during my PhD. Your passion, immense knowledge and wisdom have encouraged and inspired me during this journey. I am truly honoured to have been mentored by you, thank you. To Professor Jillian Dorrian and Dr Crystal Yates, my second and co-supervisors, thank you for the invaluable supervision and support that helped shape my academic writing and statistical analysis skills. I am so grateful to you both.

To Dr Mikaela Owens, thank you for your immense help, patience, and encouragement with statistical analysis but also for bringing the fun! To all the wonderful individuals who make up the BBB research centre, thank you. I can't name you all, but to a special few; Daria, Darren and Monique, we started our undergraduate and PhD journeys together. Your text messages, lunch catch ups, and passing comments of 'you've got this, we've got this' helped me more than you will know. Words cannot express the gratitude I have for each of you and the support and encouragement you have given me.

To all the participants and research assistants, without whom this thesis would not have been possible; thank you. I am sincerely grateful and wish also to thank the University of South Australia, and the support of the Australian Government Research Training Program Scholarship for helping to make this thesis and its associated research possible.

To professional editor, Rosemary Purcell, who provided copyediting and proofreading services according to the guidelines laid out in the university-endorsed national *Guidelines for Editing Research Theses.*

Finally, and most importantly to the three most important men in my life; my truly amazing, one of kind husband, Jamie. You have loved, encouraged and supported me more than words could describe. You have been my biggest fan and I will be eternally grateful for you. You make me want to be a better person. Sorry I got a little crazy at times on this journey. To my two sons, Matt and Will, your love, humour, smiles and tenacity to always do your best inspired me on this journey. Matt, watching you train for and undertake your triathlons with a never say die attitude truly encouraged me to keep going. Will, listening to you practise your music every day and watching you grow in the mastery of your instruments reminded me that mastery of anything, including this thesis, can only be achieved by taking on board the guidance, knowledge and wisdom of those we learn from and putting this into practise daily. Thanks guys, you are the best support team anyone could ever hope to have, and I am so very lucky and blessed that you are mine.

Funding and scholarship statement

Linda R Grosser is supported, via scholarship, by the Commonwealth Government Research Training Program (RTP) Domestic Fee Offset

The beginning is perhaps more difficult than anything else, but keep heart, it will turn out all right.

- Vincent van Gogh



Cortisol and shiftwork: A scoping review

Published Reference: Grosser, L., Knayfati, S., Yates, C., Dorrian, J., & Banks, S. (2022). Cortisol and shiftwork: A scoping review. *Sleep Medicine Reviews, 64,* 101581. https://doi.org/10.1016/j.smrv.2021.101581. (Permission to publish, Appendix 1)

Chapter 1 Cortisol and shiftwork: A scoping review

Abstract

The aim of this review was to explore the extent and nature of evidence exploring shiftwork and disruptions to cortisol. A systematic search was conducted across five databases: Medline, EMBASE, Psych INFO, Joanna Briggs Institute and PubMed between July and August 2020. Cortisol data were characterised into three main outcomes, 1) cortisol levels, 2) cortisol rhythm and 3) cortisol awakening response (CAR) during shiftwork. The main findings demonstrate that shiftwork, especially night shift, significantly disrupts the production of cortisol, the cortisol rhythm and the CAR, and irregular shift schedules produce greater disruptions to cortisol than regular shift schedules. It was difficult to draw conclusions about the impact of shiftwork on movement of the cortisol rhythm and adaptation or recovery of the cortisol rhythm to and from night shift as the literature lacks consistency in the definition of methods and variables. The literature demonstrates cortisol levels, cortisol rhythm and the CAR are all disrupted by shiftwork, but there is a lack of consistency between studies on the use of variables, and most of the literature focuses on acute disruption rather than chronic effects. It will be important for future studies to investigate possible mechanisms that link shiftwork, disruptions to cortisol and chronic health conditions prevalent in shiftworkers.

Key Words: Shiftwork, Cortisol, Cortisol Awakening Response (CAR)

1.1 Introduction

Shiftwork permits business to maximise production by enabling operations (e.g. medical, mining, manufacturing) to function around the clock. While shiftwork has become a fundamental part of many industries generating numerous advantages, there are disadvantages, such as negative impacts on physiological functioning due to disrupted daily (circadian) rhythms (Haus & Smolensky, 2006; Herichova, 2013; James et al., 2017). For this review, shiftwork includes any work schedule occurring outside traditional work hours (09:00h–17:00h) (Costa, 2003). Shiftworkers may work permanent or rotating shifts in a variety of combinations, desynchronising biological and social rhythms.

Circadian rhythms, an important element of virtually all facets of physiology (e.g. cortisol) and behaviour (e.g. sleep), are controlled by a circadian clock (suprachiasmatic nucleus-SCN) and are fundamentally essential for the preservation of optimal human health (Abbott et al., 2020; Van Dycke et al., 2015). Circadian rhythms are entrained to the light–dark cycle ensuring behavioural and physiological rhythms are appropriately timed (Haus & Smolensky, 2006; Van Dycke et al., 2015). Circadian misalignment is a consequence of shiftwork. Typically, circadian misalignment occurs when sleep and wake are inappropriately timed, that is, misaligned to the biological night. Working at night requires individuals to perform when circadian rhythms promote sleep, and sleep during the day when circadian rhythms promote wakefulness and activity (Czeisler & Gooley, 2007). Circadian misalignment contributes to the dysregulation of physiological systems like cortisol (McEwen & Karatsoreos, 2015). Changes in the circadian rhythm of cortisol have provided a physiological approach to assess shiftwork-induced disruptions (Lac & Chamoux, 2004).

Cortisol is secreted by the hypothalamic-pituitary-adrenal (HPA) axis, a key endocrine and stress response system (Koch et al., 2016; Tsigos & Chrousos, 2002). Cortisol levels are continuously monitored in the body to maintain homeostasis. Cortisol regulates its own secretion via negative feedback whereby high levels of circulating cortisol prompt the secretion of corticotrophin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) to be suppressed, which in turn moderates the production and release of cortisol (Clow, Hucklebridge, & Thorn, 2010; Heaney, 2013). Cortisol displays a distinct circadian rhythm that aligns with the daily circadian rhythm of the HPA axis, which is regulated by the body's central clock, the SCN (Liyanarachchi et al., 2017). Cortisol levels begin to slowly rise ~2–3h after sleep onset, reaching a peak early in the morning (~09:00h) with levels steadily declining across the day, until reaching a nadir around midnight when HPA axis activity is also at its lowest (Born et al., 1986; Hellhammer et al., 2009; Liyanarachchi et al., 2017). The diurnal profile can be masked by stress-induced cortisol surges (e.g. food intake, anxiety producing stressors) (Clow, Hucklebridge, & Thorn, 2010; Hellhammer et al., 2009). Under normal physiological conditions, the cortisol rhythm is constant and reproducible; however, some individuals (~10%) exhibit a flattened almost non-linear diurnal rhythm, the determining factor/s of which are unknown (Selmaoui & Touitou, 2003; Stone et al., 2001).

Morning cortisol levels show a sharp increase (~50–75%) that typically peak ~30–45 minutes post awakening, known as the CAR (Clow et al., 2004; Pruessner et al., 1997). The CAR is a distinct feature of the cortisol circadian cycle (Clow, Hucklebridge, & Thorn, 2010; Pruessner et al., 1999). It is considered to be an independent dynamic event initiated by awakening rather than a continuation of normal diurnal cortisol profile (Wilhelm et al., 2007). In contrast to the secretion of cortisol throughout the day,

the CAR is regulated by neural input from the sympathetic nervous system stimulating the adrenal cortex, which fine-tunes the CAR (Clow, Hucklebridge, Stalder, et al., 2010). Different patterns of the CAR (e.g. blunted and heightened peaks), have been associated with a range of negative health outcomes (e.g. post-traumatic stress disorder, depression) (Huber et al., 2006; Wessa et al., 2006). However, the relationship between the CAR and the underlying physiology of these conditions is unclear (Clow, Hucklebridge, & Thorn, 2010). The role of the CAR is similarly unclear, though research suggests it to be associated with adverse prior day experiences and anticipation of forthcoming challenges (Fries et al., 2009; Steptoe & Serwinski, 2016). Researchers have further suggested that the CAR may play a role in immune system balance. It has been proposed that the CAR may be the event that switches the immune system from a night-time cellular to a daytime humoral response immunity (Clow, Hucklebridge, & Thorn, 2010; Hucklebridge et al., 1999). The CAR is frequently used in psychoneuroendocrinology as a biomarker of HPA axis activity (Clow, Hucklebridge, & Thorn, 2010; Hellhammer et al., 2009).

Glucocorticoid receptors, the receptors to which cortisol binds, are present in virtually all tissues in the body. Cortisol, therefore, can affect the performance of nearly every organ system. (Kadmiel & Cidlowski, 2013; Spencer & Deak, 2017). Cortisol plays a central role in the regulation of metabolism (Liyanarachchi et al., 2017), promotes the breakdown of carbohydrate and protein (Buckingham, 2006; Chung et al., 2011), is an important regulator of immune and inflammatory processes (Munck & Náray-Fejes-Tóth, 1992), and regulates blood pressure (Buckingham, 2006). Health problems arise if cortisol levels are too high or too low. Through the process of allostasis (McEwen, 2005) the body continuously monitors and adjusts cortisol levels to ensure stability and balance. An allostatic state (e.g. a flattened cortisol rhythm following consecutive night

shifts) is the result if stability is not retained (McEwen & Karatsoreos, 2015). Chronic instability, for example cortisol levels repeatedly elevated over many days, results in allostatic overload leading to increased disease risk (McEwen & Karatsoreos, 2015).

Significant disturbances on the diurnal cortisol rhythm have been associated with pathologies such as depression and obstructive sleep apnoea (Liyanarachchi et al., 2017). Research further suggests that persistent disturbances in the cortisol rhythm, which shiftworkers regularly experience, may play a role in the pathogenesis of health burdens such as cardiometabolic disease and cardiovascular disease (Puttonen et al., 2010; Ritonja et al., 2018; Spiegel et al., 1999). These health problems translate into higher economic and social costs for the individual, organisations and society (Costa & Di Milia, 2008). The aim of this scoping review was to explore the extent and nature of evidence concerning the impact of shiftwork on cortisol.

1.2 Method

Adopting Arksey and O'Malley's (2005) and Levac et al.'s (2010) guidelines, a comprehensive scoping review on literature relating to shiftwork and cortisol was undertaken.

- Search dates: July–August 2020
- Key search terms: "shiftwork" or "night shift" or "work hours" and "cortisol" or "salivary cortisol" or "cortisol awakening response" or "CAR" and "cortisol rhythm" or "circadian misalignment" and "sleep loss" or "sleep deprivation" or "sustained wakefulness
- Electronic databases: MEDLINE, EMBASE, Psych INFO, Joanna Briggs Institute, PubMed.

- Inclusion criteria:
    - human studies with both field and/or experimental (simulated) designs investigating associations between shiftwork (i.e. work outside of the normal 9am–5pm hours)
    - sleep restriction, sleep loss, disturbed sleep
    - changes in cortisol levels/rhythms; the CAR; Healthy adults.
- Exclusion criteria:
    - use of hair and urinary cortisol (usually assessed as a 24h total, which do not capture diurnal fluctuations or rhythmicity (Manenschijn et al., 2013; Rogers et al., 2021)
    - interventions (e.g. dexamethasone, light) that significantly influence cortisol levels. Light increases morning cortisol levels (Leproult et al., 2001) and dexamethasone reduces cortisol levels by suppressing the HPA axis. If shiftworking individuals displayed elevated morning cortisol levels post dexamethasone intervention, this would intimate a disturbance in the functioning of the HPA axis rather than shiftwork, that is, circadian disruption, influencing cortisol levels (Kirschbaum & Hellhammer, 1994; Pruessner et al., 1999).
    - conference/symposium papers: exploring psychological illness and cortisol; exploring neurological illness such as Parkinson's disease and Alzheimer's disease; exploring sleep patterns, cortisol and so on in children; examining differences between a clinical population and shiftworkers.

The literature search identified 6,486 papers of which 1,728 duplicates were removed. The remaining 4,758 papers were reviewed twice against title and abstract by LG, SK, JD and SB. Following consideration of the inclusion and exclusion criteria, 158 papers were selected and assessed for full-text eligibility, with 38 satisfying the criteria for this

review. Article selection followed standard processes (Peters et al., 2020) and is illustrated in Figure 1. Papers were included if they described published/unpublished primary research or literature reviews that included shiftwork and cortisol. All studies that reported an association between shiftwork and cortisol, irrespective of study design, were eligible for this review. If the relevance of a study was unclear from the abstract, these papers were selected and assessed for full-text eligibility. During the process of study selection, numerous studies were excluded. Examples include studies that used hair or urinary cortisol, or interventions such as dexamethasone. Papers that had poor or no methodological details or were not specific to shiftwork and cortisol were excluded from the scoping review. Any uncertainties about inclusions and exclusions were discussed between LG and SB. Reference lists of identified articles were scanned to detect additional papers that may have been missed during the initial electronic database searches. None were identified. A summary of included papers is provided in Table 1.

Key information was harvested as per the guidelines suggested by Arksey and O'Malley (2005). A wide variation of terminologies has been used in the literature to report outcomes (e.g. profile, levels, rhythm, acrophase, slope). Cortisol measurement approaches in each paper were grouped into one of the three following categories:

- **Cortisol levels:** Where articles compared and/or reported differences in cortisol levels at time points specified by clock time, minutes post activity, time into shift (e.g. start versus end)
- **Cortisol rhythm:** Where articles reported changes in diurnal shape/pattern or profile, reporting variations observed in amplitude, peak and trough times, phase shifts, normal or reversed rhythms
- **CAR:** Where articles reported waking levels, morning peak, mean increase (MnInc) and/or area under the curve (AUC).

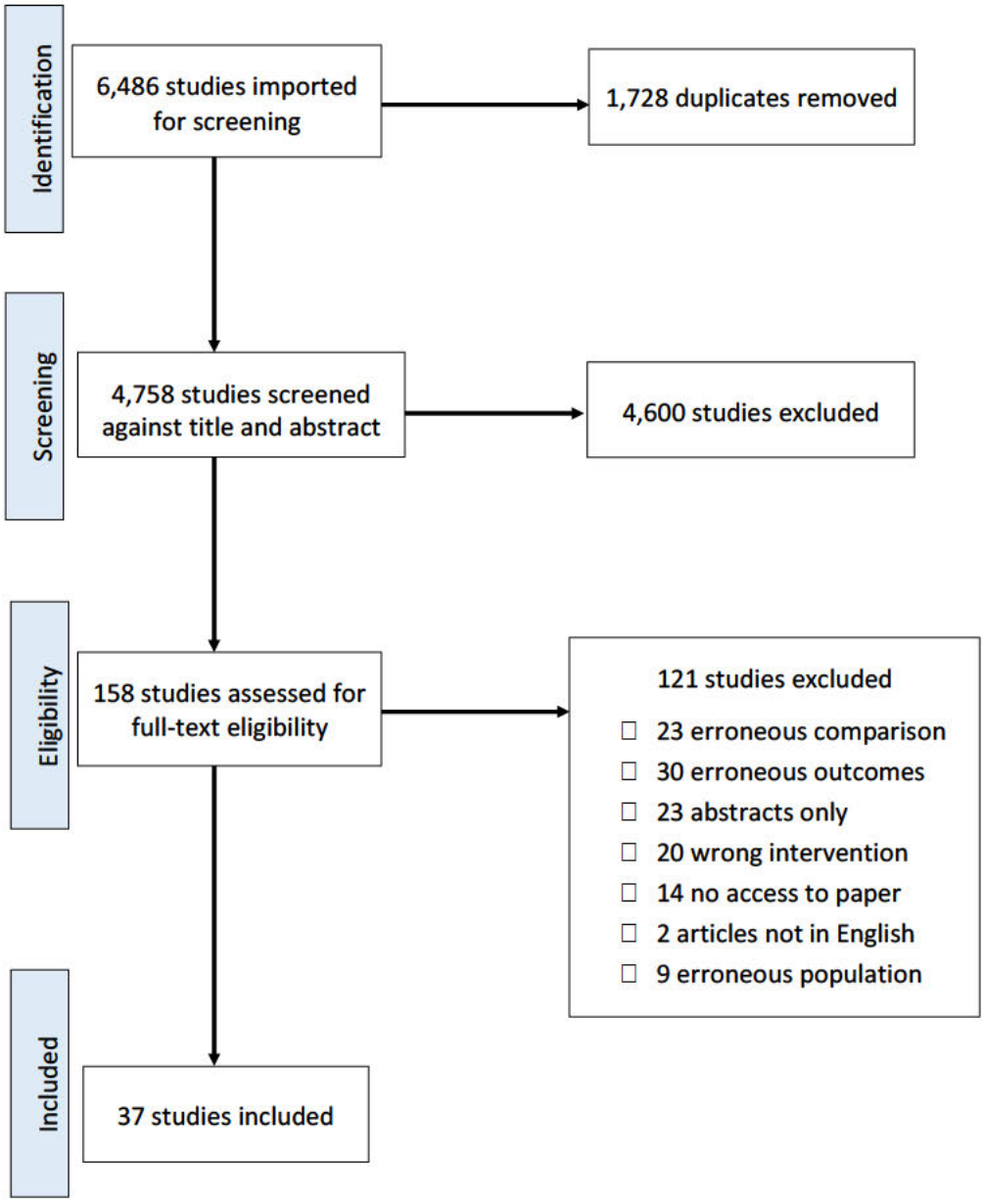


Figure 1.
PRISMA flow diagram for article selection

1.3 Findings

The 38 included studies were published between 1990 and 2020. The majority were from Europe, with the greatest number from Germany. More papers were published in the last 10 years (23 papers) compared with the previous two decades combined, with a relatively even spread of contributors from Asia, Europe, North America and Oceania (Australia). Approximately half of the studies focused on first responders and medical personnel (Table 1). Six papers were laboratory experiments simulating shiftwork hours. The majority of studies explored shift type or scheduling (see Table 2 for shift schedules). Four papers examined sleep restriction (partial or complete loss of sleep, often a result of inadequate opportunity, suboptimal sleeping conditions, and/or time of day) (Leproult et al., 2001). Cortisol was the primary outcome in 17 papers (measured via saliva = 14, blood = 1 or blood + saliva = 1), and the secondary outcome in 21 papers (measured via saliva = 16, or blood = 6). The majority of studies (30 papers) were conducted in the field with sampling conducted by participants. Eight studies were conducted in a laboratory, with sampling conducted by researchers. The majority of studies employed ≥3 sampling time points with six studies collecting samples at two time points only. Nineteen studies collected samples on awakening.

The results of the studies are presented by outcome: 1) cortisol levels, 2) cortisol rhythm and 3) CAR.

Table 1.
Research studies in review

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Cannizzaro et al., 2020	90	Security	S	NS in monitoring room vs NS inspecting streets vs DS	1,3	22:00h,06:30h, 14:00h	↑ in both NS conditions		
Choshen-Hillel et al., 2021	51	Doctors	S	26h-NS (Residents) vs no NS (Attendings)	2, 2–4	Day 1 08:00h–10:00h for no NS & start of 26h-NS. Day 2 08:00–10:00h post 26h-NS only	↑ after 26h-NS		
Hall et al., 2019	26	Firefighters	P	OC+callout vs OCnocallout vs NOC	7,7	Waking +30, +60min, 3h, 6h, 9h and 12h post waking	↓ AUC_g OC+callout		↓ peak OC+callout
Morales et al., 2019	58	Doctors	S	24h-on-call vs DS	1,2	08:00h start of workday for both conditions & 08:00h morn post 24h-on-call	↑ post 24h on-call		
Vincent et al., 2019	72	Firefighters in lab	S	OC+callout vs OCnocallout vs Control (not OC)	3,9	07:00h,07:15h, 07:30h,07:45h, 08:00h,09:30h, 12:30h,14:30h and 17:30h	↓ AUC_g in OC conditions		AUC ↓ in OC+callout peak ↑ in OC nocallout

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Li et al., 2018	70	Doctors	P	Physicians working SW or daywork only. Assessed at BL (2 consecutive days not during or on days post NS) and 12mth follow up; same procedure as BL	4,4	Waking, +4h, +8h and +16h	↑ AUC_g in SW	↑ slope in SW	Waking levels sig higher in SW group
Chang, 2018	128	Nurses	S	Slow-rotating shift: 1-mth DS, 1-mth ES, 1-mth NS. Assessed in each shift type	3,2	Waking +30min. Data collection started Day 14 of new roster to eliminate shift change influence			DS sig ↑ CAR compared with ES & NS. DS sig ↑ waking levels compared with NS
Charles et al., 2016	319	Police	P	Fixed shifts 4 days on of either DS, ES or NS followed by 4 days off	1,7	Waking, +15, +30, +45min post waking, at lunch, dinner & bed (times not defined)	Levels differed between shift type (data not provided)	↓ slope in NS	

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Hall et al., 2016	16	Lab	S	OC+callout during day vs OC+callout at night vs OC nocallout (day & night)	4,7	Waking +15, +30, +45 & 60min post waking +90min & 120min	↑ in OC+callout at night. No difference in OC+callout during day		
Jensen et al., 2016	73	Police	S	Compared 2, 4 & 7 consecutive NS with = recovery days	2,6	Last day of NS and last day of recovery. First sample on waking, last sample at bed. Remaining samples collected at 07:00h,1100h, 15:00h,19:00h, 23:00h, & 03:00h only if awake. 11:00h & 03:00h samples excluded from analyses	↑ levels post-waking in all 3 conditions, a drop & rise again at bedtime	Phase delay in each condition	

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Wolkow et al., 2016	37	Firefighters	S	3 days & 2 nights simulated firefighting work. SR(4h-TIB) vs NoSR(8h-TIB)	2,6	Waking (06:30h), 07:30h,09:00h,11:15h,13:30h, 15:30h,17:30h, 19:30h,21:30h	↑ in SR at 17:30h and 19:30h on Day 2 and at 15:30h, 17:30h, and 21:30h on Day 3. AUC ↑ in SR on Day 2 and Day 3		
Lammers-van der Holst & Kerkhof, 2015	25	Police	P	Assessed once in academy working (08:00h–16:00h) and 3 times post academy working rotating SW schedule; DS, ES & NS (no information on what shifts officers worked at follow up)	4,3	Waking, +30min, +60min post waking			Mean values ↑ from baseline to sig higher levels at ~14mths after starting SW

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Merkus et al., 2015	29	Oil rig	P	Two-week 12h shift tours of either 14-DS or 14-NS	4,3	Waking, +30min post waking and bedtime on the 1st, 4th, 7th & 11th day-off shift	NS sig smaller decline to evening levels on Day 4 and Day 11	NS sig flatter cortisol profile on 1st day off	NS decreased waking levels and post 30min on Day 4 and 7 post roster
Niu et al., 2015	62	Nurses	P	5DS+5off vs 5NS+5off vs 4DS+2off vs 4NS+2off	9,4	Days-2–5 on shift and Days-1–5 off shift. Waking, +30min, 6h and 12h post waking	↑ levels 6h and 12h post awakening in NS	Reversed rhythm by Day 4 on shift in NS. Rhythm restored by Day 2 off roster in NS	Days-3–4 on shift NS blunted CAR
Ernst et al., 2014	30	Doctors	S	1x24h-OC vs 1 x NOC	2,2	Bloods before and after OC/NOC at 07:00h & 09:00h	Morning post 24h-OC cortisol levels ↓ compared to morn before		
Guyon et al., 2014	13	Healthy participants	P	2SR(4h_TIB) vs 2NoSR(10h_TIB)	1,45	Blood and saliva at 20min intervals from 09:00h-00:00h and 14:00h–00:00h	SR displayed↑ in total cortisol levels	Amplitude across the day ↓ by ~21% in SR	

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Bostock & Steptoe, 2013	30	Pilots	S	2DS vs 2ES + 2 rest-days	2,6	Waking +30min, 2.5h, 8h, 12h and Bedtime	↑ AUC in DS with slower rate of decline compared with ES and rest-days		CAR greater in DS. DS ↓waking levels & greater peak at 30min
Fekedulegn et al., 2012	65	Police	P	DS, ES, or NS	1,4	Waking +15, +30 and 45min post waking	Levels differed sig at all time points between shift type. Total AUC lowest in NS		NS ↓ waking levels. CAR peak smaller in NS
Anjum et al., 2011	16	Nurses	S	Continuous 9 days NS with alternate DS. # of NS and DS during 9-day schedule not provided	# of sample days not advised	~8h intervals on a NS 13:00h–15:00h, 22:00h–01:00h, 05:00h–08:00. On a DS 14:00h–150:0h, 21:00–22:00h, 05:00h–06:00	NS ↓ early morning, ↑ at midnight and highest in the afternoon	Altered pattern during NS	
Carev et al., 2011 [46]	12	Doctors	S	Each participant observed on an 8h-DS and again on a 24h-shift	2,6	08:00h,11:00h, 14:00h,17:00h, 21:00h, 23:00h	Both conditions ↑ levels at 23:00h		

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Wirth et al., 2011	68	Police	P	Long-and short-term SW 4 days on, 3 days off, 3 days on, 3 days off. DS, ES or NS	1,4	On a scheduled day-off. Majority worked a DS or a day-off prior to sampling day. Waking +15, +30, +45min post waking			Waking cortisol AUC ↓ in short-term NS and ES than DS
Ulhôa et al., 2011	42	Truck drivers	S	DS (08:00h–18:00h) vs Irregular shift (20:30h–~08:00h) Irregular shift could also work a MS or AS in the same week (start and end times not provided)	2,3	Working day and a day off. Waking, +30min and bedtime	Irregular shift ↑ levels on days off compared to DS		CAR ↑ on workdays in both conditions compared to day off

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Harris et al., 2010	19	Oil rig	S	BL data: 14 days 12h fixed shift; either NS 19:00h–07:00h or DS 07:00h–19:00h. Follow-up data ~9mths after workers transitioned to Swing shift (7xNS and 7xDS)	7,5	2 days off roster 1 week before roster and 1 week post roster. During roster on fixed shifts Days 2, 7, and 14. During swing shift on Day 2 of NS, last night of NS, Day 1 after rotating and last day. Waking, +30min, 6h, 12h and bedtime	Bedtime sig ↑ post 1st NS	Flat on Day 1 of swing shift. Swing-shift rhythm readapted 1 week post roster. No readaptation in Fixed NS rhythm 1-week post roster	CAR after 1st NS sig ↓ Day 1 and last day of swing shift CAR ↓ compared to when working fixed DS
Korompeli et al., 2009	32	Nurses	S	Fixed MS only vs Irregular-rotating schedule (MS, ES & NS)	1,2	Bloods at start and end of MS shift. Bloods also at start and end of ES and NS in Irregular schedule	Irregular schedule sig greater reduction in cortisol levels		
C. Thomas et al., 2009	7,196	Shiftworkers	S	Regular NS 22:00h-07:00h at least once-a-month	1,2	45min post waking, +3h post waking	↑ levels 45min post waking		

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Griefahn & Robens, 2008	16	Healthy participants	P	Experimental SW: 3xDSvs 3xNS	3,19	24h-phase-assessment post 3xDS and post 3xNS. 07:00h after night sleep and 15:00h after day sleep + half-hourly during SW and hourly during 24h phase-assessments			CAR sig ↑ after night sleep (DS)
Vangelova, 2008	25	Sound engineers	S	Forward-rotating-schedule (MS, AS, ES, Rest, NS, Rest) vs Backward-rotating schedule (ES, AS, MS, NS, Rest, Rest) Same start and end times for shifts in each schedule	4,3	MS 06:00h, 09:00h,12:00h; AS 12:00h, 15:00h, 18:00h; ES 18:00h, 21:00h, 24:00h; NS 24:00h, 03:00h, 06:00h	Backward schedule ↑ levels at 09:00h in MS and at 03:00h NS		
Dettenborn et al., 2007	13	Healthy participants	P	SR. 3xNightly wakings at different times during the night (disturbed sleep) vs no disturbed sleep	3,2	Waking, +15min on each nightly waking and each morning	Slight ↑ on 3rd waking		No difference in morn CAR between conditions

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Kudielka et al., 2007	102	Factory workers	P	Permanent NS and DS (MS and ES) moving to Forward-rotating Schedule. Some workers remained on permanent NS or DS	2,8	Permanent NS, on Nights 1 and 5 of 6 consecutive NS and 2nd free day. Permanent DS, 2nd MS and 2nd ES and 2nd free day. Forward-rotation 2nd day of each shift. Waking, +30, +45, +60min, +4h, +8h, +12h, +16h	AUC for NS sig smaller than MS and ES. NS profile attenuated in perm DS who moved to Forward-rotation	Blunted profile during roster and days off in permanent NS. Profile stable in permanent NS workers switching to Forward-rotation	Former permanent DS who moved to Forward-rotation ↓ CAR
Karlson et al., 2006	296	Factory workers	P	24h-Fast-forward (MMAANN) vs permanent daywork or weekly changing schedule of 06:00h–14:00h and 14:00h–22:00h	2,5	Day 2 of a MS or AS and 3rd day-off. Waking, +30min, 11:00h, 8h post waking and ~21:00h	MS ↓ mean levels across the day		MS ↓ waking levels and ↓ peak
Lac & Chamoux, 2004	32	Factory workers	S	Dayworkers vs 2 different rotating schedules (3 days on, 2 days off, 3/2 and 7 days on and 5 days off 7/5)	1,8	Dayworkers assessed on Day 4 and for SW on Day 3 following a change. Every 2hrs from waking till bedtime	7/5 showed an earlier rise in levels	Both 7/5 & 3/2 disrupted profile during NS	

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Federenko et al., 2004	51	Nurses	P	Compared MS, ES and NS.	2,4	1st and 2nd day of MS, ES and NS. Waking, +30, +45, +60min	Sig ↑ in ES and NS		ES levels continued to↑ over the 60min sample period. CAR most distinct in MS
Hennig et al., 1998	24	Nurses	P	NvsD	9,9	2 consecutive MS then 7 consecutive NS. MS sampled 06:00h, 12:00h, 18:00h, 21:00h. NS sampled 21:00h, 24:00h, 03:00h, 06:00h	Morning levels lower than evening levels	Pattern change seen after 4th NS. Full reversal after 5th NS	
Weibel et al., 1996	11	Shiftworkers	P	NSvs Dayworkers	~2, 10min interval	NS workers in lab for ~40h after 4-5 consecutive NS. Blood draws 10min intervals from 20:00h (Day 1) to 23:00h (Day 2)		Acrophase shift of ~6.5h. Quiescent period shift of 3h	

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome Level	Outcome Rhythm	Outcome CAR
Roden et al., 1993	9	Shiftworkers	S	NSvs Dayworkers	1,1h intervals	NS workers in lab after 5−6 consecutive NS. 1hourly bloods from 09:00h (Day 1) to 10:00h (Day 2)		NS no phase shift evident	
Motohashi, 1992	7	Ambulance	P	DSvs 24h-shift	1, ~4hourly	~4h intervals and ~30min post ambulance activities for 24h. Times for DS not specified		Phase advance by ~2hrs in 24h	
Fujiwara et al., 1992	6	Healthy participants	S	Participants exposed to 2 consecutive DS, ES & NS. 1-week interval between each shift experience	6,4h intervals	2ml blood draws 4hourly		Significant phase delay in ES. Second peak seen at 16:00h during sleep in NS	

Author	*n*	Sample	Cortisol Primary (P) Secondary (S)	Exposure	Days, Samples	Sample times	Outcome		
							Level	Rhythm	CAR
Touitou et al., 1990	4	Oil refinery	S	Long-term Rapid-rotation SW assessed during NS and compared with Dayworkers synchronised with lights on 07:00h and off 23:00h	1,4	2hourly bloods during NS (00:00h–08:00h). Dayworker bloods same time as NS	Sig ↑ in SW between 00:00h-02:00h. Dayworker levels ↑ between 02:00h–08:00h but not in SW	Amplitude ↓ in SW	

Note: Cortisol 1 or 2, 1 = primary outcome, 2 = secondary outcome; Days, Samples, 1st# = #of sampling days, 2nd # = # of samples per day; CAR = cortisol awakening response; BL = baseline; NS = night shift; DS = dayshift; ES = evening-shift; MS = morning shift; OC = on-call; NOC = not-on-call; SR = sleep restriction; SW = shiftwork; NoSW = No shiftwork; collection method saliva unless otherwise noted, ↑ = increase, ↓ = decrease

1.4 Cortisol levels

1.4.1 Night shift

The studies explored either the acute effects of night shift or the effect of consecutive night shifts. In the studies exploring the acute effects, two reported that salivary cortisol levels in night shiftworkers were lowest in the morning, high in the afternoon and increased in the evening and at midnight (Anjum et al., 2011; Niu et al., 2015). Other studies reported specific time points across the night based on sampling times. Touitou et al. (1990) found serum cortisol levels were significantly higher in shiftworkers on a night shift between midnight and 02:00h compared with dayworkers. Cannizarro et al. (2020) found cortisol levels were significantly increased at the start (22:00h) and end (06:30h) of a night shift compared with the start and end of a day shift. Similarly, Carev et al. (2011) reported high salivary cortisol levels at 23:00h in individuals working 24h shifts. Moreover, in a study examining medical staff regularly working a 26h shift (4–8 times per month), Choshen-Hillel et al. (2021) posited individuals regularly working 26h shifts during their medical residency were chronically sleep deprived. Medical residents at baseline (post three nights of normal sleep) displayed morning cortisol levels that were lower compared to individuals who did not work these shifts. On the morning following a 26h shift, morning cortisol levels of medical residents rose to levels similar to individuals who did not work these shifts. Similarly, Ernst et al. (2014) found morning cortisol levels in doctors were lower after a night on-call compared to the morning before and with doctors not on-call.

Four studies assessed the influence of consecutive night shifts. Niu et al. (2015) found, when individuals worked five consecutive night shifts, the day-to-day levels of cortisol between Days 2 and 4 exhibited lower fluctuations than individuals working five

consecutive day shifts. Harris et al. (2010) found cortisol levels before bed were significantly higher after the first night shift; however, after working 12 consecutive night shifts cortisol levels at bedtime (i.e. morning) were similar to bedtime levels (i.e. night) in day shiftworkers but the level of decrease was low compared to day shift. Additionally, Jensen et al. (2016) compared the effect of two, four and seven consecutive night shifts with an equal number of recovery days and found all three conditions induced changes to cortisol levels. The lowest levels of cortisol were observed at 8h post awakening following two nights on and two nights off, 9.23h post awakening following four nights on and four nights off and at 11.31h post awakening after seven nights on and seven nights off. However, one short-term experimental study provides contradictory results reporting the two-day mean cortisol level during the night shift did not increase significantly (Fujiwara et al., 1992). In a longitudinal investigation of individuals who were part of a British birth cohort study (n = 7,916), it was found that people who regularly worked night-shift hours had significantly higher cortisol levels 45min and 3h post awakening (C. Thomas et al., 2009).

Ten studies investigated the acute effects of night shift and consecutive night shifts on cortisol. All except one study found that afternoon and night cortisol levels were increased. Additionally, the more consecutive night shifts individuals work the longer cortisol levels remain higher post awakening.

1.4.2 Shift schedules

A number of studies explored shift schedules and the influence of transitioning between shift types. Working on-call overnight with call-outs, two studies observed participants exhibited increased levels of basal cortisol (Hall et al., 2016; Morales et al., 2019) compared to individuals working day shift and on-call where no significant changes occurred in cortisol levels following a day callout (Hall et al., 2016). Lac and Chamoux

(2004) found individuals working a slow-rotating schedule (seven days on, five days off, 7/5) displayed an earlier rise in cortisol, at 05:00h compared to 07:00h in individuals working a fast-rotating (Table 2) schedule (three days on, two days off, 3/2). Cortisol levels were significantly higher during a night shift (23:00h–09:00h) in the 7/5 group compared to the 3/2 group. Vangelova (2008) found individuals working a backward-rotating (Table 2) schedule demonstrated higher levels of cortisol at 09:00h during a morning shift and at 03:00h on night shifts compared to individuals working the same shifts on a forward-rotating (Table 2) schedule.

Personnel who transitioned to a swing shift schedule demonstrated higher bedtime values and a lower level of cortisol decrease during the week of day shift compared to when they worked a permanent dayshift schedule (Harris et al., 2010). In contrast one study found when individuals transitioned from night shift to a day shift the levels of cortisol increased slightly during the day; however, the increase in cortisol levels were considered significant clinically but not statistically (Anjum et al., 2011). Conversely, another study reports the mean reduction of cortisol levels between the start and end of shift was statistically significant for individuals transitioning between consecutive morning, evening and night shifts on an irregular shift schedule (Korompeli et al., 2009).

The literature regarding shift schedules and cortisol levels presents varied findings depending on the shift schedule explored. Although reported cortisol levels while working the night shift were consistent with night-shift specific studies (i.e. higher levels at night and lower levels in the morning), the varied changes reported in cortisol levels overall suggest that irregular shift schedules rather than the shift type itself may influence the observed changes in cortisol levels.

1.4.3 Sleep restriction

A small number of studies assessed the relationship between sleep restriction and cortisol levels. Two studies found a significant influence following two consecutive days of sleep restriction (4h time in bed − TIB) (Guyon et al., 2014; Wolkow et al., 2016b). Sleep restriction is associated with significantly higher total cortisol levels as measured via AUC, with late afternoon and evening levels significantly elevated (Guyon et al., 2014; Wolkow et al., 2016b). Wolkow et al. (2016b) reported that after two days of sleep restriction cortisol levels at the clock times of 15:30h, 19:30h and 21:30h were higher than at these same time points on the day before exposure to sleep restriction. Guyon et al. (2014) found total serum cortisol levels were increased by ~21% and evening cortisol levels increased by ~30% after two days of sleep restriction.

There are a limited number of papers exploring sleep restriction in shiftworkers and cortisol. Findings suggest that acute sleep restriction influences cortisol levels in a similar way to that of working ~seven consecutive night shifts. That is, cortisol levels remain significantly higher for longer across the day post awakening. Irregular shift schedules may exacerbate chronic sleep restriction and associated long-term disrupted cortisol levels. Additional and larger studies conducted over more than two days are needed to advance knowledge of the magnitude of change in cortisol levels that occur the longer sleep restriction occurs.

Table 2.
Shift schedules

Term	Schedule	Shift times
Backward rotating	EAMN/RR	E = 17:30h–00:30h, A = 11:30h–19:30h, M = 05:30h–13:30h, N = 00:00h–06:00h
Forward rotating	MAE/R/N/R	M = 05:30h–13:30h, A = 11:30h–19:30h, E = 17:30h–00:30h, N = 00:00h–06:00h
24h fast-forward rotating	M/M/A/A/N/N RRRR	Shift changes at 06:00h, 14:00h and 22:00h
Fast rotating	MMM/RR EEE/RR NNN/RR	M = 04:00h–12:00h, E = 12:00h–20:00h, N = 20:00–04:00h
Slow rotating	MMM/EE/NN/RRRRR/ MM/EEE/NN/RRRRR/ MM/EE/NNN/RRRRR	M = 04:00h–12:00h, E = 12:00h–20:00h, N = 20:00–04:00h
Rapid-rotating	MMEENN/RRR	M = 06:00h–14:00h, E = 14:00h–22:00h, N = 22:00h–06:00h
Irregular schedule	MEN any combination over an irregular time period	M = 07:00h–15:00h, E = 15:00h–23:00h, N = 23:00h–07:00h
Swing shift	14-day (7N/7D)	N = 19:00h–07:00h D = 07:00h–19:00h

Note: M = morning; A = afternoon; E = evening; N = night; D = day; R = rest-day (Carev et al., 2011; Fujiwara et al., 1992; Hall et al., 2016; Lac & Chamoux, 2004; Merkus et al., 2015; Wolkow et al., 2016b)

1.5 Cortisol rhythm

1.5.1 Night shift

A total of eight studies assessed consecutive night shifts and adaptation of the cortisol rhythm (Anjum et al., 2011; Charles et al., 2016; Harris et al., 2010; Hennig et al., 1998; Jensen et al., 2016; Niu et al., 2015; Roden et al., 1993; Weibel et al., 1996). Three studies (Hennig et al., 1998; Niu et al., 2015; Weibel et al., 1996) found there was a clear reversal in the cortisol rhythm (i.e. high levels at night that would normally be low and low levels in the morning that would normally be high), after five consecutive night shifts. Hennig (1998) and Niu et al. (2015) determined that markedly increased levels at the same time points (e.g. 21:00h and 06:00h or 6h and 12h post awakening) indicated reversal of the cortisol rhythm. However, Weibel (1996) determined that a phase shift of ~3h in the quiescent period and ~6.5h in the acrophase indicated a change in the rhythm and therefore an adaptation to night shift. Harris et al. (2010) determined the cortisol curve was an indicator for changes in the diurnal rhythm and that individuals adapt to night shift within seven days; the cortisol rhythm of night shiftworkers was closer to normal day values by the seventh day of night shift.

Two studies (Anjum et al., 2011; Charles et al., 2016) showed that the percentage of hours worked on a night shift, where night shift was the dominant shift type in their schedule, altered the cortisol circadian pattern. Charles et al. (2016) reported that night shiftworkers had a significantly flatter diurnal cortisol curve compared with day shiftworkers. In contrast to this Anjum et al. (2011) found some individuals working long-term night shift on a nine-day roster where night shift was the dominant shift displayed a normal rhythm during night shift. However, when the shift rotated to a day shift a slightly reversed, but not significant, pattern was found. They concluded that alteration in the circadian pattern may develop later when the shifts were rotated.

In contrast to the above studies, two papers (Jensen et al., 2016; Roden et al., 1993) report no reversal of the cortisol rhythm or evidence of its adaptation to night shift. Jensen et al. (2016) compared the effect of two, four and seven consecutive night shifts with an equal number of recovery days. Findings demonstrated significant differences in the cortisol rhythm on all three interventions with an increasing phase delay evident with the increasing number of night shifts by ~33 minutes per day. However, Jensen et al. (2016) determined there was no adaptation of the rhythm to night shift, even after seven consecutive nights, as the curve of the cortisol rhythm did not follow the same diurnal rhythm in relation to sleep as it did on recovery days. Roden (1993), exploring night shiftworkers after their fifth or sixth consecutive night shift against day workers, found the acrophase for night shiftworkers to be similar to that of day workers, and the majority of night shiftworkers showed no evidence of a discernible phase shift of the cortisol rhythm when compared to day workers.

The literature on shiftwork, specifically night shift and changes in cortisol rhythm, is limited to a small number of papers, each with its own interpretation of when or if the cortisol rhythm adapts to night shifts. It is difficult to compare these studies due to the selection of different outcome measures to describe changes in cortisol and different statistical techniques for analysis. Thus, an area of study choosing the same outcome measure to determine reversal or adaptation of the cortisol rhythm would help to clarify the occurrence of this shiftwork-induced phenomena.

1.5.2 Shift schedules

Several studies explored the impact of shift schedules and mixed findings again emerged. Li et al. (2018) compared doctors working shifts with doctors working days only and found cortisol levels at baseline significantly predicted a steeper slope, large AUC of diurnal cortisol and significantly higher waking levels 12 months later. Touitou

et al. (1990) investigated the relationship between a fast-forward rotating shift schedule changing every three to four days, and found this schedule induced changes to the peak and trough and decreased the amplitude of cortisol with no apparent phase shift. In contrast Karlson et al. (2006) found individuals working the early morning shifts (starting at 06:00h) on a 24h fast-forward rotating shift schedule (see Table 2) clearly showed an altered rhythm with a forward-adjusted phase shift compared with regular daytime workers. Lac and Chamoux (2004) comparing a fast-rotating schedule with a slower-rotating schedule, reported the acrophase moved from 08:00h to 11:00h in the fast schedule and occurred earlier in the slower schedule, moving from 07:00h to 05:00h. Investigating sustained operations, Guyon et al. (2014) and Motohashi (1992) both reported alterations in the phase and amplitude of the cortisol rhythm. Fujiwara et al. (1992) reported decreased amplitude in experimental short-term evening and night shifts. In contrast, other studies report the amplitude remains unaffected (Jensen et al., 2016; Roden et al., 1993; Weibel et al., 1996). Switching workers to a fast-rotating schedule (see Table 2) Kudielka et al. (2007) found the cortisol profile in permanent night workers did not differ significantly; however, permanent day workers initially displayed a significantly blunted cortisol profile that normalised after a short adjustment period.

In summary, six different types of shift schedules had been investigated. Similar to results reported for shift schedules and cortisol levels, the diverse changes reported with regard to changes in the cortisol rhythm overall suggest that irregular shift schedules rather than the shift type itself may influence the observed changes in the cortisol rhythm.

1.5.3 Recovery

There is evidence that shiftwork, specifically night shift, can induce disruptions to cortisol; however, some of the studies reporting adaptation of the rhythm report mixed results on recovery of the rhythm. Harris et al. (2010) exploring 14 days of night shift and 14 days of swing shift (see Table 2) found that although the cortisol rhythm in workers on both schedules adapted to night shift after seven days, recovery of the rhythm for those working 14 consecutive night shifts had not returned to baseline values after seven days off roster. Additionally, the cortisol rhythm of workers on a swing shift (see Table 2) was flat on the first day rotating from night shift to day shift but started to revert towards a normal rhythm during the week of day shift, and was completely readapted to normal values after seven days off roster. A similar study by Merkus et al. (2015) reported that on Day 1 off roster following 14 consecutive night shifts, night shiftworkers showed a flatter cortisol profile, but no flattened profile was evident on Day 7 when compared to their baseline profile. Although the diurnal profile was evident from Day 7 the cortisol profile had not recovered, that is, it did not reflect its baseline profile by Day 11 off roster.

Following five consecutive night shifts Niu et al. (2015) found the cortisol slope of night shiftworkers compared to day shiftworkers on Day 1 off roster displayed significant differences at +30min post awakening to 6h and 12h post awakening. However, on Day 2 off roster, both night and day shiftworkers displayed similar cortisol profiles suggesting the cortisol profiles in night shiftworkers was restored. They further concluded that personnel working night shift require at least four days for the cortisol rhythm to readapt. Karlson et al. (2006), however, reported that partial adaptation of the cortisol rhythm to night work did not readapt after four days off. Additionally, Jensen et al. (2016), comparing the effect of two, four and seven consecutive night shifts with an

equal number of recovery days, reported no significant difference between the number of night shifts in the rhythm of cortisol on recovery days.

There are few studies reporting on the recovery of the cortisol rhythm and what this looks like following shiftwork-induced disruption. The lack of research and data in this area raises questions about the recovery of the cortisol rhythm, what it actually looks like and potential effects on health. Thus, similar to adaptation or reversal of the rhythm as an area of study, choosing the same outcome measure to determine reversal or adaptation of the cortisol rhythm would help to clarify the occurrence of this shiftwork-induced phenomena.

1.6 The cortisol awakening response

Sixteen studies explored the association between shiftwork and the CAR (the vigorous increase seen in cortisol levels immediately after waking, with levels peaking ~30–45min post awakening). Ten studies were conducted in the field. Of the field studies, two used objective verification of awakening time (wrist actigraphy) (Hall et al., 2019; Ulhôa et al., 2011) and texting participants the night before sampling occurred the following day to maximise adherence to the sampling schedule (Merkus et al., 2015). The remaining field studies used self-report measures (sleep diary or collection diary) for the collection and timing of samples. Validity of data collection, particularly in field studies, relies on participants accurately following a timed sampling schedule, which begins with the moment of awakening. Inaccurate sample timing can occur easily and significantly influence CAR estimates (e.g. timing and dynamic of the peak) (Stalder et al., 2016).

While all studies report shiftwork significantly influences waking levels of cortisol (i.e. the first sample sychronised with the moment of awakening) and the dynamic of post-

awakening cortisol secretion, reported as either CAR_i (identifies changes in the CAR overtime but is sensitive to the first awakening sample) or AUC in relation to ground (AUC_g) mixed findings have again emerged.

Though waking cortisol samples were obtained in all studies, not all papers reported an outcome for this measure. Waking levels are considered the starting point of the CAR period (Stalder et al., 2016). Four studies (Chang, 2018; Fekedulegn et al., 2012; Ulhôa et al., 2011; Wirth et al., 2011) found waking levels to be significantly higher in day shiftworkers compared to night shift and afternoon shiftworkers. In contrast, Bostock and Steptoe (2013) and Federenko et al. (2004) found individuals waking prior to 05:30h displayed lower waking levels. Additionally, after one week off roster post 14 consecutive night shifts, individuals displayed significantly lower waking levels of cortisol compared to their baseline (Merkus et al., 2015). Further, Lammers-van der Holst and Kerkhof (2015) found the MnInc values (based on the average of all post awakening samples) of post awakening cortisol secreted across the CAR period (i.e. samples at 15min or 30min intervals in the first hour post awakening) in individuals transitioning from regular day work hours to a rotating shift schedule was significantly higher between four and 14 months after transitioning compared to baseline levels. For some participants, these levels reverted to a smaller baseline level cortisol response at ~20 months after commencing a shiftwork schedule. The authors suggest this reversal to baseline may be indicative of a process of recovery or development of a tolerance to shiftwork.

The CAR itself was reported to be higher on workdays in day shift and irregular shiftworkers compared to their days off (Ulhôa et al., 2011). The CAR is evident in night shiftworkers (sleeping during the day) but was found to be significantly smaller compared with day shiftworkers (Griefahn & Robens, 2008; Kudielka et al., 2007),

while the CAR was most pronounced when awakening occurred very early in the morning (e.g. 04:00h) for individuals working an early shift (Federenko et al., 2004). Additionally, after the first night of night shift on a 14-day night-shift roster the CAR was significantly lower, but on the last night of night shift (Night 14) there were no differences in the CAR compared to individuals working day shift. In contrast, on the last day of a swing shift (see Table 2) the CAR was low compared to day shiftworkers (Harris et al., 2010). For individuals working on-call and responding to call-outs, the CAR peak the following morning was attenuated with lower post awakening cortisol levels (Hall et al., 2019). In contrast, Dettenborn et al. (2007) report the CAR occurs across the night and the morning after following a night of interrupted sleep (three 15min awakenings across the night). No differences were seen in the CAR following each awakening and the CAR the following morning did not differ from the CAR on the morning following a night of undisturbed sleep. Notably, only two saliva samples were collected on each night awakening and the following morning, that is, immediately on awakening and 15min post awakening. The impact of these awakenings may have been more evident had additional samples been collected over the full CAR period (30, 45 and 60min post awakening). Furthermore, working long-term night shift is associated with an attenuated CAR, but not significantly (Fekedulegn et al., 2012; Wirth et al., 2011).

CAR_i was found to be significantly lower in afternoon and night shiftworkers compared to day shiftworkers (Chang, 2018; Ulhôa et al., 2011), while early shiftworkers (waking before 05:30h) displayed a higher CAR_i compared with night shiftworkers and workers on their day off (Bostock & Steptoe, 2013; Federenko et al., 2004). Night shift, afternoon shift, and on-call workers who respond to call-outs during the night additionally display significantly lower mean values for total cortisol output over the

entire CAR period (AUC_g), compared with day shiftworkers (Fekedulegn et al., 2012; Hall et al., 2019; Vincent et al., 2019; Wirth et al., 2011). Individuals working five consecutive night shifts, by Days 3 and 4, displayed significantly flatter waking to +30min (CAR_i) compared to day shiftworkers (Niu et al., 2015).

The literature regarding the CAR and shiftwork-induced disruptions is very limited. Waking levels appear to reflect 'normal' diurnal levels, that is, individuals waking prior to 05:00h, late morning or mid to late afternoon all display low levels, which coincide with the diurnal levels of cortisol at these times. However, CAR and its associated measurements (CAR_i and AUC_g) all display a significant degree of attenuation in late evening and night shiftworkers but not in individuals waking extremely early (e.g. 0400). Additionally, data collection methodology (the number, timing of samples and number of consecutive days) should be carefully considered when interpreting data. Non-compliance with sampling time instructions has been linked with results in false estimates of high waking levels and low estimates of CAR. Additionally, collection of only two post awakening samples cannot accurately capture CAR (Stalder et al., 2016).

1.7 Discussion

This scoping review provided an overview of the shiftwork-induced disruptions to cortisol. The findings demonstrate that: 1) shiftwork, especially night shift, induces significant disruptions to cortisol production, the cortisol rhythm and CAR such that a) levels at night are high when they would normally be low, b) levels remain higher across the day the more consecutive night shifts individuals work, c) the cortisol slope flattens over consecutive night shifts and d) the dynamic of CAR is attenuated; 2) irregular shift schedules may produce greater disruptions to cortisol than regular shift schedules; 3) data on the acute and ongoing effects of sleep restriction on cortisol in shiftworkers is lacking and 4) there is conflicting data and no consistency in the choice

of a reliable outcome measure or number of days on shift/off shift used to conclude adaptation and/or recovery of the cortisol rhythm to and from night shift.

Findings of this review demonstrate that shiftwork, especially night shift, significantly disrupts the production of cortisol. When this is chronic, it can induce allostatic overload (McEwen & Karatsoreos, 2015), predisposing individuals to poor health (McEwen, 2005). Importantly, of the 38 studies reviewed, 24 investigated the effect of shiftwork on cortisol over one or two days. This scoping review indicates that more longitudinal research is needed, investigating the cumulative effects of repetitive disruption to cortisol secretion in shiftworkers. Evidence from the reviewed studies suggests that irregular shift schedules, that is, rotating from one shift type to another over several days, may produce sustained changes in cortisol secretion due to circadian dysregulation which may, in the long run, result in chronic long-term maladaptive cortisol levels. Research should explore whether repeated disruptions to cortisol may be a physiological mediator for increased chronic health risks in shiftworkers, such as cardiovascular disease and metabolic syndrome, which additionally increases the risk for type 2 diabetes (Anagnostis et al., 2009; Knutsson, 2003; Puttonen et al., 2010). In addition to sleep disruption, restriction and circadian misalignment, shiftwork results in altered meal timing. (Haus & Smolensky, 2006; Herichova, 2013; Knutsson, 2003). This scoping review reveals limited research exploring behaviours like meal timing and cortisol levels in shiftworkers. Such behavioural changes likely, to some extent, explain or exacerbate shiftwork-related alterations to physiological systems that underlie glucose tolerance, insulin sensitivity and alteration in inflammatory markers. All these systems are influenced or regulated by cortisol (Casement et al., 2006; Knutson et al., 2007; Leproult & Van Cauter, 2010), and increased circulating levels of cortisol, similar to that reported in some of the reviewed studies, may contribute to disease development

(Anagnostis et al., 2009; Manenschijn et al., 2013; Rogers et al., 2021). There is urgent need for research to better understand the relationship between sleep restriction, meal timing, cortisol levels and associated alterations to physiological systems that are regulated by cortisol. Such research would be foundational for developing interventions for improved health in shiftworkers.

The reviewed literature on shiftwork and the adaptation and recovery of the cortisol rhythm to night shifts is limited to a small number of papers with variation in methods, schedules and interpretation of when or if the cortisol rhythm adapts to and or recovers from consecutive night shifts, making comparison difficult. To better understand and accurately identify these shiftwork-induced phenomena, it is suggested that as an area of study, clearer and more consistent definitions of reversal, adaptation, or recovery of the cortisol rhythm would be of great benefit.

The majority of reviewed studies focus on sleep disruptions in health professionals and protective service workers, which are naturally stressful vocations. Of the 38 studies reviewed only one study examined changes in cortisol as a function of different job roles on a night shift. The cortisol levels of security guards inspecting properties at night increased significantly before and after their night shift compared to security guards based in a monitoring room (Cannizzaro et al., 2020). The work environment, the job role and their related stressors are likely to trigger the HPA axis influencing the release of cortisol (Lundberg, 1999). Evidence suggests the impact of stress, either psychological and or physical may be additive to the circadian changes associated with shiftwork (Cannizzaro et al., 2018, 2020; Wolkow et al., 2016b). It would be useful to disentangle stress and circadian disruption. Therefore, it is recommended that future research take this into consideration and investigate vocations with varying stress profiles, possibly assessing shiftworkers during vacation time versus work time/duties,

to provide a more comprehensive understanding of the degree of disruption to cortisol. Such research may steer the development of different targeted interventions to shiftworker health.

1.8 Conclusion

In conclusion, shiftwork, especially night shift, induces significant disruptions to the production of cortisol, the cortisol rhythm and CAR. There is evidence that shiftwork disrupts the production of cortisol and that the level of disruption varies according to shift type, irregular schedules and sleep restriction. The implications of long-term recurring and cumulative disruptions are not well understood. The significance of these changes must be established by more longitudinal research. Observing such changes will help to develop a comprehensive knowledge of the impact of repeated system disruptions in shiftworkers, and establish the role of cortisol in future health risks.

Practice points

1. Irregular shift schedules rather than the shift type itself may influence the observed changes in the cortisol rhythm.
2. There is a need for clearer and more consistent means to accurately identify reversal, adaptation and recovery of the cortisol rhythm.
3. A more comprehensive understanding of the degree of disruption to cortisol in vocations with varying stress profiles is needed.

Research agenda

1. Further research is need to better understand the relationship between sleep restriction, meal timing, cortisol levels and associated alterations to physiological systems regulated by cortisol.
2. Research demonstrates the acute impact of shiftwork-induced disruption to cortisol, but longitudinal research is needed, to investigate the cumulative effects of repetitive disruption to cortisol secretion in shiftworkers.

Conflicts of interest

The authors do not have any conflicts of interest to disclose.

What would life be if we had no
courage to attempt anything

- Vincent van Gogh



Narrative review:
Sleep deprivation and cortisol

Chapter 2 Narrative review: Sleep deprivation and cortisol

Abstract

Sleep has a large effect on physiological functions, ensuring there exists an optimal internal environment for physiological processes to occur during sleep. Much current literature links sleep loss and poor physical and mental health, and cortisol has been hypothesised by many to be a mechanistic link. Sleep deprivation is considered a physiological stressor with negative impacts on the function of physiological systems such as the endocrine system. However, results of research on sleep deprivation and cortisol is mixed, likewise the notion that it is a physiological stressor. Some researchers report an effect of sleep loss on cortisol with total sleep deprivation (TSD) demonstrating negative effects on evening and morning cortisol levels. But others report no effect.

Equally the literature is mixed regarding sleep and the CAR, with very little research on whether the CAR exists without waking. The CAR is frequently used as a biomarker of HPA axis function but there is no clear understanding as to the role of the CAR or how it responds during and post repeated episodes of sleep deprivation.

The implications of long-term recurring disruptions to the cortisol rhythm and the CAR are poorly understood. It is therefore important for research to further explore and understand the degree of disruption to the cortisol rhythm and CAR during periods of TSD in field and laboratory settings. Such knowledge will contribute to the development of future strategies (e.g. sleep education) and interventions aimed at improving the health and wellbeing of populations who frequently experience sleep deprivation, inform workplace policy (e.g. improved rostering) and encourage further research to continue exploring sleep duration and health. This chapter presents a

narrative review of the literature focusing on the implications of TSD on cortisol and the CAR.

2.1 Introduction

2.1.2 Sleep deprivation

> *"Sleep is the golden chain that binds health and our bodies together" (Thomas Dekker, 1609).*

Modern civilisation has become a 24h society in which time devoted to sleep and recognition of the importance of sleep to health, and wellbeing has declined (Ferrara & De Gennaro, 2001). Sleep and sleep duration are strongly influenced by two separate biological processes, sleep–wake homeostasis and the circadian process, working together to create a balanced sleep–wake cycle (Borbély, 1982). Sleep–wake homeostasis generates sleep pressure; the longer an individual is awake greater is the desire for sleep. This pressure dissipates during sleep. The circadian process regulates the body's sleep pattern. Sleep deprivation occurs when individuals fail to obtain an adequate amount of sleep (Abrams, 2015; Banks & Dinges, 2011). While inconsistencies exist in the literature as to how much sleep is deemed to be adequate, typically, 7h–8h of sleep is required (Ferrara & De Gennaro, 2001; Watson et al., 2015). However, adequate sleep is influenced by sleep behaviour, which is influenced by circumstances such as work hours (e.g. shiftwork), personal obligations (e.g. caring for elderly relatives), poor sleeping habits and medical problems (e.g. sleep apnoea) (Banks & Dinges, 2011). A recent population survey of 1,011 Australian adults reported poor sleep habits were common among adults with 12% reporting less than 5.5h sleep per night and 8% over 9h of sleep per night, and 26% reporting internet use every night prior to bed and 16% doing work just prior to bed (Adams et al., 2017). Such behaviours contribute to sleep loss and therefore sleep deprivation.

Sleep has a large effect on physiological functions ensuring there exists an optimal internal environment for processes occurring during the circadian rest phase (Borbély et

al., 2016). Some of the detrimental consequences of sleep deprivation are due to disruption of the balance between the homeostatic and circadian process. For example, scheduling sleep during the day and working (awake) at night alters the timing of light exposure affecting the circadian process.

Sleep also plays a major role in the function of the endocrine system (Leproult & Van Cauter, 2010). The onset of sleep displays a temporary inhibition of cortisol secretion while awakening consistently demonstrates an increase in cortisol levels. Some researchers have found an effect of sleep loss on cortisol, with TSD demonstrating negative effects on both evening and morning cortisol levels (Leproult et al., 1997; Wright et al., 2015). However, others have found no effect (Salín-Pascual et al., 1988) Any stimuli, physical or psychological that disrupts homeostasis results in a stress response (Chu et al., 2021). Therefore, sleep deprivation that disrupts the balance between the homestatic and circadian process may be a physiological stressor (Wright et al., 2015) impacting the brain and many physiological systems such as the HPA axis (McEwen, 2006).

2.1.3 Cortisol – the stress hormone

Cortisol is widely known as the stress hormone. It gets this name as it is the primary hormone released to help regulate the body's stress response (Charmandari et al., 2005; Chu et al., 2021). Cortisol is a steroid hormone produced by the zona fasciculata of the adrenal cortex in the adrenal gland. The release of cortisol is controlled by the HPA axis. Synthesis and secretion of cortisol are regulated by the CRH secreted by the paraventricular nucleus of the hypothalamus. CRH stimulates receptors in the anterior pituitary triggering the synthesis and release of ACTH, which in turn stimulates the adrenal cortex to produce cortisol. Cortisol levels are continuously monitored and regulated, to maintain homeostasis, via feedback inhibition. Sufficient cortisol levels

inhibit the release of both CRH and ACTH (Buckley & Schatzberg, 2005; Katsu & Baker, 2021; Thau et al., 2021). Both high and low levels of cortisol have been identified in the pathogenesis of various diseases like Cushing's syndrome, Android obesity, cardiovascular disease and Addison's disease (Anagnostis et al., 2009; Khani & Tayek, 2001; Manenschijn et al., 2013).

Glucocorticoid receptors (the receptor to which cortisol binds) are found in nearly every cell in the body, but not the SCN (Balsalobre et al., 2000). Cortisol has diverse effects on numerous functions throughout the body. For example, cortisol is a distinct regulator of inflammation. It attenuates signalling pathways, which suppresses production of inflammatory mediators (Cain & Cidlowski, 2017; Stalder & Kirschbaum, 2013; Yeager et al., 2011). It also interacts with significant neurotransmitter systems, for example dopaminergic and serotonergic (Dedovic et al., 2009), influencing a variety of neuronal processes like cognitive functions and memory consolidation (Wolf, 2009). Additionally, cortisol plays a key role in converting protein to glycogen that hormones, like glucagon, act on to increase glucose levels (Khani & Tayek, 2001). Moreover, cortisol displays a clear circadian rhythm aligned with the daily circadian rhythm of the HPA axis (Liyanarachchi et al., 2017), and plays a fundamental role as an internal time giver synchronising the circadian function of many peripheral organs and several brain regions (Oster et al., 2017).

2.1.4 The cortisol awakening response

Upon awakening, cortisol levels demonstrate an acute increase, which typically peaks approximately 30–45min post awakening (Pruessner et al., 1997). This is known as the cortisol awakening response (CAR). The CAR was first described by Pruessner and colleagues (1997) and has since been examined extensively in a range of healthy and clinical populations, and in field and laboratory environments, with a variety of

findings. The CAR is initiated by the process of awakening masking the pre-awakening cortisol increase of the circadian cortisol rhythm (Clow, Hucklebridge, & Thorn, 2010; Hellhammer et al., 2009; Wilhelm et al., 2007). It occurs under the influence of the HPA axis, and is regulated by neural input from the sympathetic nervous system stimulating the adrenal cortex, which fine-tunes the CAR response (Clow, Hucklebridge, & Thorn, 2010).

The CAR is present in infants from as early as two months of age (Stalder et al., 2013), and observed in ~75% of healthy adults (Wust et al., 2000). However, patients with hippocampal region damage do not display a CAR (Buchanan et al., 2004) suggesting the CAR is dependent on hippocampal integrity. The CAR displays considerable intra-individual variability with state factors (e.g. time of awakening) being more influential on its expression than trait influences like sex and genetic heritability (Hellhammer et al., 2007; Law et al., 2013). In a study exploring repeated assessments of the CAR over multiple months it was found that >70% of the variability in the CAR was attributable to day-to-day fluctuations rather than trait influences (Ross et al., 2014).

There is consensus that exposure to bright light (~800 lux) upon waking (Leproult et al., 2001; Scheer & Buijs, 1999), being woken unexpectedly (Born et al., 1999), anticipation or expectation of wake up (Born et al., 1999) and the time of awakening, specifically earlier awakening (Edwards et al., 2001; Stalder et al., 2009) increase intensity of the CAR. Conversely, waking in darkness or dim light attenuates the CAR (Scheer & Buijs, 1999), while sleep restriction and mild sleep disturbances appear to have little impact on the CAR (Elder et al., 2014).

Changes in the typical pattern of the CAR, for example consistent blunting, assessed over consecutive days, are considered to reflect dysfunction in neuroendocrine

processes (Stalder et al., 2016). The CAR has been associated with a variety of negative health outcomes including depression, autoimmune disease, chronic fatigue and post traumatic stress disorder (Kudielka & Kirschbaum, 2003; Pruessner et al., 1999; Roberts et al., 2004; Wessa et al., 2006). However, there is variation across studies as to whether the CAR is blunted or increased in different health disorders. Fatigue, post traumatic stress disorder and exhaustion are characterised by a reduced CAR; however, depression has been associated with both increased and decreased CAR (Chida & Steptoe, 2009).

The function of the CAR itself is still be determined. It has been suggested that a function of the CAR could be managing anticipated daily life demands of the upcoming day by providing sufficient resources (increased energy needs) as individuals transition from a rest to an active state in order to cope with the day ahead (Schulz et al., 1998). Powell and Schlotz (2012) explored this concept, reporting the CAR increase was associated with successful coping with same day daily life stress.

The literature also demonstrates CAR to be associated with adverse prior day experiences (Fries et al., 2009; Steptoe & Serwinski, 2016). Such findings suggest that prior day experience and anticipation for the current day ahead separately, and possibly together, influence the CAR. The CAR might be an adaptive response assisting with re-establishment and provision of energy needs to ensure optimal functioning. Additionally, self-report levels of arousal have been positively associated with changes in the MnInc levels of the CAR (Thorn et al., 2009), suggesting it may assist in stimulating arousal post awakening and reduce sleep inertia. It has been speculated that the CAR may play a role in immune system balance by switching the daytime immune system on (Hucklebridge et al., 1999).

2.1.5 Cortisol awakening response measurement

The CAR itself is typically assessed using salivary cortisol samples, ideally over two or more consecutive days (Stalder et al., 2016). Two main measurement components are required to accurately capture and assess the CAR: 1) a starting point, the first sample (S1) collected immediately on waking and considered to reflect the endpoint of the diurnal pre-awakening cortisol increase and 2) the actual increase of cortisol post awakening captured by repeated sampling at ~15min intervals across the ensuing post awakening time period of 30–60min (Stalder et al., 2016). It is recommended that S1 and the CAR be reported separately. Typically, the CAR has been reported as AUC in relation to increase (AUC_i) revealing the change in cortisol over time and AUC_g giving a measure of overall cortisol secretion during the post awakening period (Elder et al., 2014; J.C.Pruessner et al., 2003; Stalder et al., 2016).

While Chapter 1 examined the literature on shiftwork and cortisol (Grosser et al., 2022), the aim of this narrative review is to look specifically at total sleep deprivation (TSD) and the impact it has on cortisol and the CAR. To examine TSD and cortisol a systematic search was undertaken to examine the literature. The literature on CAR and TSD is so small that all the studies examining the impact of TSD on CAR were reviewed. In the following section this literature will be discussed in detail.

2.2 Sleep deprivation and cortisol

The literature on TSD and cortisol is broad and diverse with a range of methodologies used and concomitant range of results reported. A systematic search was used to examine the literature and a narrative style employed to discuss the studies.

Papers were included in this narrative review if they described published/unpublished primary research that included TSD and cortisol and the CAR. All papers that reported

an association between TSD and cortisol and the CAR, irrespective of study design were eligible for this review. Papers included were field or laboratory studies exploring the impact of ≥24h TSD on the cortisol rhythm or the CAR as assessed by blood, urine, hair or salivary cortisol analyses.

Studies have explored the impact of sleep deprivation and circadian misalignment on cortisol (Table 3). All of the studies except one (Kant et al., 1984) used blood measures of cortisol (Kant used urinary measures). All of the studies except one (Song et al., 2015) were conducted in the laboratory, with participants who had undergone health screening. Only three studies included female participants.

Of the studies reviewed, eight explored the effect of 24h TSD on cortisol with four studies exploring, 36h, 48h, 64h and 72h of TSD. Ten studies reported a significant influence of TSD on cortisol levels. Seven studies reported only an increase in levels at different times of the night or morning.

Leproult et al. (1997) found cortisol levels during a constant routine (CR) protocol of 24h TSD were significantly increased between 18:00h and 23:00h. Song et al. (2015), employing an ambulatory protocol, found levels were higher at 08:00h at the end of 24h TSD compared to 08:00h on the morning prior. Weibel et al. (1995), also during a CR protocol of 24h TSD, found cortisol levels increased between 23:00h and 03:00h while Chapotot et al. (2001) found that during a CR protocol of 24h TSD cortisol levels increased at 06:00h, 07:30h, 08:30h, 10:00h, 13:00h and 17:00h compared to participants in the sleep condition. Weitzman et al. (1983), did not report specific times of increase, only that cortisol levels, during 24h ambulant TSD, were increased during the first half of the night. Schüssler at al. (2006) assessed cortisol levels at baseline, that is, prior to individuals commencing 24h TSD and post 24h TSD during a recovery

sleep, and reported cortisol AUC levels were elevated during the second half of the night. Wright et al. (2015) reported cortisol levels during 24h CR TSD increased during the first half of the night compared to the corresponding time of night 24h earlier when individuals were permitted to sleep. Additionally cortisol levels were also higher after the night of TSD for some timepoints during the day, especially morning hours.

One study reported an increase in night-time levels at 02:00h and a decrease in morning levels at the end of a 24h ambulant TSD period at 07:00h (von Treuer et al., 1996) while two studies reported only a decrease in cortisol levels. Following 48h ambulant TSD, Åkerstedt et al. (1980) reported a decrease in morning levels at 08:00h compared to 08:00h 48h earlier and Kant et al. (1984) reported that 72h TSD resulted in a decrease in urinary cortisol levels.

In contrast to the above studies, two papers (Dinges et al., 1994; Salín-Pascual et al., 1988) reported no change in cortisol levels. Following 64h ambulant TSD Dinges et al. (1994) reported cortisol levels varied systematically across the deprivation period, and determined the pattern was not associated with sleep deprivation; cortisol levels were higher on the baseline day and recovery days between 22:00h and 23:00h than on any sleep deprivation day at the same time. Exploring 36h TSD, Salín-Pascual et al. (1988) reported cortisol levels changed significantly over time but there were no differences between pre and post TSD conditions by time point and there were no significant changes in the amplitude or rhythm of cortisol secretion.

The reported disparities in the findings above may be attributed to differences in the number, time and type (i.e. blood vs. urine) of sample collected and study methodology (CR or ambulatory). Four of the studies assessed the effect of TSD on cortisol post TSD, that is, obtaining cortisol samples either at the end of the TSD period or during a

post recovery sleep (Åkerstedt et al., 1980; Salín-Pascual et al., 1988; Schüssler et al., 2006; Song et al., 2015). Assessing the effects of TSD on cortisol post the TSD period does not provide an opportunity to explore how the cortisol rhythm responds during TSD. Notably it does provide an indication as to how long the effects of TSD may impact the cortisol rhythm and how long it may take for the cortisol rhythm to recover. Leproult et al. (1997) reported that post TSD (32h), the normal day-long decline occurred at a slower rate demonstrating a delay of ~12–24h before the return of the quiescence of cortisol resulting in an elevated evening cortisol levels.

Eight studies employed continuous blood sampling ≥ every 10min across the hours of TSD, which ranged from 24h to 36h (Chapotot et al., 2001; Leproult et al., 1997; Salín-Pascual et al., 1988; Schüssler et al., 2006; von Treuer et al., 1996; Weibel et al., 1995; Weitzman et al., 1983; Wright et al., 2015). Of the eight, Salín-Pascual et al. (1988) was the only study to not utilise continuous blood draws during TSD; instead it employed continuous blood draws on a baseline sleep night and a recovery sleep night. In contrast to the seven studies employing continuous blood draws during the TSD period and reporting significant change in cortisol levels, Salín- Pascual et al. (1988) reported no change in cortisol levels. Three studies obtained blood samples at specific time points during TSD, for example 22:00h on Day 1, Day 2 and Day 3 (Dinges et al., 1994) or single time points pre and post TSD, for example 08:00h (Åkerstedt et al., 1980; Song et al., 2015), one of which reported no change in cortisol levels (Dinges et al., 1994), while one study utilised 24h urine cortisol levels.

Continuous sampling provides an opportunity to examine circadian rhythmicity and how this may be impacted by TSD, whereas single timepoints provide only information for change that may or may not occur at that point in time. Urinary cortisol is a useful index of unbound cortisol produced over the course of a complete circadian cycle

(Levine et al., 2007); however, it does not capture diurnal fluctuations that may occur during periods of sleep deprivation.

In summary, the literature on TSD and its impact on cortisol has found varying results. Of the 12 papers reviewed in Table 3, 10 report significant effects of TSD on cortisol; however, results are mixed with some papers reporting impacts at different times of day, increase only, increase and decrease or decrease only and two reporting no effect of TSD on cortisol. A mix of CR and ambulant protocols were utilised across the studies, so too a mix of sampling methods (continuous vs. single point, during TSD or post TSD) and type of sample (blood or urine). The lack of consistency in results suggest more research is required with rigorous methodology to investigate the effect of sleep deprivation on cortisol.

Table 3
Studies exploring total sleep deprivation and cortisol

Author(s) and date	*n*	Method	Sampling	TSD	Changes in cortisol
Song et al. (2015)	149[a]	AMB	2 samples: 08:00h baseline & post TSD	24h	Sig. ↑ post-TSD
Wright et al. (2015)	17 (3f)	CR	During BL sleep. Every 30min during TSD	40h	Sig. ↑ in the early evening and early morning during TSD
Schüssler et al. (2006)	8	CR	Every 30min between 20:00h and 22:00h then every 20min between 22:00h and 07:00h	24h	AUC sig. ↑ during the second half of the night post-TSD, during REC sleep. No difference between BL and REC night at time points 05:35h and 06:15h respectively
Chapotot et al. (2001)	12	CR	Every 10min between 18:00h and 18:00h	24h	Sig. ↑ between 23:00h-18:00h. Sig. higher between 06:00h-07:30h, 10:00h-13:00h & 17:00h during TSD vs. control. Mean secretory amplitude in TSD group sig. increased by 18.5%
Leproult et al. (1997)	17	CR	Every 20min from18:00h Day 1 to 02:00h Day 3	32h	Sig. ↑ between 18:00h and 23:00h on TSD Day 2 vs. Day 1 (mean increase ~45%). Post-TSD delay of ~12h–24h in the return to typical evening cortisol levels. Typical day-long decline post TSD occurred at slower rate
von Treuer et al. (1996)[b]	9	AMB	Hourly draws	36h	Sig. ↑ levels at 02:00h and greater AUC post-TSD. Sig. ↓ levels at 07:00h post TSD
Weibel et al. (1995)	14	CR	Every 10min from 23:00h Day 1 to 23:00h Day 2	24h	Amount secreted in first 4 h ~60% greater during TSD. Daytime sleep did not inhibit secretion post-TSD
Dinges et al. (1994)	20 (7f)	AMB	5 samples: BL 22:00h & 22:30h. TSD 22:00h Day 1, Day 2 & Day 3	64h	No apparent association between levels and TSD. Only significant difference occurred between at 22:00h Day 1 of TSD and 22:00h first day post REC sleep
Salín-Pascual et al. (1988)	12 (3f)	NR	During pre and post TSD sleep. Hourly between 22:00h and 06:00h	36h	No sig. AUC change post-TSD
Kant et al. (1984)[c]	6	AMB	Samples 3x24h blocks from 08:00h–07:59h	72h	↓ slightly during TSD. No sig. effect across days
Weitzman et al. (1983)	6	AMB	Every 20min	24h	Sig. ↑ during the first half of the night during TSD

Author(s) and date	*n*	Method	Sampling	TSD	Changes in cortisol
Åkerstedt et al. (1980)	12	NR	2 samples: 08:00h the day before and after TSD	48h	No sig. change post TSD

Note: ↑ = increased; ↓ = decreased; TSD = total sleep deprivation, participants are all male except where specified, f = female; [a]participants are military servicemen – all other studies include laboratory participants; Ambulant (AMB) = participants permitted to walk and move about laboratory; CR = constant routine, participants remained recumbent during experimental period; BL = Baseline; REC = recovery; [b]participants permitted 1 caffeine drink every 3h if habitual coffee drinkers; participants in lab for first 24h of TSD but permitted to leave for remaining 12h of TSD and return that night; [c]Caffeine & tobacco permitted, only study to use urinary (rather than blood) measures; NR = not reported..

2.3 Sleep deprivation and the cortisol awakening response

Studies investigatin CAR post sleep deprivation are minimal. Only one study to date (Vargas & Lopez-Duran, 2017b) has specifically explored the CAR and TSD (see Table 4). Results showed that participants displayed 'blunted' cortisol (CAR) with no significant increase on the morning following 29h ambulant TSD compared to participants in the control who, following an 8h sleep opportunity, demonstrated the traditional rise and fall of cortisol in the 2h post awakening (Vargas & Lopez-Duran, 2017b). Interestingly the authors did not assess presentation of a CAR prior to participants entering the lab nor assess presentation of a baseline CAR following an adaptation night

Also only one published study, has assessed the CAR pre and post a two-week offshore night-shift schedule (Harris et al., 2010). Results showed that the CAR had not recovered to baseline levels (pre night-shift schedule) one week after returning home from a two-week offshore tour (Harris et al., 2010) suggesting the CAR had possibly adapted to the night-shift schedule and re-adaptation/recovery to baseline levels may take considerably longer than one week.

There have been studies that have explored sleep duration (e.g. varying nap times and lengths versus night sleep) and its relationship with CAR. Results from these studies are mixed. Federenko et al. (2004) found students napping for approximately 1h in the early evening (between 18:45h and 20:30h) demonstrated no CAR on awakening. The authors concluded that waking up, by itself, is not sufficient to stimulate the CAR. Similarly, Devine and Wolf (2016) found no CAR in participants after a 50min nap, but participants who had a 90min nap either in the morning or the afternoon, displayed a CAR on awakening. However, the magnitude of the CAR was not as great compared to

participants who awoke from a 6h night sleep. The authors here concluded that sleep duration influences presentation and magnitude of the CAR.

Furthermore, exploring forced/surprised awakenings during the night, Dettenborn et al. (2007) found no presentation of a CAR during these awakenings and concluded a lack of HPA axis activation to the forced awakening, whereas Born et al. (1999) reported a greater increase in cortisol concentrations after a surprise awakening. In contrast to these studies, exploring the alerting effect of bright light on participants exposed to 36h sleep deprivation Leproult et al. (2001) found a stimulatory effect on cortisol levels in the absence of a sleep–wake transition. The elevation in cortisol levels between 05:00h and 05:15h for individuals exposed to bright light in the morning during 36h sleep deprivation was significantly larger than increases occurring at the same clock time relative to baseline. These observations demonstrate that the CAR or the acute cortisol rise observed in the morning does not necessarily require the awakening process to stimulate it.

The literature suggests that approximately 23% of individuals do not show an increase in cortisol levels, that is, the CAR, after awakening (Wust et al., 2000). Additionally, participants in the TSD condition were kept in constant lighting conditions of <40 lux while participants in the control transitioned from a dark to light environment upon awakening. The literature demonstrates exposure to light in the early morning increases cortisol levels (Leproult et al., 2001; Scheer & Buijs, 1999). It is therefore possible that the significant difference in morning cortisol levels could be attributed to the transition from a dark to a light environment.

The literature exploring the impact of TSD on CAR is small. Previous research demonstrates the CAR has been predictive of same day cognitive flexibility/ performance (Hodyl et al., 2016; Law et al., 2015), response inhibition (i.e. conflict monitoring) (Shi et

al., 2018) and differences in synaptic plasticity in the brain (Clow et al., 2014). Collectively, these studies might suggest that without the CAR individuals may experience a decline in cognitive flexibility and behavioural performance. Over time, these implications may translate into higher economic and social costs for individuals, organisations and society. It is therefore important to look at the CAR pre, during and post sleep deprivation. Without the waking process and the CAR it is possible there may be flow on effects that could be studied with further research. The lack of studies exploring the CAR during and post periods of sleep deprivation highlights a gap in the literature that warrants further investigation.

Table 4.
Studies exploring the cortisol awakening response and total sleep deprivation

Author(s) and date	Study sample	Field or laboratory	Biological sampling	Protocol	Results
Vargas & Lopez-Duran (2017b)	*n* = 40	Lab	Saliva: 5 time points, waking, +30, +45 +60, +120, +150min post waking	Ambulatory 24h TSD	Participants in the TSD condition had a blunted CAR as indicated by a flatter initial acceleration and less acute deceleration

Note: TSD = total sleep deprivation; CAR = cortisol awakening response; inclusion criteria = field or laboratory studies exploring the impact of TSD on the CAR as assessed by salivary cortisol levels.

2.4 Conclusion

The narrative in this chapter provided an overview of TSD-induced disruptions to cortisol and the CAR. The findings demonstrated that TSD induces significant disruptions to cortisol; however, the results are inconsistent with regard to whether cortisol levels increase, decrease or both, and the time of day this occurs. Additionally only one study has explored TSD and the CAR, with results suggesting the CAR, too, is impacted by TSD.

Cortisol influences the performance of many physiological systems. It is an important hormone vital for the protection of overall health and wellbeing (Kadmiel & Cidlowski, 2013; Spencer & Deak, 2017). High and low levels of cortisol have been identified in the pathogenesis of various diseases and TSD displays negative effects on both evening and morning cortisol levels. TSD and disrupted sleep across the night produce a smaller CAR. As unhealthy states may be associated with circadian misalignment, the CAR may play a role in physiology closely tuned to circadian rhythms, and unhealthy states could be detected by examining the CAR (Clow, Hucklebridge, Stalder, et al., 2010) during and post periods of sleep deprivation.

The implications of long-term recurring disruptions to the cortisol rhythm and the CAR are poorly understood. It is therefore vital for research to further explore and understand the degree of disruption to the cortisol rhythm and CAR during periods of TSD in field and laboratory settings. Observing and understanding the impact TSD has on the cortisol rhythm and CAR will help to advance knowledge and contribute to the development of future strategies (e.g. sleep education) and interventions aimed at improving the health and wellbeing of populations who regularly experience sleep deprivation.

Science is a great game. It is inspiring and refreshing. The playing field is the universe itself .

- Isidor Isaac Rabi



Thesis Aims

Literature summary and thesis aims

A 24h society necessitates a 24h workforce. It is therefore important to understand the impact of night shift and sleep deprivation and the concomitant behavioural changes that occur with altering sleep–wake patterns, such as eating during the night, on cortisol rhythms. Such knowledge will aid in developing future strategies and interventions aimed at improving the health and wellbeing of people impacted by night shift and sleep deprivation.

The overarching aim of this thesis is to investigate the impact that TSD or working night shifts has on the cortisol rhythm. The four specific research aims addressed in Chapters 4, 5 and 6 of this thesis are outlined below.

Study 1, Chapter 5: Cortisol regulates multiple body systems and displays a circadian rhythm aligned with the sleep–wake and dark–light cycle. Shiftwork disrupts the normal 24h light–dark exposure, sleep–wake cycles and the circadian regulation of behavioural and physiological processes like cortisol secretion. Cortisol is often considered a stress hormone and elevated levels of cortisol at night could be a mechanism linking shiftwork and negative health outcomes such as weight gain and impaired glucose metabolism (Grant et al., 2017; Salgado-Delgado et al., 2010). Inconsistencies have been reported in the literature on the impact of night shift on cortisol; some report an increase in levels and reversal of the rhythm, while others report no change. Behaviours that change concomitantly with night shift include timing of eating. The extent to which altered patterns of eating during night shift impact cortisol is largely unknown, but it could be another way in which cortisol is elevated during night shift. Chapter 4 investigates the impact of four consecutive night shifts on the cortisol rhythm. Therefore Chapter 4 has two research aims.

Research Aim 1: Determine the impact of four consecutive simulated night shifts on the cortisol rhythm.

Research Aim 2: Examine the impact of eating a meal or snack, or not eating on cortisol during four consecutive simulated night shifts.

Study 2, Chapter 6: Sleep deprivation is considered a physiological stressor impacting the brain and many physiological systems (Wright et al., 2015; McEwen, 2006) but many inconsistencies have been reported in the literature on the effects of sleep deprivation on cortisol. Some studies have found an effect of sleep loss on cortisol with total sleep deprivation demonstrating negative effects on evening and morning cortisol levels. But others report no effect. Such inconsistencies may be due to differences in methodology and regularity of sampling making it difficult to determine if sleep deprivation does increase this stress hormone. Therefore Chapter 5 has one research aim.

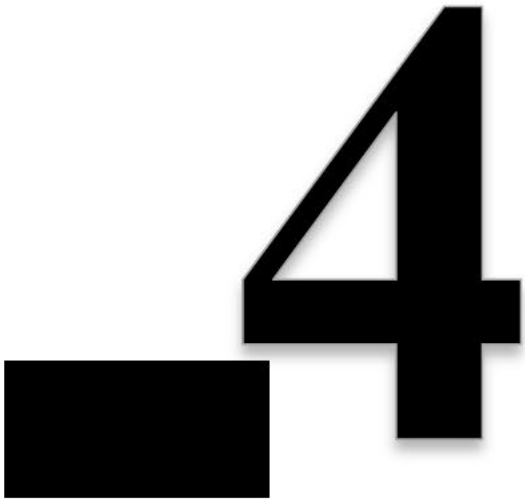
Research Aim 3: Investigate the impact of 62h of TSD on cortisol rhythms.

Study 2, Chapter 7: The CAR is a key reference point of a healthy circadian cortisol rhythm and is influenced by, among other factors, the sleep–wake cycle. The literature shows that sleep, pre-awakening cortisol levels and the awakening process influence the presence and magnitude of the CAR. What is not clear is how morning cortisol levels and the CAR is affected during and post periods of acute sleep deprivation and how the CAR responds post sleep deprivation. Therefore Chapter 6 has one research aim.

Research Aim 4: Examine if there is a sudden morning rise in salivary cortisol that mimics CAR when no awakening occurs, and investigate recovery sleep CAR after a period of 62h TSD.

I am always doing what I cannot do yet, in order to learn how to do it.

- Vincent van Gogh



Methods

Chapter 4: Method

Two controlled laboratory studies were conducted, the results forming the foundation of this thesis. Each study explored the hormone cortisol and its response to shiftwork, the intake of food during night shift and sleep deprivation. Study 1 was a cross-sectional experimental study exploring cortisol's response to four consecutive night shifts and food intake at night. Study 2 was a cross-sectional experimental study designed to explore how the circadian cortisol rhythm and the CAR respond to 62h TSD. Study 1 is reported in Chapter 5. While reported across Chapters 6 and 7, corresponding to Research Aims 3 and 4 respectively, Study 2 was a single 62h TSD study.

4.1 Ethics and informed consent

4.1.1 Ethics approval

Study 1 and Study 2 were approved by the University of South Australia's (UniSA) Human Research Ethics Committee (Study 1: 0000033621); Study 2 (202112) in accordance with the Declaration of Helsinki. Study 1 was registered with the Australia and New Zealand Clinical Trials Registry (ACTRN12615001107516). To ensure participant confidentiality, participant ID numbers were allocated to participants in both studies with all associated data coded accordingly.

4.1.2 Informed consent

Participants were provided with a comprehensive participant information form when first expressing interest in participating in either Study 1 or Study 2 and again at their first in-laboratory screening session for either study. The information form advised participants on the study protocol, potential risks and honorarium details (Appendix 3 – Study 2; Appendix 4 – Study 2). Participants were encouraged to read the information handout again at the in-laboratory screening session and to ask any questions. Informed consent

was obtained after participants were asked if they wished to proceed with the study and they indicated their desire to do so (Appendix 5 – Study 1; Appendix 6 – Study 2).

4.2 Recruitment

Participants were recruited using advertising flyers that summarised the studies and honorarium. Flyers were displayed on community and university notice boards, social media sites and classified advertisements posted on a free online marketplace (Gumtree Australia) (Appendix 7 – Study 1; Appendix 8 – Study 2). Individuals wishing to participate contacted researchers via email. A researcher then replied via email requesting further information from the potential participant regarding age, gender, height, weight, contact details and preferred day and time for contact to be made. The participant information form was included with this email. Upon receipt of the requested information from potential participants a researcher would call and conduct a comprehensive telephone screening session taking ~30min to complete. Questions in this screening session addressed some of the inclusion criteria (Table 5) in addition to providing participants with a more detailed overview of the study and screening processes. Individuals deemed eligible for the study were then scheduled for the first of two in-laboratory screening sessions.

Table 5.
Inclusion criteria

Criteria	Measured	Study 1	Study 2
Healthy adults (18–40 years)	Initial email Participant demographic information form	•	•
Physically healthy	Comprehensive telephone screen Participant demographic information form Confidential medical screen Complete blood profile	•	•
Habitual nightly sleep 6–9h	Comprehensive telephone screen Actigraphy and sleep diary for 2 weeks prior to study Pittsburgh Sleep Quality Index (Buysse et al., 1989)	•	•
No lights out earlier than 21:00h on weeknights	Comprehensive telephone screen	•	•
No morning wake later than 09:00h on weekdays	Comprehensive telephone screen Pittsburgh Sleep Quality Index	•	•
No habitual napping	Comprehensive telephone screen Actigraphy and sleep diary for 2 weeks prior to study	•	•
No caffeine intake >400mg daily (8 caffeinated sodas or 4 coffees)	Comprehensive telephone screen Participant demographic information form	•	•
No cardiovascular disease	Comprehensive telephone screen Participant demographic information form	•	•
No neurological disorders	Comprehensive telephone screen Participant demographic information form	•	•
No sleep disorder	Pittsburgh Sleep Quality Index Sleep–wake survey	•	•
No pulmonary disease requiring daily inhaler use	Comprehensive telephone screen Participant demographic information form	•	•
No liver disease or abnormalities	Comprehensive telephone screen Participant demographic information form Confidential medical screen	•	•
Psychologically healthy	Comprehensive telephone screen Beck Depression Inventory (Beck, et al., 1996)	•	•
Non-smoker	Comprehensive telephone screen Participant demographic information form	•	•
No excessive alcohol consumption (>14 drinks per week)	Comprehensive telephone screen Participant demographic information form	•	•
No illicit drug use	Comprehensive telephone screen Participant demographic information form Urinalysis	• • •	•

Criteria	Measured	Study 1	Study 2
Not pregnant	Comprehensive telephone screen Complete blood profile	•	•
No BMI ≥ 30kg/m2	Comprehensive telephone screen Confirmed on 1st in lab screening Participant demographic information form	•	•
No corticosteroid or anti-inflammatory medications	Comprehensive telephone screen Participant demographic information form Confidential medical screen	•	•
No transmeridian travel in previous 2 months	Comprehensive telephone screen Participant demographic information form	•	•
No blood donation in the previous month	Comprehensive telephone screen Participant demographic information form	•	•
Suitable veins for cannulation	Examination during blood collection	•	
No shiftwork in the past 6 months	Comprehensive telephone screen Participant demographic information form	•	•
Not recently commenced (<12 months) transgender hormone therapy	Comprehensive telephone screen		•
No extreme exercise routine	Comprehensive telephone screen	•	•
No food difficulties (e.g. vegetarian, gluten intolerance)	Comprehensive telephone screen Participant demographic information form	•	•

Note: mg = milligrams; kg = kilograms; m = metres; BMI = body mass index

4.2.1 Screening session one

Screening session one required individuals to attend the sleep laboratory where the study would be conducted. Individuals were given a tour of the sleep laboratory and had the purpose and requirements of the study explained to them (verbally and in writing). Prior to completing a battery of questionnaires individuals gave written consent. Questionnaires included a subject enrolment and demographic information form, Pittsburgh Sleep Quality Index (PSQI), sleep–wake survey, Composite Morningness and Eveningness Scale, Beck Depression Inventory (BDI), Confidential Medical Screen, Berlin Questionnaire (Study 2 only), Stop Bang Questionnaire (Study 2 only) and Handedness Questionnaire.

For Study one, participants were screened for drugs (urine sample) that may influence usual sleep–wake behaviour. Urine samples were collected and analysed using a Split Specimen Cup (LaneWorkSafe, Australia) with all participants meeting the required urinalysis criteria (Table 6) Additionally for Study 1, to ensure the participant's veins were suitable for intravenous cannulation during the study, and to assess the physiological health of participants, a blood sample was taken. This sample was sent to SA pathology for analysis.

Table 6.
Screened urinalysis criteria

Drug	Maximum Level
Amphetamine	300 ng/ml
Methamphetamine	300 ng/ml
Cocaine	300 ng/ml
Marijuana	50 ng/ml
Benzodiazepines	200 ng/ml
Morphine	300 ng/ml

In Study 2, participants were not drug tested nor assessed for intravenous cannulation; however, to assess physiological health prior to commencing the study participants attended Clinpath Laboratories where a blood sample was obtained and analysed. Dr Gary Wittert reviewed and approved blood results for both Study 1 and Study 2, ensuring participants results fell within the reference interval (Table 7).

Table 7
Screened bloods criteria

Blood Parameter	Units	Reference Interval
Biochemistry		
Sodium	mmol/L	135–145
Potassium	mmol/L	3.5–5.5
Chloride	mmol/L	95–110
Bicarbonate	mmol/L	20–32
Urea	mmol/L	2.5–6.5
Creatine	umol/L	45–85
eGFR	ml/min	<59
Uric acid	mmol/L	0.15–0.40
Calcium	mmol/L	2.15–2.55
Corrected calcium	mmol/L	2.12–2.55
Phosphate	mmol/L	0.7–1.5
Total bilirubin	mmol/L	3–15
Alk Phos	U/L	20–105
Gamma GT	U/L	5–35
LDH	U/L	120–250
AST	U/L	10–35
ALT	U/L	5–30
Total protein	g/L	68–85
Albumin	g/L	37–48
Globulin	g/L	23–39
Cholesterol	mmol/L	3.5–5.5
Glucose fasting	mmol/L	3.6–5.4
Haematology		
Haemoglobin	g/L	119–160
Red Cell count	x10^12/L	3.8–5.8
Haematocrit		0.35–0.48
MCV	fL	80–100
MCH	pg	27.0–32.0
MCHC	g/L	310–360
RDW		10.0–15.0
White cell count	x10^9/L	4.0–11.0
Neutrophils	x10^9/L	1.7–7.5
Lymphocytes	x10^9/L	1.0–4.0
Monocytes	x10^9/L	0.0–1.0
Eosinophils	x10^9/L	0.0–0.5
Basophils	x10^9/L	0.0–0.3

Blood Parameter	Units	Reference Interval
Platelets	x10^9/L	150–450
Thyroid Function		
TSH	mU/L	0.40–3.50

Note: mmol/L = millimoles per litre; g/L = grams per litre; umol/L = micromoles per litre; U/L = units per litre; fL = femtolitre; pg = pictogram; mlU/L = milli International Unit per litre; eGFR = estimated glomerular filtration rate; Gamma GT = gamma-glutamyl transferase; LDH = lactate dehydrogenase; AST = aspartate aminotransferase; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration; RDW = red cell distribution width; TSH = thyroid stimulating hormone

In addition to questionnaires, drug screening and blood tests, participants were required to take home and complete some tasks prior to attending the second screening session. For Study one this included a seven-day sleep–wake diary and a four-day food diary to provide understanding of their normal dietary intake. Participants were also required to wear an activity monitor, the data from which were used to confirm self-reported sleep–wake schedules. For Study 2 participants wore a GeneActiv watch and completed a seven-day sleep–wake diary.

4.2.2 Screening session two

Participants deemed eligible to continue with the screening process were contacted via email and/or phone soon after screening session one and booked in for screening session two, which took place one week prior to commencement of the laboratory study. During session two, participants were given another tour of the sleep laboratory and again had the purpose and requirements of the study explained to them (verbally and in writing). For Study 1 and Study 2, participants were provided with a list of foods that were to be included in the menu during the study and were asked to confirm they were willing to consume the foods listed. Additionally, participants were provided with a list of things they would need to bring/pack for their time in the laboratory, and a list of pre-study reminders.

For Study 2, participants were provided with a GeneActiv watch and a take home pack of tasks they were to complete and bring with them on Day 1 of the laboratory study. Detailed information regarding these tasks are provided in Chapters 5 and 6.

4.3 Participants

Data from 52 participants in Study one were used in the final analysis exploring four consecutive simulated night shifts with varied eating patterns on the cortisol rhythm.Data from 21 participants in Study 2 were used in the final analysis exploring the cumulative effect of 62h of TSD on the cortisol rhythm and investigating the effect of 62h TSD on the CAR. Table 8 displays participant demographics for both studies.

Table 8
Participant demographics for each study

Study	Condition	*n* (M, F)	Age[a] *M* (*SD*)	BMI[b] *M* (*SD*)
Study 1	Condition 1	14 (M8, F6)	24 (3.66)	25.28 (3.19)
	Condition 2	19 (M11, F8)	24.74 (5.41)	23.75 (2.31)
	Condition 3	16 (M8, F8)	25.44 (5.60)	23.62 (1.94)
Study 2	TSD	21 (M10, F11)	22.80 (4.40)	21.98 (3.64)

Note: [a] = years; BMI = body mass index; [b] = kg/m2; TSD = total sleep deprivation

4.4 Study design

4.4.1 Laboratory setting

For both studies, participants resided in the Sleep and Chronobiology Laboratory (SCBL) at UniSA. This laboratory has no clocks, is windowless and sound attenuated. Study one was conducted at two sites with the first seven study runs conducted at the SCBL, City East campus of UniSA where participants had their own bedroom but shared bathroom and lounge facilities. The laboratory floor plan is depicted in Figure 2.

Testing rooms 1 and 2 were used for blood collection, aliquoting and storage. Two blood-taking stations were set up in each room. Partitions were erected between each station to provide participants with privacy and to reduce possible anxiety issues during cannulation.

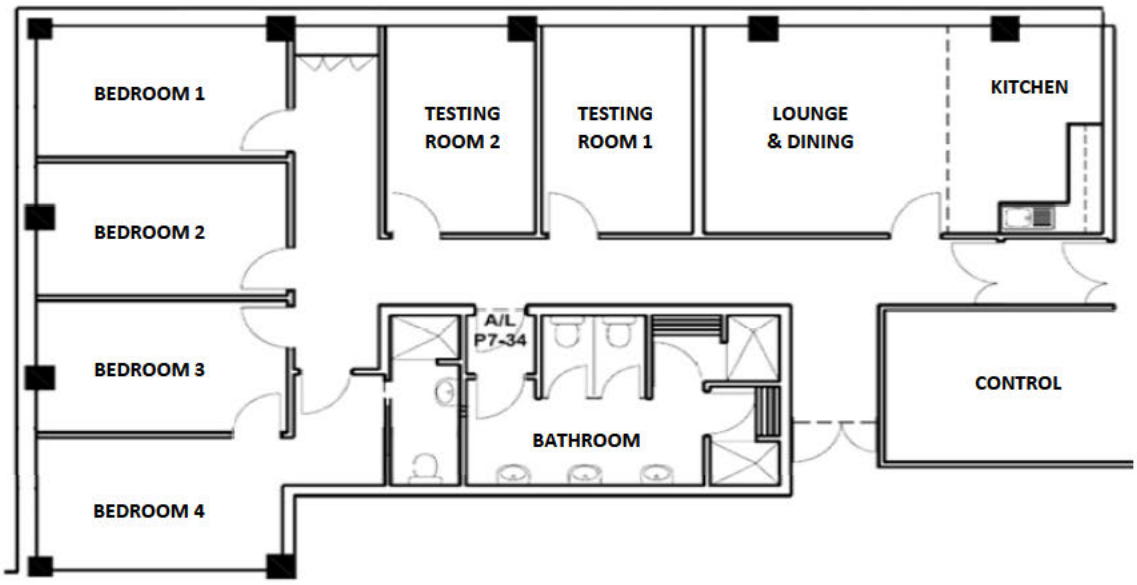


Figure 2.

Floor plan of Sleep and Chronobiology Laboratory (City East campus)

The remaining eight study runs were undertaken at the new SCBL, Magill campus of UniSA where participants had their own bedroom with ensuite and shared lounge facilities. The laboratory floor plan is depicted in Figure 3. The dedicated wet lab/treatment suite was used for blood collection, aliquoting and storage. Four blood-taking stations were set up with pull-around curtains providing partitioning between participants. All study runs for Study 2 were undertaken at the SCBL, Magill campus of UniSA.

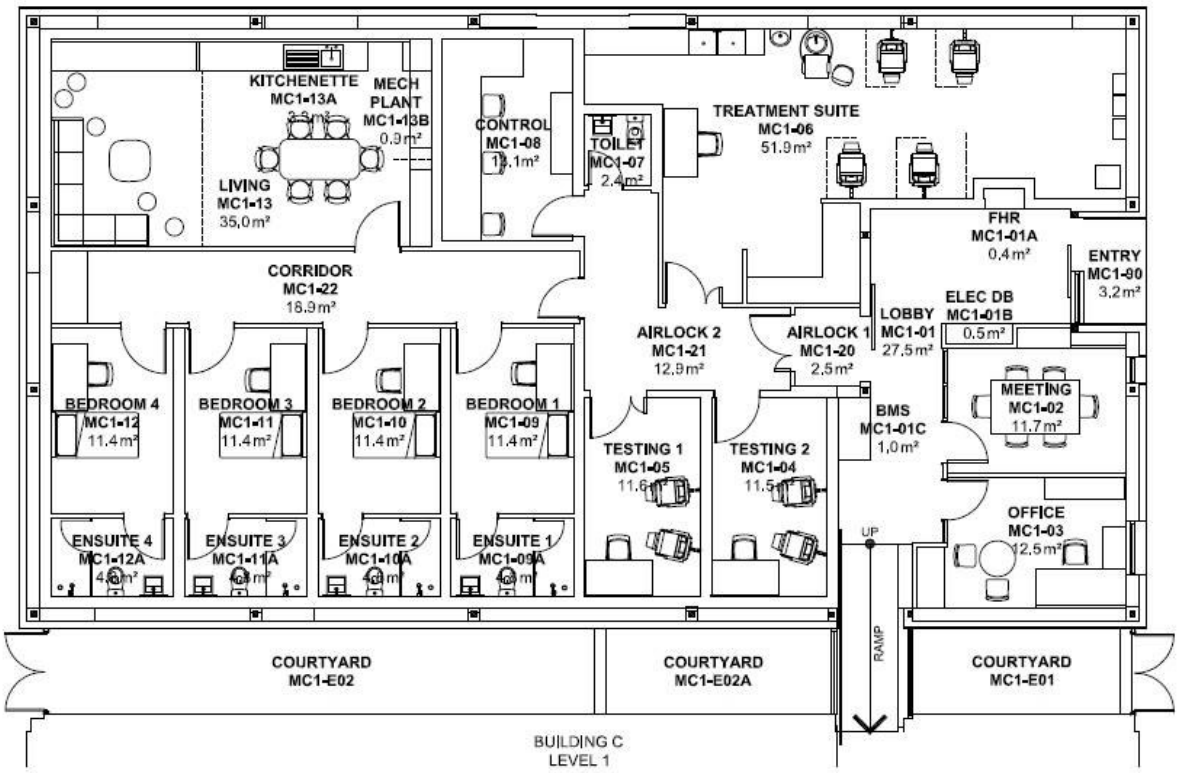


Figure 3.

Floor plan of Sleep and Chronobiology Laboratory (Magill campus)

A constant room temperature of 22 (±1ºC) was maintained in the laboratory at all times. For both study protocols, light intensity during scheduled wake periods was set to 100 lux at eye level to simulate an office work environment and <1 lux (darkness) during scheduled sleep periods.

Participants were each assigned their own room and were prohibited from entering other participant's rooms. Participants' rooms were equipped with computers for undertaking cognitive tests and driving simulators. Computers were not connected to the internet and were not permitted for use outside of testing times.

4.4.2 Laboratory protocol

Participants involved in the study and research staff were the only individuals permitted entry into the sleep laboratory during the dedicated study period. All research staff wore a standard uniform and were required to avoid wearing or carrying articles (e.g. sunglasses) that may provide information as to the time of day. Participants were required to surrender all electronic devices that would provide information as to time of day (smart watches, phones, laptops etc.) upon entry to the laboratory; these were returned to participants at the end of the study.

When not undertaking neurobehavioural assessments or having biological samples taken, participants were encouraged to engage in leisure time in the shared lounge facility where they could interact with fellow participants and research staff, watch DVDs, read, do puzzles or play board or card games.

Participants were monitored at all times (except when using bathroom facilities) by research staff who took detailed notes relating to participant behaviour. Additionally, all bedrooms were equipped with closed-circuit television cameras that were operational only during scheduled sleep times to monitor participants for safety reasons and ensure compliance with the study protocol during sleep periods.

All participants were required to make their own transport arrangements to and from the sleep laboratory. Participants were strongly advised not to drive themselves to or from the facility but rather take public transport, a cab or have someone drive them.

4.4.3 Food intake

Participants were instructed that no food was to be brought into the laboratory as all meals and snacks were provided for the duration of each study. For Study one and Study 2, the energy requirements for each participant were calculated using Harris Benedict

equations (Harris & Benedict, 1919), and the macronutrient content of meals was based on the average Australian diet and was controlled within each meal (Australian Bureau of Statistics, 1997). Chapters 5, 6 and 7 cover meal timing and composition. All meals were consumed in the participant's bedroom. Participants were encouraged to eat all food presented to them and instructed that no food was to be kept for later. Research staff prepared all meals in a kitchen facility that was screened off from participants. Research staff were required to weigh and record each food item pre and post consumption.

For Study 1, the timing of food intake during the simulated shiftwork protocol varied between conditions to accommodate testing schedules. The meal at night group consumed food at 19:00h (dinner), 00:30h (meal at night) and 07:00h (breakfast). The snack at night group consumed food at 19:00h (dinner), 00:30h (snack at night), 07:00h (breakfast), 17:00h (snack on waking). The no meal at night group consumed food at 19:00h (dinner), 07:00h (breakfast), 09:30h (snack before bed) and 17:00h (snack on waking). Participants in Study one were permitted to only drink water; consumption of caffeine was prohibited.

For Study 2, the timing of food intake remained constant in each study run: Breakfast at 08:00h, lunch 12:00h, dinner 18:00h and a snack at 00:30h. During Study 2, participants were permitted to drink water or non-caffeinated herbal tea; consumption of caffeine was prohibited.

4.5 Materials

4.5.1 Biological outcome measures

Saliva samples were collected using the Salivette® (Sarstedt, Numbrecht, Germany) with cotton swab inserts. Participants were instructed to place the cotton swab in their mouth without touching it and roll (not chew) it around in their mouth for 2min. After 2min participants were instructed to carefully spit/return the cotton swab back into the salivette,

again without touching it with their hands. The Salivettes® tubes were then stored at −80ºC prior to analysis. Chapters 4 and 5 and 6 discuss saliva sampling times.

4.5.2 Assay procedure

Saliva cortisol was determined at the Adelaide Research Assay Facility by Enzyme Linked Immunosorbent Assay (ELISA) (1-3002, Salimetrics, State College PA, USA). Samples were assayed directly as per the manufacturer's protocol.

Saliva cortisol was determined by adding 25 µl of sample to a 96 well plate coated with anti cortisol monoclonal antibodies, and 25 µl of a 1:1600 dilution of cortisol conjugated to horse radish peroxidase (HRP). The plate was incubated for 60min, during which time cortisol from the standards and unknowns competed for antibody binding sites with the HRP conjugated cortisol. The plate was then washed to remove unbound cortisol. Bound cortisol peroxidase was measured by the reaction of the HRP enzyme with the substrate tetramethylbenzidine (TMB). This reaction was stopped by adding sulfuric acid and optical density was then read on a Biotek Synergy H1 plate reader (Winooski, Vermont, USA) at 450 nm. The amount of antibody bound cortisol-HRP detected, as measured by the intensity of colour, is inversely proportional to the amount of cortisol present. The amount of cortisol present in samples was determined by interpolation against the standard curve using the Biotek plate-reader analysis software package, Gen5. Standards and controls were assayed in duplicate, samples were assayed in single.

The lower limit of quantitation (LLoQ) for the assay was 0.012 ng/ml. The intra-assay coefficient of variation of the assays was 10.3%. The inter-assay coefficient of variation of the assays at the low-quality control level (0.108 ng/ml) was 10.5%. The inter-assay coefficient of variation of the assays at the high-quality control level (1.09 ng/ml) was 6.0%.

It is looking at things for a long time that ripens you and gives you deeper meaning.

- Vincent van Gogh



Circadian and behavioural impacts on cortisol during simulated night shifts

Chapter 5: Circadian and behavioural impacts on cortisol during simulated night shifts

Abstract

Cortisol regulates multiple body systems and displays a circadian rhythm aligned with the wake–sleep and light–dark cycle. There is little information on the cumulative effect of consecutive night shifts on the pattern and timing of cortisol changes, and how this may be affected by the food intake during the night shift.

This study aimed to determine the impact of four consecutive simulated night shifts with varied eating patterns at night on the cortisol rhythm. Participants completed four in-laboratory consecutive night shifts (Nights 1−4 20:00h–05:30h). Participants were assigned to a meal at night (n = 17), snack at night (n = 16), or no food at night (n = 19) condition. Saliva was collected across 15 time points between 20:00h and 05:30h per night shift. Mixed-effects Analysis of Variance (ANOVA) tested for fixed effects of night, time, condition and their interactions on cortisol, and cortisol AUC, AUC_g and AUC_i, post meal snack and the circadian morning cortisol rise (03:00h–05:30h) with subjects as a random effect, each with their own intercept.

There was a significant night-by-time interaction ($p < 0.001$), with higher cortisol levels at 20:00h and lower levels at 05:30h on each consecutive night shift. There was also a significant 3-way night, time, and meal condition interaction ($p = 0.029$) on the morning cortisol rise, with greater decreases in the meal and no-meal-conditions with each consecutive night shift.

Both night shift and eating during the night shift induce changes in cortisol. Further research is needed to better understand relationships between altered sleep–wake cycles,

meal timing, and cortisol rhythm changes on cortisol-regulated health outcomes in shiftworkers.

5.1 Introduction

Shiftwork refers to work done outside the traditional hours of 09:00h–17:00h (Costa, 2003), and is a recognised risk factor for chronic health conditions including metabolic syndrome, type 2 diabetes and cardiovascular disease (Knutsson, 2003; Puttonen et al., 2010; Rogers et al., 2021). Shiftwork disrupts the normal 24h light–dark exposure and wake–sleep cycles, and the circadian regulation of behavioural, cellular and metabolic and physiological processes, including cortisol secretion (Hastings et al., 2018; Reppert & Weaver, 2002).

Cortisol displays a distinct circadian rhythm. Cortisol importantly regulates intermediary metabolism (Hiller-Sturmhöfel & Bartke, 1998), immune and inflammatory processes and cognition (Buckingham, 2006; Kadmiel & Cidlowski, 2013; Munck & Náray-Fejes-Tóth, 1992; Oster et al., 2017). Secretion is controlled by the HPA axis and levels are continuously adjusted via a negative feedback system to maintain homeostasis (Clow et al., 2010; Heaney, 2013). The cortisol rhythm has a close temporal association with the sleep−wake and night−dark cycles. Cortisol levels are normally at their lowest and begin to gradually rise ~2−3h after sleep onset, abruptly rise to a peak within the first hour post awakening, then steadily decline across the day (Born et al., 1986; Hellhammer et al., 2009; Liyanarachchi et al., 2017). The diurnal rhythmicity of cortisol can be masked by food consumption (Legler et al., 1982; Van Cauter et al., 1992) and shifted by altered sleep−wake cycles (Leproult et al., 1997) such as those experienced by night shiftworkers.

A growing body of evidence from cross sectional and clinical studies demonstrate that high and low levels of circulating cortisol and persistent subtle disturbances in the cortisol rhythm can lead to dysfunction of systems influenced by cortisol, which may play a role in the pathogenesis of health burdens. For example, recent research describes cortisol as

one of the key factors in the pathogenesis of metabolic syndrome (highly associated with shiftwork and hypercortisolism) and its symptoms are similar to those of Cushing's syndrome, a disease associated with a disturbed circadian rhythm and hypercortisolism (Chung et al., 2011; Macfarlane et al., 2008; Walker, 2006). Likewise, long-term elevated cortisol levels are associated with cardiovascular disease and high blood pressure (Iob & Steptoe, 2019; Manenschijn et al., 2013; Walker, 2007).

Cortisol has frequently been explored in shiftworkers, with studies exploring the impact of shiftwork schedules, particularly night shift, on cortisol levels and its rhythm (see review in Grosser et al., 2022). However, previous studies are constrained regarding the number and timing of samples collected (blood or saliva), number of night shifts explored and sample size. A 24h CR study minimises exposure to stimuli that evoke response(s) in the cortisol rhythm (Fujiwara et al., 1992; Roden et al., 1993; Weibel et al., 1996). Participants during a CR protocol are exposed to continuous ambient light, evenly distributed meals throughout the day, a constant posture and wakefulness over a 24h time period (Czeisler & Gooley, 2007). Although not a reflection of real-world light and dark, a wake−sleep exposure protocol associated with night shiftwork is ideal to assesses the endogenous circadian phase and amplitude of the cortisol rhythm over a 24h period. Field studies that capture a more real-world experience have often only explored a single night shift with insufficient cortisol sampling to assess the cumulative effects of night shift on cortisol (Cannizzaro et al., 2020; Choshen-Hillel et al., 2021; Morales et al., 2019). Other studies have collected samples at the start, in the middle and on the last night of a night-shift roster over more than seven days (Harris et al., 2010) or on the last night only (Jensen et al., 2016), thereby potentially under-sampling when cortisol changes may have occurred within and between days. Laboratory studies that simulate shiftwork across consecutive night shifts, to allow for more frequent cortisol sampling in a tightly

controlled behavioural and lighting environment, permit opportunity to better understand the cumulative effect of night shifts on cortisol.

Given no evidence to support that energy intake systematically differs between traditional day workers and shiftworkers (Bonham et al., 2016; de Assis et al., 2003), there is a need for shiftworkers to fit mealtimes around their shift schedules, which may simply be to alter their mealtimes without substantially changing macronutrient intake. Consequently, night shiftworkers often eat during the night and distribute their daily food intake by eating smaller meals and snacks over a greater time frame (Reeves et al., 2004).

Shifting food intake to the night, a time normally primed by the circadian system for rest and low cardiometabolic activity, coincides with the cortisol nadir when glucose tolerance may become problematic (Gur et al., 2014; Plat et al., 1996; Wehrens et al., 2017). Such behaviours, out of synchrony with underlying circadian regulation of metabolism may, to some extent, explain or exacerbate shiftwork-related alterations to physiological systems influenced by cortisol (Leung et al., 2021).

Recent evidence from rodent and human studies supports that eating food during the body's natural rest phase and circadian nadir of cortisol, promotes glucose intolerance, weight gain and obesity (Damiola et al., 2000; Morris et al., 2015; Salgado-Delgado et al., 2010). Moreover, Salgado-Delgado et al. (2010) showed that when rats were fed during their rest phase (day), mean corticosterone levels were high compared with rats fed during the active phase (night). They concluded that high levels of corticosterone, known to increase lipogenesis and stimulate abdominal fat (Wajchenberg, 2000), may likely contribute to weight gain and abdominal fat in rats fed during their rest phase. Corticosterone, like cortisol in humans, is the predominant glucocorticoid produced after stimulation of the HPA axis across many species including mice and rats, and regulates

diverse physiological processes such as immune, metabolism and stress responses (Koren et al., 2012; Ramamoorthy & Cidlowski, 2016).

While many studies have investigated energy intake, sleep restriction and altered mealtimes on outcomes such as weight gain and glucose tolerance (Dinneen et al., 1993; Gu et al., 2020; Rizza et al., 1982; Van Cauter et al., 1992), to what extent altered patterns of food intake during night shift impact cortisol is unknown. Therefore, using four consecutive simulated night shifts, with varied eating patterns on the cortisol rhythm, the aims of this study were to determine 1) the impact of four consecutive simulated night shifts on the cortisol rhythm and 2) the impact of eating a meal or snack, or not eating on cortisol during four consecutive simulated night shifts.

5.2 Ethical considerations

This study was approved by UniSA's Human Research Ethics Committee (#0000033621) and was prospectively registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615001107516). All participants were provided with comprehensive information sheets detailing the study procedures prior to providing written informed consent and contributing de-identified data.

5.3 Participants

Healthy, non-shiftworking individuals were recruited for this study via advertising flyers (Appendix 7) and website postings summarising the study and associated honorarium. An initial telephone screen collected self-reported sleep patterns (~bed and wake times), general health status, height/weight, age, smoking status, daily/weekly alcohol and caffeine intake. If deemed eligible, participants then attended face-to-face screening sessions where they completed a general health questionnaire providing any clinical history, the PSQI (Buysse et al., 1989), the composite Morningness−Eveningness

questionnaire (Horne & Östberg, 1976) and the BDI (Beck et al., 1996) to determine psychological health. To further determine good physical health and assess morning fasting blood glucose, all participants underwent a fasting blood screen. Individuals were excluded from participating if they met any of the exclusion criteria, for example, food allergies, dieting or sleep disorders (Table 9). To control for changes in circulating cortisol levels, sleep quality and basal metabolism, female participants were only scheduled to participate during the luteal phase of their menstrual cycle (Baker & Driver, 2004; Curtis et al., 1996; Driver et al., 2008; Hamidovic et al., 2020). In the week (seven days) prior to commencing the study, participants were required to adhere to a strict sleep schedule (verified by wrist actigraphy and a sleep diary) of retiring between 22:00h and 23:00h and waking between 06:00h and 07:00h, and to abstain from caffeine, alcohol and napping.

Table 9.
Exclusion criteria

Criteria	Assessed by
Known sleep disorder or sleep disorder breathing	Self-report general health questionnaire
Sleep disturbance or habitual napping	Self-report telephone screen and score > 6 Pittsburgh Sleep Quality Index
Food allergies / intolerance / restricted eating	Self-report general health questionnaire
BMI > 30kg/m2	Objective height/weight measurements
Use of prescription or over-the-counter medication known to affect cortisol (contraception was permitted)	Self-report general health questionnaire
Illicit drug use or active smoker	Self-report general health questionnaire. Urine toxicology performed immediately prior to commencement of study
Not engaged in shiftwork or transmeridian travel in the 60 days prior to the study (assessed to ensure stable sleep–wake patterns and no circadian misalignment)	Self-report telephone screen and general questionnaire
Excessive alcohol consumption (>2 standard drinks per day)	Self-report telephone screen and general health questionnaire
Excessive caffeine consumption (>2 cups per day)	Self-report telephone screen and general health questionnaire
Pregnancy	Self-report telephone screen and blood results
Abnormal blood chemistry	Fasting blood test results and confirmed by a registered General Practioner
Psychological health	Self-report telephone screen and Beck Depression Inventory (score ≥ 14)
Neurological disorders	Comprehensive telephone screen
Pulmonary disease requiring daily inhaler use	Comprehensive telephone screen

Note: BMI = body mass index

5.4 Study protocol design

This study was conducted at the SCBL at UniSA. This laboratory has no clocks, is windowless, sound attenuated and maintained at a constant temperature of 22 (±1ºC). Light intensity during scheduled wake periods was set to 100 lux at eye level to simulate an office work environment and <1 lux (darkness) during scheduled sleep periods. Participants were each assigned their own bedroom with ensuite and were prohibited from entering other participant's rooms. Participant rooms were equipped with computers for undertaking cognitive driving simulator tests. Computers were not connected to the

internet and were not permitted for use outside of testing times. Participants remained in the laboratory for seven consecutive days and were randomly assigned at the study run level to one of three conditions: meal at night (n = 17), snack at night (n = 16), or no eating at night (n = 19). Participants remained blinded to the presence of different meal conditions. Participants entered the laboratory on Day 1 to familiarise themselves with the laboratory environment. The protocol (Figure 4) included a baseline sleep opportunity of 8h time in bed (TIB), before participants transitioned to the simulated night-shift schedule. All participants completed four in-laboratory consecutive night shifts (Nights 1−4, 20:00h–05:30h) with a daily sleep opportunity (10:00h–17:00h). During waking hours, saliva samples (assayed for cortisol) were collected hourly and at 30min, 60min and 120min post 00:30h test meal/snack. Participants in the no eating at night condition were also sampled at these times. Participants had a recovery sleep opportunity of 8h TIB, exiting the laboratory on Day 7.

Participants were continuously monitored by research assistants during all periods of wakefulness. A number of neurobehavioural test batteries (cognitive performance and questionnaires) and driving simulation task (reported in previous publications Grant et al., 2017; Gupta et al., 2017) were completed during the night shift. When not undertaking neurobehavioural assessments, driving simulation or providing biological samples, participants were encouraged to engage in leisure time in the shared lounge facility where they could interact with fellow participants and research staff, watch DVDs, read, do puzzles, or play board or card games. Vigorous exercise was not permitted given known stimulus effects of exercise on the HPA axis that increase cortisol levels (Hill et al., 2008).

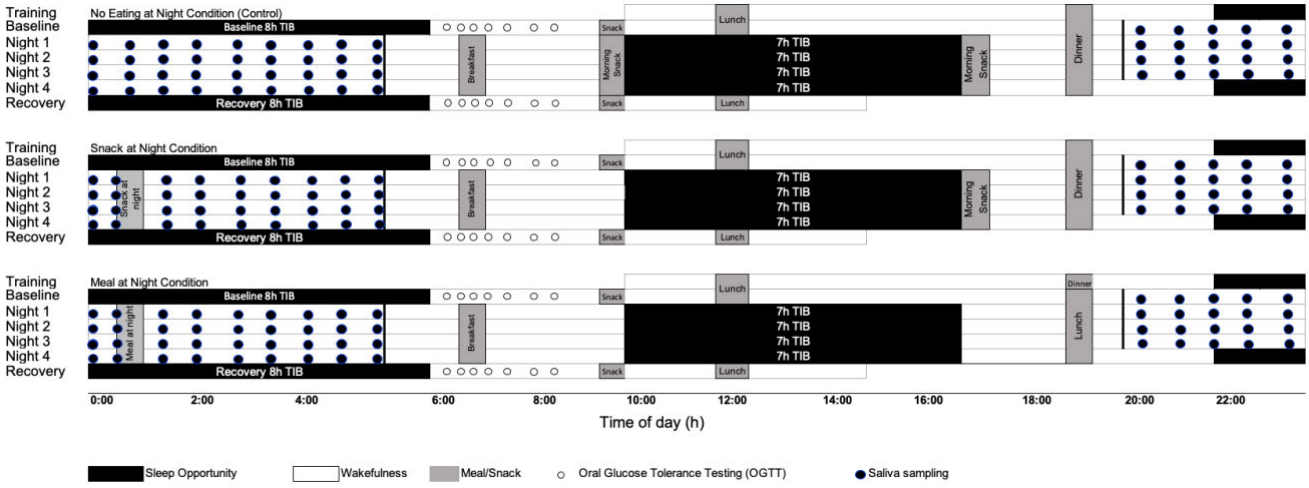


Figure 4.

Protocol schematic displaying sleep periods, general mealtimes, test meal/snack time and saliva collection times across time of day

Note: (x-axis; midnight 00:00h through to 23:00h) and day (y-axis).Black blocks = sleep opportunity; light grey blocks = general mealtimes and test meal/snack times; white blocks = periods of wake; black dots = saliva sampling times; white dots = oral glucose tolerance testing. Night shift was from 20:00h–05:30h. During this time participants also performed several neurobehavioural test batteries and driving simulation tasks. Participants had free/leisure time outside of the night-shift hours.

5.4.1 Food intake

Food intake and mealtimes were strictly controlled during each study run. Participants were not permitted to bring food into the laboratory and all meals and snacks were provided for the duration of the study. The energy requirements for each participant were calculated using the validated Harris Benedict equations (Frankenfield et al., 2003; Harris & Benedict, 1919) and rounded to the nearest 500 kJ, ensuring the energy content maintained body weight for each subject. Regardless of which condition participants were allocated to, the macronutrient content of meals was based on the average Australian diet (Australian Bureau of Statistics, 1997). Meal plans for participants were created using FoodWorks version 8 (Xyris Pty Ltd, 2015) with data sourced from the AusBrands 2017 and AusFoods 2017 databases. Figure 5 shows mealtimes and an example meal plan for each condition.

The timing of food intake during the simulated shiftwork protocol varied between conditions to accommodate testing schedules. The meal at night group consumed food at 19:00h (dinner), 00:30h (meal at night) and 07:00h (breakfast). The snack at night group consumed food at 19:00h (dinner), 00:30h (snack at night), 07:00h (breakfast), 17:00h (snack on waking). The no meal at night group consumed food at 19:00h (dinner), 07:00h (breakfast), 09:30h (snack before bed) and 17:00h (snack on waking). On baseline and recovery days, participants in all conditions completed an oral glucose test (10% of energy requirements on these days was accounted for by the glucose drink). Participants consumed all meals and snacks in their bedrooms. Water was permitted as required during waking periods, but the consumption of caffeine was prohibited.

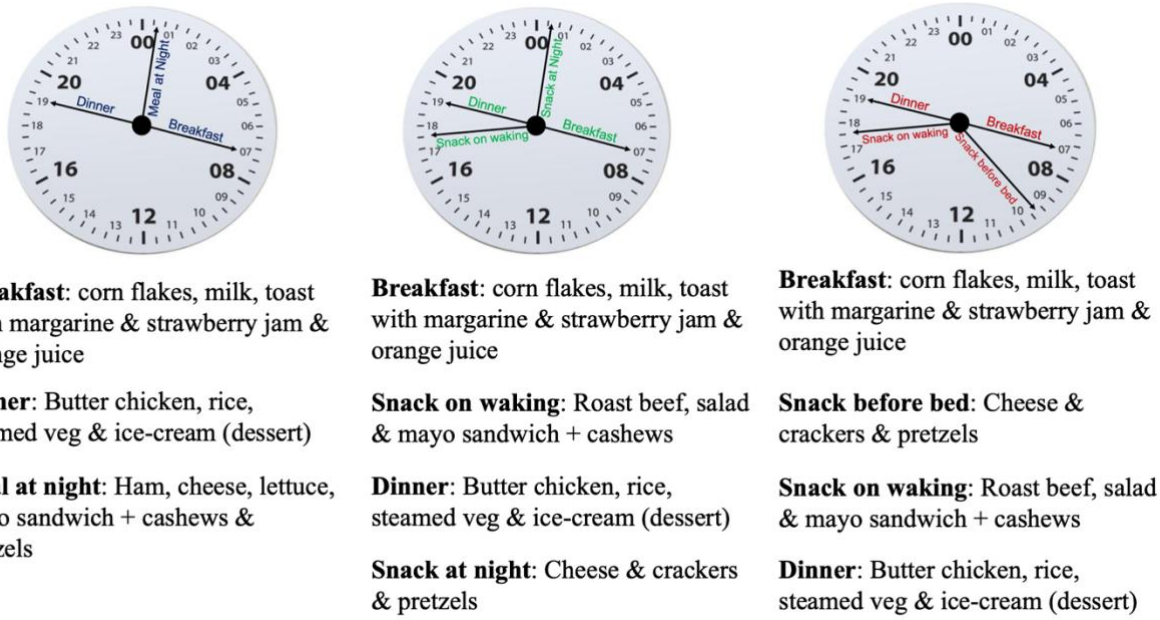


Figure 5.
Eating behaviour varied between conditions: example meal plan for each condition

5.4.2 Saliva collection

Saliva samples were collected 15 times per day across the simulated night-shift hours at: 20:00h, 21:00h, 21:30h, 22:30h, 23:30h, 00:00h, 00:30h, 01:00h, 01:30h, 02:30h, 03:00h,

03:30h, 04:00h, 05:00h and 05:30h using the Salivette® (Sarstedt, Numbrecht, Germany) with cotton swab inserts. Participants were instructed to place the cotton swab in their mouth without touching it and to roll (not chew) it around in their mouth for 2min. After 2min participants were instructed to 'spit' the cotton swab back into the salivette, again without touching it. The Salivette® tubes were then stored at −80◦C prior to analysis. A total of 3,120 samples (60 per participant) were collected and assayed for cortisol.

5.4.3 Saliva assay analysis

Saliva Cortisol was determined at the Adelaide Research Assay Facility by ELISA (1-3002, Salimetrics, State College PA, USA). Samples were assayed directly as per the manufacturers' protocol.

In brief, saliva cortisol was determined by adding 25 µl of standards, controls, or sample to a 96 well plate coated with anti cortisol monoclonal antibodies, and 25 µl of a 1:1600 dilution of cortisol conjugated to HRP. The plate was incubated for 60min, during which cortisol from the standards and samples compete for antibody binding sites with the HRP conjugated cortisol. The plate was then washed to remove unbound cortisol. Bound cortisol peroxidase was measured by the reaction of the HRP enzyme with the substrate TMB. This reaction was stopped by adding sulfuric acid and optical density was then read on a Biotek Synergy H1 plate reader (Winooski, Vermont, USA) at 450 nm.

The amount of antibody bound cortisol–HRP detected, as measured by the intensity of colour, is inversely proportional to the amount of cortisol present. The amount of cortisol present in samples was determined by interpolation against the standard curve using the Biotek plate-reader analysis software package, Gen5. Standards and controls were assayed in duplicate, samples were assayed in single. All cortisol results are presented as ng/ml.

The LLoQ of the assay was 0.12 ng/ml. The intra-assay coefficient of variation of the assays was 4.0%. The inter-assay coefficient of variation of the assays at the low-quality control level (0.93 ng/ml) was 12.7%. The inter-assay coefficient of variation of the assays at the high-quality control level (10.8 ng/ml) was 4.5%.

5.5 Statistical analyses

Analyses were completed using IBM SPSS Statistics for Macintosh, Version 28.0. (Armonk, NY: IBM Corp). To examine if and when circadian misalignment with simulated night shift alters the cortisol rhythm, average cortisol values across the 15 collection points per night shift (20:00h–05:30h) were analysed for the no meal at night condition (avoiding the impacts of eating on night-time cortisol values). Mixed-effects ANOVA was conducted, specifying salivary cortisol (ng/ml) as a dependent variable, with fixed effects of night (Nights 1−4), time (20:00h, 21:00h, 21:30h, 22:30h, 23:30h, 00:00h, 00:30h, 01:00h, 01:30h, 02:30h, 03:00h, 03:30h, 04:00h, 05:00h and 05:30h), a night-by-time interaction, and subject ID as a random effect on the intercept.

Cortisol indices of reactivity and peak reactivity, which quantify cortisol reactivity in response to the night shift, were also calculated using Khoury et al.'s (2015) formula. Reactivity measures change in cortisol between the start /baseline (20:00h) and end (05:30h) of the night shift (i.e. 05:30h cortisol value minus the 20:00h cortisol value). Peak reactivity measures the change between baseline and peak value (i.e. peak value minus baseline 20:00h value). Peak value is defined as the highest cortisol value across the period of sampling (Khoury et al., 2015).

ANOVA was also used to examine the impact of eating during the night and meal size on cortisol levels, cortisol output post test meal or snack (00:30h–02:30h) during each night shift and cortisol output during the typical circadian morning cortisol rise (03:00h–

05:30h) were analysed for all conditions. During the post meal/snack and morning cortisol periods, AUC with respect to AUC_g and AUC_i were calculated using J.C Pruessner et al.'s (2003) formula (derived from the trapezoid formula). AUC_g measures total cortisol output encapsulating intensity and sensitivity (i.e. the difference between the single measurements from each other and the distance of these measures from the ground), whereas AUC_i measures change in cortisol over repeated samples calculated with reference to the first value ignoring the distance from ground for all measurements (J.C Pruessner et al., 2003). Where data were missing, the value from the sample directly prior and after the missing data point were averaged (interpolated). Separate mixed-effects ANOVA specified dependent variables of 1) AUC_g and AUC_i post meal/snack, 2) AUC_g during the circadian morning rise. Models also specified fixed effects of condition (meal at night, snack at night, no meal at night), night (Nights 1−4), a condition by night interaction, and subject ID as a random effect on the intercept.

Partial eta-squared (η^2_p) effect sizes are also reported alongside significance tests. Since traditional effect sizes are not calculated due to the way in which variance is apportioned in mixed-effects ANOVA, effect sizes were estimated based on the effects from simple repeated measures ANOVA (i.e. without the random effect). As a comparison, thresholds are typically set at small = 0.01, medium = 0.06 and large = 0.14).

5.6 Results

5.6.1 Participants

Four hundred and twenty-three participants were screened for eligibility from which 55 healthy participants consented and commenced, with 52 (*n* = 29 male; *n* = 23 female) subsequently completing the study (Figure 6). A total of 17 participants were in the meal at night condition (*n* = 10 male, *n* = 7 female, age 23.5 ± 3.5 years; BMI 24.8 ± 2.6

kg/m^2), 16 participants were in the snack at night condition (n = 8 male, n = 8 female, age 25.4 ± 5.6 years; BMI 23.8 ± 2.6 kg/m^2), and19 participants were in the no eating at night condition (n = 11 male, n = 8 female, age 24.7 ± 5.4 years; BMI 23.4 ± 2.4 kg/m^2). Of the 23 female participants, 9 were using oral contraceptives (3 in the meal at night condition, 4 in the no meal at night condition, 2 in the snack at night condition) and all were in the luteal phase of their menstrual cycle when participating during the in-laboratory study. All participants were intermediate chronotypes. Descriptive statistics for sleep variables prior to participants commencing the in-laboratory study are shown in Table 10.

Table 10.
Descriptive statistics for sleep variables prior to commencing in-laboratory study

	All participants (n = 52) ***M* (*SD*)**	**Meal at night (n = 17)** ***M* (*SD*)**	**Snack at night (n = 16)** ***M* (*SD*)**	**No eating at night (n = 19)** ***M* (*SD*)**
Total sleep time (mins)	483.67 (72.40)	502.40 (81.90)	482.40 (70.16)	469.63 (69.52)
Sleep efficiency (%)	85.77 (8.80)	86.64 (7.50)	85.43 (9.53)	85.46 (9.58)

Note: Values are average of estimated total sleep time, and sleep efficiency for home sleep for the week prior to the study calculated from actigraphy. A repeated measures ANOVA was conducted and there was no statistically significant difference between the means for total sleep time (p = .583) or sleep efficiency (p = .939).

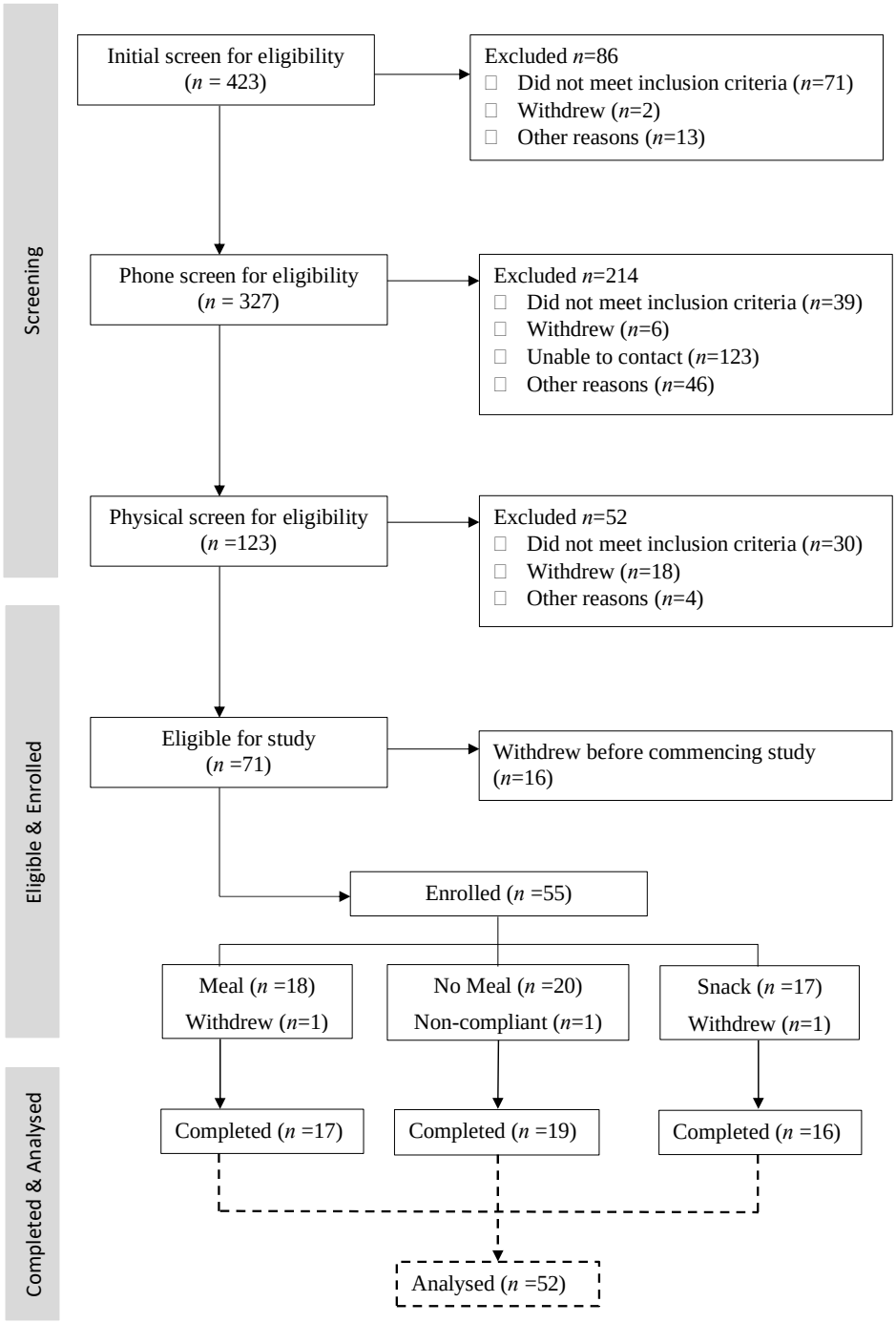


Figure 6.
Consort diagram

Note: Shows the number of participants who were screened, enrolled, consented to participate, completed lab protocol and had data analysed

5.6.2 The effects of simulated night shifts on the cortisol rhythm

There were significant effects of time ($F_{14,1055} = 37.74$ $p < .001$, $\eta^2_p = 0.446$) and night*time ($F_{42,1055} = 3.12$, $p < 0.001$, $\eta^2_p = 0.169$), such that, compared to the same times on Night 1, by Night 4, the mean cortisol values were significantly higher at 20:00h (Night 1 = 1.49 ± 0.78, Night 4 = 0.86 ± 0.58 ng/ml, $p < 0.01$) and significantly lower at 05:30h (Night 1= 1.07 ± 0.67, Night 4 = 2.11 ± 1.17 ng/ml, $p < 0.001$). The difference between values at the start and end of each night and the peak reactivity (i.e. peak cortisol value) were smaller across each night shift (Figure 7, Table 11).

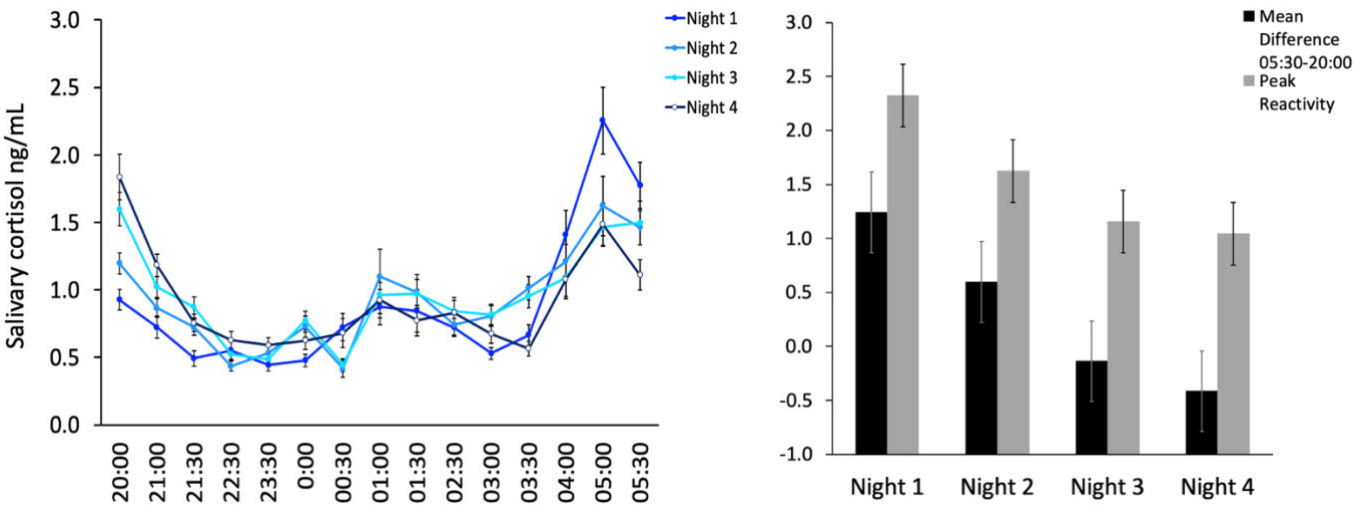


Figure 7.
Overnight cortisol across the four simulated night shifts (Nights 1−4) in the no meal at night group

Note: Left panel shows *M* ± SEM salivary cortisol concentrations (y-axis) at 15 sample points (non-equidistant, x-axis) across four consecutive night shifts for the no eating at night condition only. Right panel shows summary reactivity indicators (Khoury et al., 2015) for each night shift. The black bars show cortisol reactivity to the night shift, that is, the difference in cortisol values between the start (20:00h) and end (05:30h) of the night shift, and the grey bars are an indicator of peak reactivity (the difference between baseline (20:00h) and peak cortisol values for each night shift. Error bars indicate *SE*.

Table 11.
Reactivity changes in cortisol across four consecutive simulated night shifts

Cortisol ng/ml	Night 1	Night 2	Night 3	Night 4
Reactivity	1.24	0.60	–0.14	–0.14
Peak Reactivity	2.32	1.63	1.16	1.05

Note: Reactivity measures change in cortisol between the start (20:00h) and end (05:30h) of the night shift (i.e. 05:30h cortisol value minus the 20:00h cortisol value) in the no meal at night condition. Peak reactivity measures the change between baseline and peak value (i.e. peak value minus baseline 20:00h value). Peak value is defined as the highest cortisol value across the period of sampling (Khoury et al., 2015).

5.6.3 The impact of eating at night and meal size on cortisol response

Post food intake there was a significant effect of condition ($F_{2,49} = 3.59$, $p = 0.035$, $\eta^2_p = 0.117$, AUC_g $p < 0.001$) and night (AUC_i $p < 0.001$). The meal and snack conditions, respectively (AUC_g $p = 0.001$, AUC_g $p < 0.001$) had significantly greater total cortisol output between 00:30h and 02:30h compared to the no meal condition. Cortisol levels in the snack condition were significantly higher ($p = 0.013$) compared to the no meal condition. There were no significant differences in cortisol levels between the snack and meal condition ($p = 0.440$). The magnitude of the cortisol increase was significantly greater on Nights 2 and 3 (AUC_i $p < 0.001$) compared to Night 1, and greater on Night 3 (AUC_i $p = 0.004$) compared to Night 4, but no significant difference between Night 4 and Night 1 ($p = 0.559$). Table 12 shows mean cortisol concentrations per condition across each night shift. The effect of condition*night was not significant ($F_{6,770} = 1.07$, $p = 0.378$, $\eta^2_p = 0.046$) (Table 12 and Figure 8).

Table 12.
Mean cortisol concentrations per condition 00:30h–02:30h across each night shift

	Time	Meal Cortisol levels ng/ml *M* (*SD*)	Snack Cortisol levels ng/ml *M* (*SD*)	No meal Cortisol levels ng/ml *M* (*SD*)
Night 1	00:30	0.72 (0.66)	0.89 (1.08)	0.59 (0.36)
	01:00	1.14 (0.91)	1.00 (1.31)	0.53 (0.43)
	01:30	0.88 (0.70)	1.13 (1.75)	0.58 (0.70)
	02:30	0.54 (0.29)	0.88 (0.51)	0.75 (0.58)
Night 2	00:30	0.29 (0.19)	0.66 (0.59)	0.32 (0.43)
	01:00	1.28 (1.30)	1.61 (2.08)	0.50 (0.41)
	01:30	1.42 (0.86)	1.13 (1.21)	0.44 (0.32)
	02:30	0.88 (0.47)	0.88 (0.71)	0.51 (0.33)
Night 3	00:30	0.38 (0.31)	0.63 (0.37)	0.34 (0.28)
	01:00	1.13 (1.20)	1.16 (1.04)	0.65 (0.64)
	01:30	1.13 (0.76)	1.09 (0.76)	0.73 (0.76)
	02:30	0.90 (0.90)	1.06 (0.74)	0.61 (0.48)
Night 4	00:30	0.77 (1.23)	0.68 (0.40)	0.60 (0.54)
	01:00	1.14 (1.26)	1.10 (0.88)	0.58 (0.50)
	01:30	0.73 (0.49)	1.10 (1.23)	0.53 (0.45)
	02:30	0.64 (0.42)	1.25 (0.79)	0.65 (0.51)

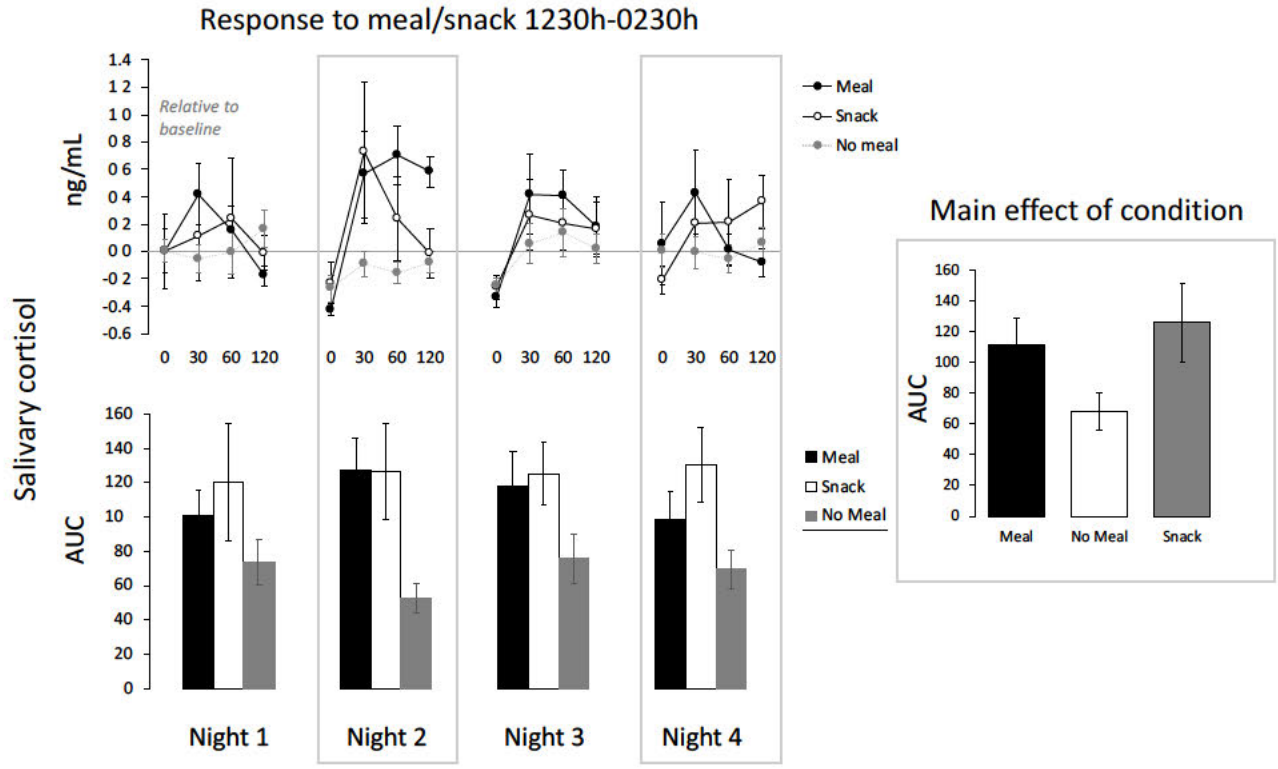


Figure 8.
Cortisol following meal, snack, or no meal (00:30–02:30h) across four simulated night shifts (Nights 1–4)

Note: Upper panel shows absolute saliva change from baseline in response to meal, snack, or no meal at 0min pre-meal, snack, or no meal and at 30, 60 and 120min post meal snack, or no meal. Cortisol levels peak approximately 30min post meal/snack consumption and remain high for ~1h (60min) post meal/snack consumption before declining. Lower panel shows AUC_g in response to the meal, snack, or no meal condition. Total cortisol output in the meal and snack conditions on all 4 nights is significantly higher than the no meal condition. Results indicate that food consumption on the night shift significantly influences cortisol levels. Error bars indicate standard error. Right inset shows the main effect of condition on AUC_g.

5.6.4 The impact of eating at night and meal size on the circadian morning cortisol rise

There was a significant and large effect of night ($F_{3,963} = 11.11$, $p < 0.001$, $\eta_p^2 = 0.117$, AUC_g $p = 0.002$, AUC_i $p < 0.001$), and night-by-time by condition ($F_{24,935} = 1.63$, $p = 0.029$). Total cortisol output decreased significantly (AUC_g $p < 0.001$) as did the magnitude of decrease (AUC_i $p < 0.001$) with each successive night shift (Figure 9, Table 13). Total cortisol output on Night 4 was significantly less (AUC_g $p < 0.001$) compared to Night 1 but not significantly different to Nights 2 and 3 respectively ($p = 0.232$, $p = 0.367$). Cortisol levels on Night 4 at 03:00h were significantly less than Nights 1, 2 and 3 respectively ($p = 0.014$, $p = 0.010$) and at 03:30h significantly less than Nights 1 and 3

respectively ($p = 0.010$, $p = 0.007$). Cortisol levels at 05:30h on Night 4 were significantly less than Nights 1, 2 and 3 respectively ($p < 0.011$, $p = 0.001$, $p < 0.001$). Cortisol levels on Night 4 in the snack condition were significantly greater than those in the meal and no meal conditions ($p < 0.001$). There were no main effects of condition ($F_{2,48} = 1.75$, $p = 0.184$) (Table 13 and Figure 9).

Table 13.
Mean AUC_g and AUC_i for cortisol across each condition and night shift

	MEAL		SNACK		NO MEAL	
00:30h–02:30h	**AUC_g *M* (*SE*)**	**AUC_i *M* (*SE*)**	**AUC_g *M* (*SE*)**	**AUC_i *M* (*SE*)**	**AUC_g *M* (*SE*)**	**AUC_i *M* (*SE*)**
Night 1	100.43 (15.31)	14.31 (19.68)	120.49 (34.27)	13.76 (23.96)	73.44 (13.06)	2.58 (8.91)
Night 2	126.97 (18.54)	91.75 (16.48)	126.42 (27.41)	47.30 (20.79)	52.62 (8.29)	14.03 (6.13)
Night 3	117.71 (20.12)	71.75 (14.64)	125.03 (18.46)	49.58 (18.88)	75.52 (14.73)	34.85 (10.30)
Night 4	97.84 (17.05)	5.02 (24.04)	130.22 (21.74)	48.69 (16.79)	69.75 (11.08)	−1.93 (11.55)
03:00h–05:30h						
Night 1	186.83 (38.08)	92.95 (34.62)	205.60 (33.98)	80.65 (29.35)	245.54 (27.15)	129.60 (23.50)
Night 2	162.39 (21.81)	−11.79 (16.20)	188.06 (26.96)	−2.25 (29.11)	206.16 (35.40)	59.61 (15.98)
Night 3	197.13 (27.19)	22.96 (23.41)	176.01 (27.72)	−19.82 (25.01)	163.74 (23.68)	15.64 (19.47)
Night 4	128.93 (28.07)	31.84 (25.56)	181.73 (24.68)	4.02 (27.95)	155.41 (18.32)	34.49 (10.94)

Note: AUC_g = area under the curve with respect to ground, reflecting total cortisol output; AUC_i = area under the curve with respect to increase, however, during the time 03:00h–05:30h results returned negative values and in these cases the results can also be regarded as an index of decrease rather than area (J.C Pruessner et al., 2003); participants in the meal and snack conditions received a meal or snack at 00:30h; *M* and SE values are raw cortisol data measured as ng/ml.

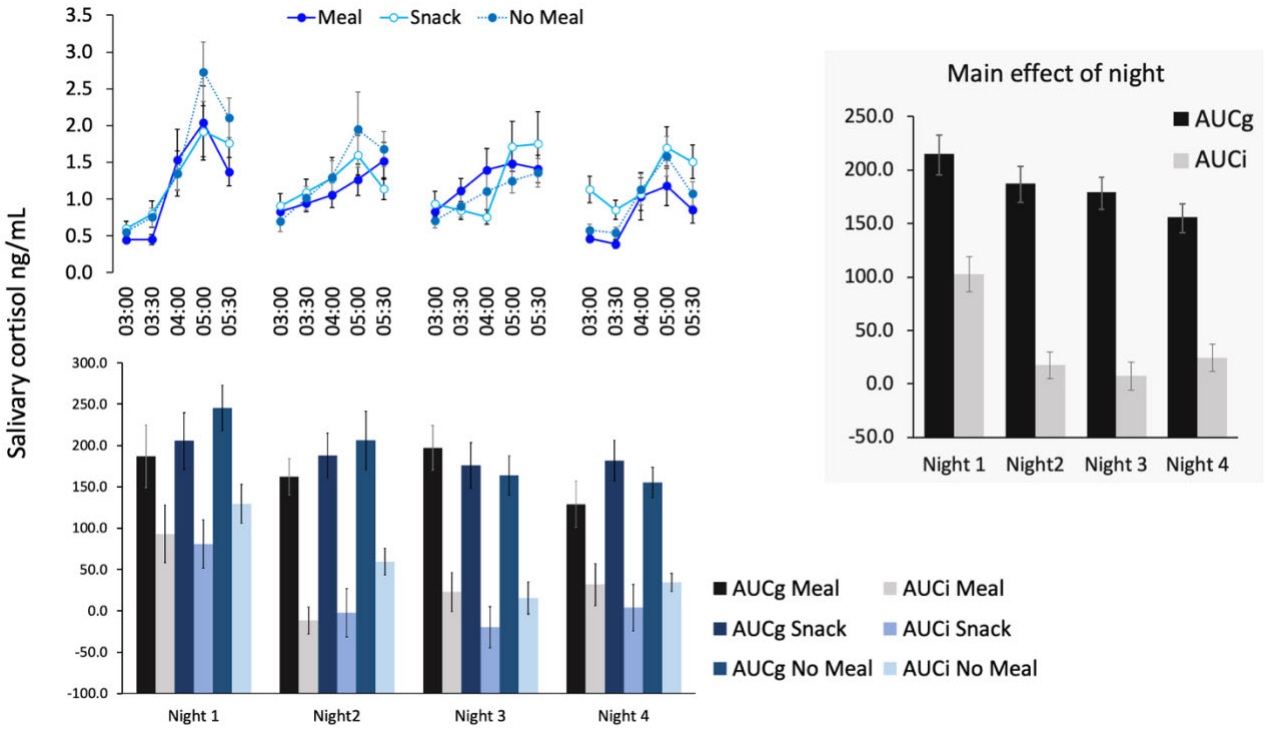


Figure 9.
Cortisol outcomes per condition between 03:00h and 05:30h across the four night shifts

Note: Upper panel shows impact of meal, snack, or no meal at night on the circadian morning cortisol rise (03:00h–05:30h). Lower panel shows AUC_g and AUC_i per condition for the circadian morning cortisol rise 03:00h–05:30h. Results show cortisol levels between 03:00h and 05:30h decrease in all conditions with each successive night shift regardless of whether food was consumed or not. Error bars indicate standard error. Right inset shows the main effect of night on AUC_g and AUC_i

5.7 Discussion

This study investigated the impact of consecutive simulated night shifts, with varied eating patterns, on cortisol concentrations. We found that four consecutive simulated night shifts with varied eating patterns significantly changed the cortisol profile. Changes were apparent from Nights 2–4 at the start (20:00h) and end (05:30h) of each night shift, and eating a meal/snack at 00:30h produced significant increases in cortisol levels 2h post meal. By the end of the shiftwork period, relative to the first night, cortisol levels at 20:00h (start of a night shift) were higher than morning levels at 05:30h (end of night shift). Other shiftwork studies have referred to this as a reversal effect (Hennig et al., 1998; Niu et al., 2015). Assessing salivary cortisol 3-hourly in nursing staff across seven consecutive night shifts, Hennig et al.

(1998), similar to the current study, reported a change in cortisol levels at the start (21:00h) and end (06:00h) of the night shift. However, change was only evident after the fifth consecutive night when cortisol levels at 21:00h were higher than those at 06:00h which suggested a reversed rhythm. Niu et al. (2015) collected samples four times daily (on awakening, +30min, +6h and +12h post awakening) over four days (Days 2–5) of a five-day consecutive night-shift roster in nursing staff, and reported that cortisol profiles in the afternoon and night on Day 4 were higher than those recorded in the morning, which also indicated a reversed circadian rhythm. The current study sampled every 2h across each of the four night shifts, and changes in cortisol levels were evident at the start (20:00h) and end (05:30h) of the second night shift, and continued to change with each consecutive night shift. Results from this study are in line with those of Harris et al. (2010) who reported disturbances in the cortisol rhythm during the second night shift in workers on an offshore oil rig. Though cortisol levels at 20:00h and 05:30h in the current study exhibited change with levels at 20:00h being higher than those at 05:30h by the third night shift, levels were still low around midnight and demonstrated the typical circadian early morning rise at ~03:00h. Weitzman et al. (1971) established that the diurnal cortisol rhythm, in non-shiftworking individuals, is characterised by low levels around midnight with levels beginning to then increase around 02:00h–03:00h. Our results suggest a temporal shift in the rhythm rather than a reversal, as the observed changes in cortisol did not profoundly alter levels during the circadian nadir of the typical diurnal cortisol rhythm.

The observed change in cortisol levels as early as Night 2 demonstrate that the HPA axis responds quickly to the night-shift environment. Cortisol has many actions and is vital for maintaining physiological homeostasis (Gross & Cidlowski, 2008), reduces inflammation (Cain & Cidlowski, 2017), helps the body manage stress, and is critical for functions of the central nervous system as well as digestive and renal systems (Gross & Cidlowski, 2008). It

is therefore reasonable to suggest this rapid biological response to change in behavioural activities occurs to assist the body achieve stability, that is, maintain homeostasis, by itself changing to cope with the situation. Thus, night shift is a factor that may alter the response of the HPA axis to circadian cues responsible for the pattern of the diurnal cortisol curve. As these results reflect an acute disruption, and cortisol plays a central role in the regulation of several physiological systems, it will be important for future studies to investigate the effects of repeated acute disruptions on cortisol in healthy volunteers and in shiftworkers with and without the chronic health conditions prevalent in shiftworkers.

To our knowledge this is the first study exploring consecutive simulated night shifts together with the impact of altered patterns of food intake on cortisol. Results suggest that eating a meal or snack at 00:30h produces an acute increase in cortisol levels directly after eating. Eating at night on the night shift, irrespective of meal size, during the body's normal rest phase acutely increased cortisol levels. In the current study participants in the eating conditions displayed a significant increase in cortisol levels approximately 30min post food intake, with levels remaining high for approximately 1h post food intake. Previous studies have found cortisol levels increase approximately 30min post the midday meal (Quigley & Yen, 1979; Rosmond et al., 2000; Slag et al., 1981; Stimson et al., 2014). Other studies have looked at glucose and insulin response to altered meal times, while concurrently examining cortisol (Chellappa et al., 2018; Holmbäck et al., 2002; Van Cauter et al., 1992). However, these studies did not directly explore the cortisol response to altered meal times (Chellappa et al., 2018), or did not report the cortisol response to a nocturnal meal (Van Cauter et al., 1992). Holmbäck et al. (2002), however, described the cortisol response to meals during a 24h, laboratory-based CR protocol, reporting acute increases in cortisol levels post meals consumed at 08:00h and 14:00h. While CR studies strictly control for external variables and provide an opportunity to understand mechanisms, the protocol itself limits generalisability

of the findings, particularly given the ablation of sleep is likely to substantially alter the normal CAR. The current study used a more realistic and pragmatic design compared to the CR protocol. It explored different meal sizes, eating versus not eating at night and participants were engaged in simulated night work undertaking various study tasks during the night shift. This study shows that eating meals during the night shift acutely increases cortisol levels when they would normally be low.

The duration and magnitude of the cortisol rise post night meal or snack is potentially important as increased levels of cortisol for lengthy durations can produce adverse metabolic effects (Walker, 2007) and potentially lead to adverse health outcomes (Stimson et al., 2014). While there is a paucity of studies exploring food intake during the night shift on cortisol our results are consistent with rodent studies exploring food intake during the rest phase. Rats fed during the rest phase demonstrated high mean cortisosterone levels and displayed a propensity to obesity characterised by accumulation of abdominal fat. (Salgado-Delgado et al., 2010). Collectively, these findings suggest eating at night may be a likely contributing factor for cardiometabolic health outcomes in shiftworkers (Sletten et al., 2020).

Behavioural changes of altered eating patterns may cause or exacerbate shiftwork-related alterations to physiological systems influenced by cortisol, for example glucose tolerance, in response to eating at night (Mason et al., 2020; Qian et al., 2018). Repeated disturbances in the cortisol rhythm can induce allostatic overload (McEwen, 2005). An increase in allostatic load can lead to allostatic overload and changes in physiologic function (McEwen & Karatsoreos, 2015), which may contribute to the pathogenesis of health burdens such as cardiometabolic disease and type 2 diabetes (Ritonja et al., 2018; Spiegel et al., 1999). Therefore, findings from this study suggest cortisol may be an underlying mechanism to disruptions in these systems.

As evidenced by the results of this study, not eating at night maintains low cortisol levels, which might be important to reduce disease risk. However, the practicalities of not eating at night need to be tested in a shiftworking population. Night shiftworkers, like traditional day workers, need to meet daily nutritional needs appropriate for their energy demands during wake and sleep. This needs to be considered if suggesting food avoidance across the biological night when individuals working the night shift are awake. Additionally, macronutrient content and frequency of meals need also to be considered, as different dietary components and meal frequency induce different effects on cortisol levels (Ishizuka et al., 1983; Jenkins et al., 1989; Martens et al., 2010; Slag et al., 1981; Stimson et al., 2014).

This study explored healthy, non-shiftworking individuals in a controlled laboratory environment where food intake and mealtimes were strictly controlled, and energy requirements for each participant calculated to accommodate daily energy needs, which limits the generalisability of our findings. Shiftworking populations include individuals with health issues such as gastrointestinal disease, obesity and type 2 diabetes, which are more common among shiftworkers than non-shiftworkers (Knutsson, 2003) who may respond differently. This study did not explore the cortisol response to food intake during a 'normal' workday and so could not determine if the magnitude or duration of the cortisol response to food intake at night was greater or equivalent to that of food intake during the day. Additionally, shiftworkers may not take, or have the time, to prepare suitable meals. However, control over these variables increases the internal validity of this study.

5.8 Conclusion

In conclusion, results from this study show the endocrine system responds quickly to changes in human behaviours. Four consecutive night shifts (altered sleep–wake cycles) produced rapid change in the cortisol profile at the start and end of each consecutive shift. While there

was no evidence of changes in cortisol levels stabilising by the end of the four night shifts, it is plausible that consistent and gradual ongoing adaptation may have continued to occur with circadian adjustment likely to take considerably longer than four days to complete. Eating at night produced an acute increase in cortisol levels. While this acute increase is a normal response to eating, persistent, cumulative effects of repetitive disruption to the cortisol rhythm from the night-shift schedule and eating at night may contribute to allostatic load and chronic disease risk in night shiftworkers. This study demonstrated the acute impact of eating at night and cortisol disruption. However, longitudinal research, with real-world shiftworkers, is needed to investigate the cumulative effects of repetitive disruption to cortisol from eating at night on the night shift. Further research is needed to better understand the relationship between altered sleep–wake cycles, meal timing, changes to the cortisol rhythm and associated alterations to physiological systems regulated by cortisol such as metabolism. Observing such changes will help to develop more comprehensive knowledge of the impact of repeated system disruptions in shiftworkers, and establish the potentially important role of cortisol in future health risks.

Research, like art, demands constant observation.

- Adapted: Vincent van Gogh



The impact of 62h sleep deprivation on cortisol

Chapter 6: The impact of 62h sleep deprivation on cortisol

Abstract

Sleep has a large effect on physiological functions, ensuring there exists an optimal internal environment for physiological processes to occur during sleep. Sleep deprivation is considered a physiological stressor. It has negative impacts on many physiological systems such as the endocrine system. Numerous studies have explored the impact of TSD on cortisol, but inconsistencies in the findings have been reported and the exact impact of sleep deprivation on cortisol is unclear.

This study investigated the cumulative effects of 62h of TSD on cortisol and considered whether sleep deprivation is a physiological stressor as assessed by changes in salivary cortisol levels. Participants (n = 21) completed 62h of TSD. Saliva was collected across 34 time points between (07:00h Day 2 – 20:00h Day 4). Mixed-effects ANOVAs tested for fixed effects of time and day and their interaction on cortisol and cortisol AUC_g with participant ID as a random effect each with their own intercept. Additional growth models were conducted exploring the rate of change in cortisol across the 62h of TSD using maximum likelihood estimation.

There was a significant time-by-day interaction ($p < 0.001$), with higher cortisol levels at 18:00h and 20:00h on Days 2 and 3 respectively compared to Day 1. The linear and quadratic effect of time on cortisol reflected the initial increase in cortisol levels across the waking day with a subsequent deceleration of this increase as wakefulness across the TSD period continued.

Results demonstrate that sleep deprivation impacts cortisol, with a small increase in the early evening. Findings suggest that sleep deprivation is a mild physiological stressor. Acute sleep deprivation is common in modern work environments and disruptions in the circadian rhythm

of cortisol can result in disruptions to signals the cortisol rhythm sends to cortisol-sensitive tissue. Further research is required to better understand the impact of TSD on individuals who, due to work or lifestyle factors, frequently experience periods of sleep deprivation.

6.1 Introduction

Sleep deprivation is considered a physiological stressor associated with increased cortisol levels (Wright et al., 2015) that impacts the brain and many physiological systems. A physiological stressor can be defined as any external or internal condition that disrupts homeostasis (Chu et al., 2021; Kagias et al., 2012; Russell & Lightman, 2019). A stressful situation, whether environmental or psychological, can activate a cascade of stress hormones that produce physiologcial changes (Charmandari et al., 2005; Chu et al., 2021; Russell & Lightman, 2019). A stress response encompasses two stages mediated by two systems. First, a fast acute response mediated by the sympathetic-adreno-medullar axis resulting in increased secretion of epinephrine and norepinephrine. Second, a slower response, approximately 15–20min after the onset of stress. This response is mediated by the HPA axis, a key system that synchronises the stress response with circadian regulatory processes, resulting in increased secretion of cortisol levels that can remain high for several hours (Chu et al., 2021; Dedovic et al., 2009; Hannibal & Bishop, 2014; Russell & Lightman, 2019). Cortisol displays a distinct circadian rhythm, but when it is generated outside of circadian or pulsatile conditions, cortisol release is deemed specific to stress (Herman et al., 2005).

Cortisol's distinct circadian rhythm provides signals to multiple peripheral biological systems regarding time of day that is essential for the synchronisation of physiological functions (e.g. metabolism) and general healthy functioning (e.g. inflammation and immune response) (N. Smyth et al., 2013). Cortisol is vulnerable to sleep deprivation (Chapotot et al., 2001; Leproult et al., 1997; Meerlo et al., 2008; Weibel et al., 1995; Wright et al., 2015). Disturbances in the circadian rhythm of cortisol can result in disruptions to signals the cortisol rhythm provides to peripheral systems that may affect their functioning, disrupt pathology and impact health (Adam et al., 2017; Cuesta et al., 2015; Karatsoreos & McEwen, 2011)

Laboratory studies in healthy individuals examining the impact of sleep deprivation from 24h (Chapotot et al., 2001; Schüssler et al., 2006; Song et al., 2015; Weibel et al., 1995; Weitzman et al., 1971) to 72h of wake (Kant et al., 1984) on cortisol report mixed results. Some studies report a significant increase in cortisol levels (Chapotot et al., 2001; Leproult et al., 1997; Song et al., 2015; von Treuer et al., 1996; Weibel et al., 1995; Weitzman et al., 1971; Wright et al., 2015) while others report decreased (Åkerstedt et al., 1980; Kant et al., 1984) or no significant change in cortisol (Dinges et al., 1994; Salín-Pascual et al., 1988).

The inconsistencies in the results may be due to differences in methodology, such as CR versus ambulant; a CR protocol permits assessment of circadian rhythms by reducing the effect of exogenous and activity time cues. Participants experience ~24h constant wakefulness while resting in a supine position exposed to continuous dim ambient light with small meals spread evenly throughout the protocol (Czeisler & Gooley, 2007). Addtionally, the type of sample (blood or urine) and sampling protocol, such as frequent versus infrequent sampling or sampling during TSD versus sampling post TSD can have an effect. As cortisol release is pulsatile, the lack of regular sampling may miss a cortisol pulse, which reduces the opportunity to examine circadian rhythmicity of cortisol and how this may be impacted by TSD. Additionally, infrequent sampling may miss important changes in cortisol that could inform how and or why changes in a peripheral system synchronised /influenced by cortisol occurred.

When interpreting these studies it is important to consider the type and method of sampling employed to assess the impact of TSD on cortisol. Approximately 90% of circulating cortisol is classified as bound cortisol, that is, cortisol that has attached itself to cortisol binding globulin protein (Hindmarsh & Geertsma, 2017). Approximately 5–10% is classified as free cortisol, that is cortisol not attached to cortisol binding globulin protein (Hindmarsh & Geertsma, 2017). Free cortisol is the biologically active component of cortisol. It is free to

bind to target cortisol receptors in tissue to elicit its effects. Moreover it permits assessment of cortisol's circadian rhythm (Hellhammer et al., 2009; Hindmarsh & Geertsma, 2017; Kudielka et al., 2012). Urinary cortisol (usually assessed as a 24h total) is a useful index of unbound cortisol produced over the course of a complete circadian cycle (Levine et al., 2007); however, it does not capture diurnal fluctuations that may occur during periods of sleep deprivation. Additionally, renal conditions can impact cortisol metabolism and urinary clearance of cortisol metabolites affecting urinary cortisol levels (Hellhammer et al., 2009; Sagmeister et al., 2022). Blood sampling typically measures total cortisol, that is, the sum of the bound and unbound components (Bozovic et al., 2013; Levine et al., 2007). Also, blood sampling is invasive, accompanied by additional stress effects of blood sampling, and requires trained personnel.

Salivary cortisol reflects the unbound or circulating free biologically active component of cortisol and is considered more meaningful in terms of cortisol activity (N. Smyth et al., 2013) as it gives an opportunity to look at dynamic change in cortisol over the entire day as well as over short periods of time within a day (Kirschbaum & Hellhammer, 1994). Salivary cortisol values are highly correlated (≥0.90) to serum unbound cortisol concentrations in healthy men and women with a time lag of <5min for salivary cortisol values to reflect serum cortisol values (Vinning et al., 1983). Moreover salivary cortisol values are not impacted by the use of oral contraceptives (Kirschbaum & Hellhammer, 1999). However, women in the follicular phase (the start of menstruation to the moment of ovulation) of their menstrual cycle display higher cortisol levels than women in the luteal phase (after ovulation) of their cycle (Hamidovic et al., 2020). Collection of salivary cortisol is non-invasive, avoids the possible stress effects of blood sampling and is a good reflection of HPA axis responsivity (Bozovic et al., 2013; Jessop & Turner-Cobb, 2008).

Therefore the research aim of this study was to investigate the impact of 62h of TSD on the cortisol rhythm.

6.2 Ethical considerations

Approval was granted by the UniSA Human Research Ethics Committee (202112). Written consent was obtained prior to commencement and participants were aware they could withdraw at any time. Participants were compensated financially for their time. To ensure participant confidentiality, participant ID numbers were allocated to participants with all associated data coded accordingly.

6.3 Participants

A total of 21 healthy, non-shift-working, participants (n = 10 male; n = 11 female, mean age 22.8 ± 4.40 years) were recruited from the community via advertising flyers displayed on community and university notice boards, social media sites (Facebook) and classified posts on a free online marketplace (Gumtree Australia). Individuals wishing to participate underwent detailed screening (telephone and face-to-face) to ensure they were of good physical and mental health (confirmed by a confidential medical screening, fasting blood test and the BDI) (Beck et al., 1996) and did not use prescription or over-the-counter medications known to affect cortisol levels (e.g. steroid creams) or sleep. Participants were excluded if they had engaged in transmeridian travel, smoking or illicit drug use, excessive alcohol (>2 standard drinks/per) or caffeine consumption (>2 cups/day) in the two months prior to the study.

In the week prior to entering the laboratory participants completed a sleep–wake diary and wore a wrist activity monitor, GENEActiv (*Activinsights Ltd. GENEActiv: The Original, Wrist-Worn Raw Data Accelerometer*, 2021) to ensure a minimum of 8h sleep per night was obtained. Participants reported a stable habitual sleep duration between 7 and 8h a night

(average total sleep time 7.6h ± 0.9h). Participants were instructed to abstain from napping, caffeine and alcohol in the week prior to the study.

6.4 Study protocol

This five day, within-groups, repeated measures study was conducted in the sleep laboratory at the Behaviour-Brain-Body Research Centre at UniSA. The laboratory has no clocks, is windowless, sound attenuated, room temperature maintained at a constant 22 (± 1)°C and light intensity was set to 50 lux at head height (slightly dimmed light) during scheduled wake periods and approximately <0.03 lux (darkness) during all scheduled sleep periods. Each bedroom contained a desktop computer for use during cognitive testing. These computers did not have time displays and were not permitted to be used by participants outside of scheduled testing times. Participants did not have access to mobile phones, internet, or any other electronic devices that may provide time of day/social time cues. Participants remained in the laboratory for five consecutive days and were not permitted to leave, except in the case of an emergency.

A detailed study protocol is shown in Figure 10. Participants entered the laboratory at 10:00h on Day 1, were familiarised with their surroundings and cognitive test batteries. Prior to commencing their 62h of sleep deprivation, participants received an 8h TIB sleep opportunity from 23:00h to 07:00h. Participants then commenced 62h of sleep deprivation (07:00h Day 2–21:00h Day 4). This time period was chosen to allow for two full 24h circadian cycles of data collection. Throughout the sleep deprivation period a number of cognitive test batteries and driving simulation tasks, reported in Marando et al., 2022 and Volmer et al., 2022, were completed at regular intervals. When participants were not undertaking scheduled cognitive testing, they were free to read, watch DVDs, play board/card games, and interact with each other and the research assistants in the shared living space. Participants were not permitted to

engage in vigorous physical activity/exercise as exercise can act as a stimulus on the HPA axis producing significant increases in cortisol levels (Hill et al., 2008). Food intake and mealtimes were strictly controlled during each study run. All meals and snacks were provided for the duration of the study. The energy requirements for each participant were calculated using Harris Benedict equations (Harris & Benedict, 1919), and the macronutrient content of meals was based on the average Australian diet and was controlled within each meal (Australian Bureau of Statistics, 1997). The consumption of caffeine was not permitted. Research assistants monitored participants throughout the protocol to ensure protocol compliance and that participants remained awake. On completing 62h of sleep deprivation participants received a 9.5h TIB recovery sleep opportunity (21:30h–07:00h).

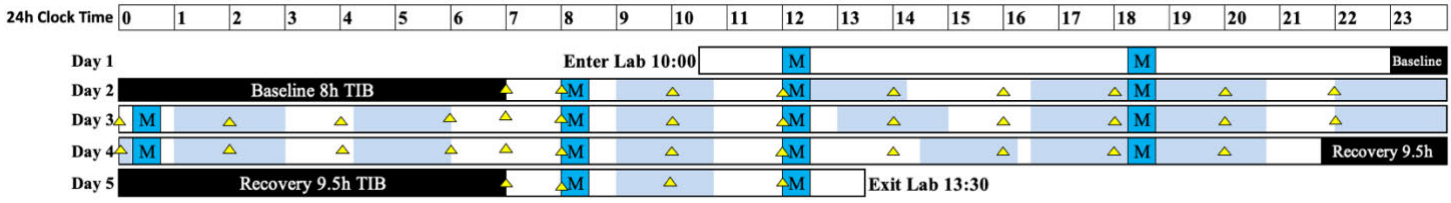


Figure 10.
Protocol schematic

Note: Black bars = scheduled sleep opportunities, white bar = wake periods, light blue bars = cognitive testings, yellow triangle = saliva samples, blue box with M = meals/snacks.

6.5 Materials

6.5.1 Saliva sampling

Saliva samples were collected using the Salivette® (Sarstedt, Numbrecht, Germany) with cotton swab inserts. Participants were instructed to place the cotton swab in their mouth without touching it and roll (not chew) it around in their mouth for 2min. After 2min participants were instructed to 'spit' the cotton swab back into the salivette, again without touching it. The Salivettes® tubes were then stored at −80ºC prior to analysis.

Sampling commenced at 07:00h on Day 2 when participants awoke from their sleep, and again at 08:00h. This permitted an opportunity to capture the endpoint of the circadian pre-awakening increase (Stalder et al., 2016) and ensure avoidance of the CAR, the marked increase in cortisol secretion over the first 30–45 min after morning awakening. To maintain continuity of sampling, the 07:00 and 08:00 samples were collected across the 62h of sleep deprivation in addition to samples collected every 2h across the deprivation period.

6.5.2 Assay procedure

Saliva cortisol was determined at the Adelaide Research Assay Facility by ELISA (1-3002, Salimetrics, State College PA, USA). Samples were assayed directly as per the manufacturer's protocol. Saliva cortisol was determined by adding 25 µl of sample to a 96 well plate coated with anti cortisol monoclonal antibodies, and 25 µl of a 1:1600 dilution of cortisol conjugated to HRP. The plate was incubated for 60 minutes, during which cortisol from the standards and unknowns competed for antibody binding sites with the HRP conjugated cortisol. The plate was then washed to remove unbound cortisol. Bound cortisol peroxidase was measured by the reaction of the HRP enzyme with the substrate TMB. This reaction was stopped by adding sulfuric acid, and optical density was then read on a Biotek Synergy H1 plate reader (Winooski, Vermont, USA) at 450 nm. The amount of antibody bound cortisol-HRP detected, as measured by the intensity of colour, is inversely proportional to the amount of cortisol present. The amount of cortisol present in samples was determined by interpolation against the standard curve using the Biotek plate-reader analysis software package, Gen5. Standards and controls were assayed in duplicate, samples were assayed in single. The LLoQ for the assay was 0.012 ng/ml. The intra-assay coefficient of variation of the assays was 10.3%. The inter-assay coefficient of variation of the assays at the low-quality control level (0.108 ng/ml) was 10.5%. The inter-assay coefficient of variation of the assays at the high-quality control level (1.09 ng/ml) was 6.0%.

6.6 Statistical analyses

Analyses were completed using IBM SPSS Statistics for Macintosh, Version 28.0. (Armonk, NY: IBM Corp). To examine the effect of 62h TSD on the circadian cortisol rhythm, average cortisol values from 31 collection time points between (08:00h Day 2 and 20:00h Day 4) were analysed. Where data were missing, the datum from the sample directly prior and datum after the missing data points were averaged (interpolated).

Cortisol data were positively skewed and hence were log transformed to meet the assumption of normality. An ANOVA was conducted with salivary cortisol as a dependent variable. Sample time (00:00h, 02:00h, 04:00h, 06:00h, 08:00h, 10:00h, 12:00h, 14:00h, 16:00h, 18:00h, 20:00h, 22:00h), day and a day-by-time interaction were treated as fixed effects, and participant ID as a random effect. AUC_g, calculated using Pruessner et al.'s (2003) formula, was used to assess total cortisol output 08:00h–08:00h Day 2 to Day 3 and 08:00h–08:00h Day 3 to Day 4. Bonferroni corrections were used for post hoc pairwise comparisons.

In addition to the mixed ANOVA assessing the difference in cortisol across days and time, additional growth models were conducted exploring the rate of change in cortisol across the 62h of TSD using maximum likelihood estimation. A step-by-step process was used for building the model starting with the least complex model (null model) with only the outcome variable (cortisol) and the random variable (participant ID) included, to the most complex model where no further significant model improvements were found. Each model had a gradual increase in complexity from the addition of random slopes, polynomial predictors (e.g. linear and quadratic) and interactions (Table 14). Consistent with growth models the autoregressive (1) – heterogenous (AR (1): heterogenous) covariance was used.

To determine model fit the change in −2 Log Likelihood (−2LL) of two consecutive models, likelihood ratio tests was used in addition to visual comparisons of the Akaike's information

criterion (AIC). The −2LL, also referred to as the 'deviance', is a measures of fit between the proposed model and the data, with lower scores indicating better fit (Williams, 2015). AIC is a measure of 'goodness of fit'. Typically AIC is more suitable in finding the best model for prediting future observations (Chakrabarti & Ghosh, 2011).

For all models, the linear fixed effect of 'Day' (median centred at study Day 2) represents the average change from study Day 1 to study Day 3 and the linear (Time) and quadratic ($Time^2$), and fixed effects of time of day represents the direct and overall trend across time awake respectively.

The proportion of variance in the model (i.e. the effect size) was calculated using RStudio, Version 1.2.5033 as SPSS does not calculate effect size for growth models. Partial eta-squared (η^2_p) effect sizes are reported alongside significance tests. As a comparison, thresholds are typically set at <0.01 = very small, 0.01–0.06 = small, 0.16–0.14 = medium, and >0.14 = large (Field, 2013).

6.7 Results

6.7.1 Participants

Twenty-three healthy, non-shiftworking, participants were enrolled in the study. After commencement of the study one participant withdrew due to illness, while another participant withdrew on training Day 1 for personal reasons. A total of 21 participants completed the study (n = 10 male; n = 11 female, age 22.8 ± 4.40 years; Figure 11). No female participants were using oral contraceptives and all were in the luteal phase of their menstrual cycle when participating during the in-laboratory study. All participants were intermediate chronotypes.The following ethnic backgrounds were included in this study; Asian (29%), Indian (29%) and Caucasian (42%).

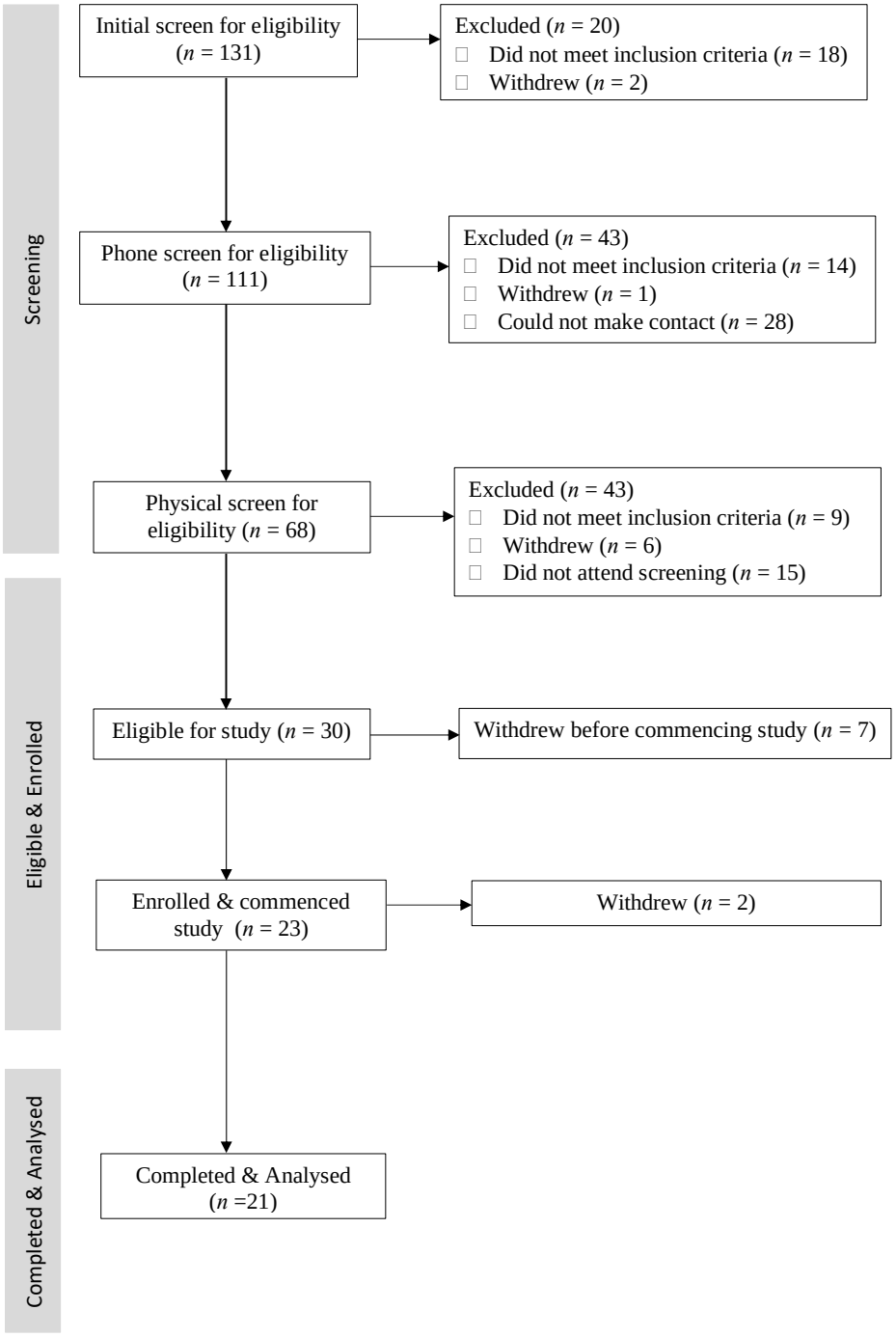


Figure 11.

Consort diagram of participants who were screened, determined for eligibility, completed laboratory protocol and data analysed

6.7.2 Area under the curve

The cortisol AUC_g was not significantly different across days (p = 0.808) 08:00h–08:00h Day 2 to Day 3 19.2 ± 6.1 ng/mL and 08:00h–08:00h Day 3 to Day 4 19.4 ± 7.4 ng/ml.

6.7.3 The effects of 62h total sleep deprivation on the cortisol rhythm

Cortisol levels on Day1 increased post waking, peaking at 08:00h and decreasing across the rest of the day. On Days 2 and 3 cortisol levels started low, peaking between 6:00h and 10:00h (Figure 12). The linear mixed-effects model (Table 14, Model 1b) yielded significant (p < 0.001), large effects of time (η^2_p = .653) day (η^2_p = .700) and time*day (η^2_p = 0.923). Compared to the same times on Day 1, cortisol levels were significantly lower at 08:00h (p = 0.031) and significantly higher at 18:00h and 20:00h (p <= 0.007) on Days 2 and 3. Cortisol levels were also significantly higher at 22:00h on Day 2 (p = 0.023) compared to the same time on Day 1 (Figure 12).

Investigation of non-linear models yielded the best fit for Model 3, which included the quadratic term for Time ($Time^2$), and had the lowest −2LL and AIC values, and a significantly improved fit relative to the less complex model (Model 3 vs. Model 2a, p = 0.026, Table 14). This model yielded significant effects of Day (p =0 .032), Time (p < 0.001), Day*Time (p = 0.019), and the quadratic term $Time^2$ (p <0 .001), and is illustrated in the dotted lines on Figure 12.

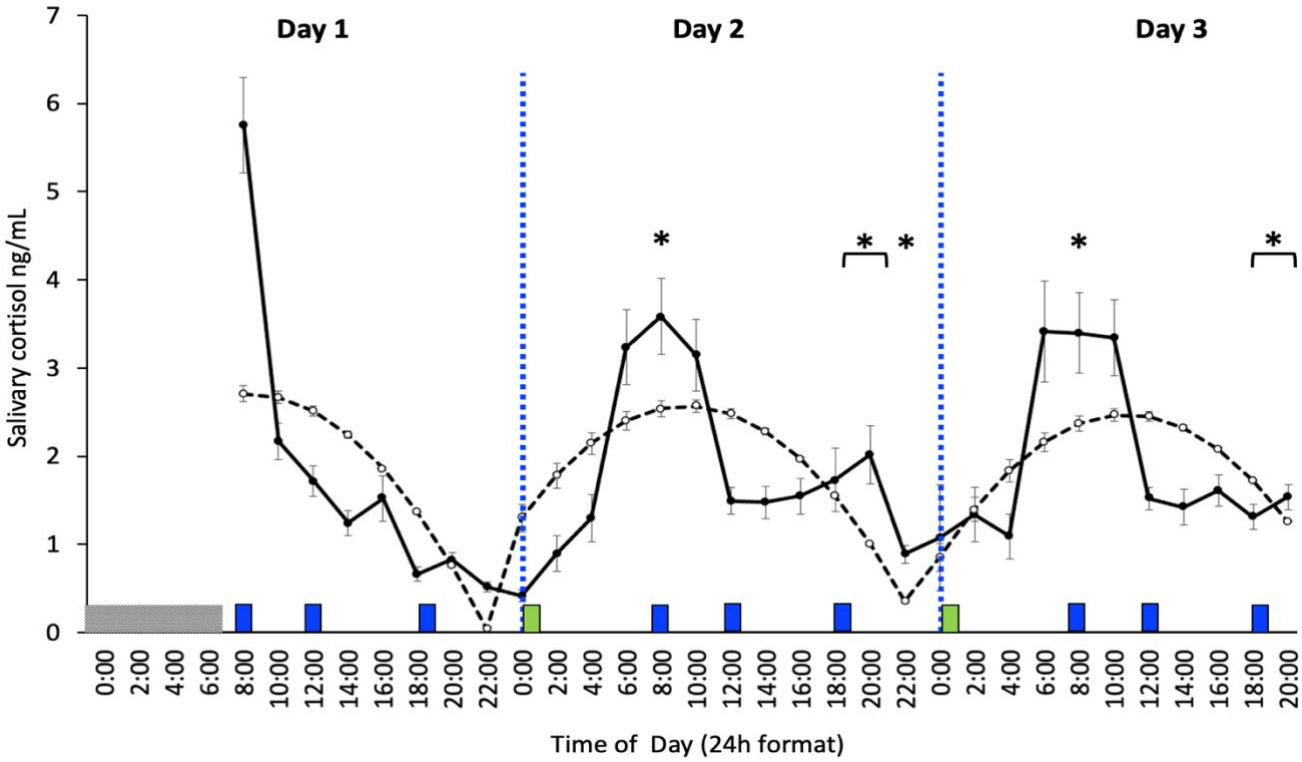


Figure 12.
Predicted salivary cortisol levels (Model 3; black dotted line) vs. actual cortisol values (black solid line) across 62h total sleep deprivation

Note: *M* ± SEM salivary cortisol concentrations (y-axis) at 31 sample points. Participants awakened 07:00h on Day 1 and remained awake for 62h (07:00h Day 1–22:00h Day 3). The cortisol spike at 08:00h on Day 1 reflects the peak of the cortisol awakening response. The blue lines denote midnight; the end of one day and commencement of the next. Grey box denotes sleep period. Blue boxes denote meals, breakfast, lunch and dinner. Green boxes denote snack. Error bars = *SE* *$p < 0.05$, significance in cortisol levels from Day 1.

Table 14.
Models for the effect of 62h total sleep deprivation on cortisol

Model	Day β3 β (*SE*) *p* value	Time β1 β (*SE*) *p* value	Day*Time β4 β (*SE*) *p* value	Time2 β2 β (*SE*) *p* value	Day*Time2 β5 β (*SE*) *p* value	−2 Log LL	AIC	Model comparison vs	Model comparison Δχ2(df)
Model 0a						2555.7	2561.7		
Model 1ab	−0.04 (0.09) 0.627	−0.08 (0.02) < 0.001				2542.4	2552.4	M0	13.36 (2), $p < 0.001$
Model 1bc	−1.12 (0.21) <0.001	−0.11 (0.02) <0.001	0.17 (0.03) <0.001			2511.4	2523.4	M1a	31.03 (1), $p < 0.001$
Model 2ad	−0.44 (0.21) 0.038	0.536 (0.08) <0.001	−0.44 (0.03) 0.025	−0.06 (0.01) <0.001		2442.8	2456.8	M1b	68.53 (1), $p < 0.001$
Model 2be	−0.57 (0.33) 0.084	0.52 (0.08) <0.001	0.12 (0.11) 0.273	−0.06 (0.01) <0.001	−0.00 (0.01) 0.612	2442.6	2458.6	M2a	0.26 (1), $p = .692$
Model 3f	−0.45 (0.21) 0.032	0.54 (0.08) <0.001	0.07 (0.03) 0.019	−0.06 (0.01) <0.001		2436.9	2454.9	M2a	5.89 (2), $p = 0.026$
Model 4g	−0.45 (0.21) 0.033	0.54 (0.08) <0.001	0.07 (0.03) 0.021	−0.06 (0.01) <0.001		2435.0	2455.0	M3	1.90 (1), $p = 0.111$

Note: β = Beta; AIC = Akaike's information criterion; BIC = Schwarz's Bayesian Criterion; Model 0a = null model; Model 1ab random intercept (ID) with predictors of time and day; Model 1bc random intercept (ID) with predictors of time, day, and time*day; Model 2ad random intercept (ID) with predictors of day, linear and quadratic time and time*day; Model 2be random intercept (ID) with predictors of day, linear and quadratic time and linear time*day and quadratic time*day; Model 3f random intercept (ID) with predictors of day, linear and quadratic time and time*day in which the slopes for linear time were free to vary (random slopes); Model 4g random intercept (ID) with the main effects of linear & quadratic time & day and the interaction between day and time (linear & quad) as predictors in which the slope for linear time as free to vary (random slope).

6.8 Discussion

This study investigated the cumulative effects of 62h of TSD on salivary cortisol in healthy participants under controlled laboratory conditions. It also considered if sleep deprivation was a physiological stressor. Results demonstrated the cortisol rhythm is largely unchanged during sleep deprivation, apart from a small increase in the early evening on Day 1.

These results are in line with other studies demonstrating the cortisol rhythm largely is resistant to acute changes in sleep–wake behaviour (Åkerstedt et al., 2009; Dinges et al., 1994; Leproult et al., 1997; Song et al., 2015; Weitzman et al., 1983; Wright et al., 2015). Similar to the current study, Leproult et al. (1997) reported increases in cortisol levels between 18:00 and 23:00h following 24h TSD. The observed increases early in the night may suggest a mild activation of the HPA axis but like the current study the rise was small and temporary. Leproult and colleagues suggested that cortisol levels are minimally modulated by sleep and sleep loss. Results from the current study demonstrate changes in HPA axis activity to be small in response to sleep deprivation. However, there is increasing evidence to suggest that the cumulative effect of small deviations in cortisol levels may play a role in the pathogensis of health burdens such as cardiometabolic and cardiovascular disease (Jacobsen, 2005; Puttonen et al., 2010; Ritjona et al., 2018; Spiegel et al., 1999), which provides further support for the importance of the small changes observed in the current study.

Other sleep deprivation studies have reported no effect at all of sleep deprivation on cortisol. In studies by Dinges et al. (1994) and Åkerstedt et al. (1980) sleep deprivation was not found to increase cortisol; rather, cortisol levels were higher at baseline than on any sleep deprivation day. These results may be due to the frequency of sampling.

Dinges et al. (1994) collected blood samples at two timepoints, 22:00h and 22:30h on the day prior to commencing the study, and on each of the sleep deprivation days, that is, after 15h, 39h and 63h of sleep deprivation and following a recovery sleep. Åkerstedt et al., (1980) collected blood at 08:00h the day prior to commencing the study and 08:00h the morning participants completed the study. The current study sampled frequently. Other sleep deprivation studies (Chapotot et al., 2001; Leproult et al., 1997; von Treuer et al., 1996; Weibel & Brandenberger, 1998; Weitzman et al., 1971; Wright et al., 2015) employing frequent blood samples (every 10, 20 or 60min) across the sleep deprivation period reported significant albeit small increases in cortisol levels. More regular sampling is likely more sensitive to capturing these secretory episodes and therefore capture the small changes in cortisol levels that might otherwise be missed with infrequent/single timepoint assessment.

Measuring salivary cortisol in the current study permitted the opportunity to measure the circulating free biologically active component of cortisol, which is meaningful in terms of cortisol activity and a better measure of adrenocortical function (Hellhammer et al., 2009). Measuring cortisol in saliva removed the invasive measures and additional stress that can be induced by blood sampling. Additionally, because many studies have found that consuming food (Quigley & Yen, 1979; Rosmond et al., 2000; Stimson et al., 2014) and mental workload/cognitive effort (Chapotot et al., 2001; Gaillard & Wientjes, 1994; J. Smyth et al., 1998) temporarily increase cortisol levels, the present study controlled for as many extraneous factors as possible, specifically timing and quantity of food intake, physical activity and prohibiting the intake of caffeine.

This study explored the acute effect of 62h of TSD on cortisol in healthy well-rested participants under controlled laboratory conditions. Further research is required in individuals who are sleep deprived, for example shiftworkers, assessing if there are

changes in overall cortisol levels in response to real-world sleep deprivation. Workers experience many different kinds of stressors, which cannot be simulated in the laboratory environment, that may impact cortisol levels. This study did not collect salivary samples before and after meal consumption or cognitive testing. Nor were self-reported perceptions of stress and workload levels examined. Additional salivary samples could progress understanding as to the impact of food and workload on cortisol levels during periods of sleep deprivation. Understanding the interaction between cortisol, sleep loss, workload and food intake, would help to disentangle the mechanisms contributing to the increased risks for poor health in individuals whose occupation/s expose them regularly to periods of sleep deprivation.

6.9 Conclusion

In conclusion, this study found that sleep deprivation is a mild stressor increasing cortisol levels in the early evening. Acute sleep deprivation is common in modern work environments and disruptions in the circadian rhythm of cortisol can disrupt the circadian function of systems influenced by cortisol which may impact health (Oster et al., 2006). Slight deviations in cortisol levels, may amount to significant cortisol load causing the body to move from a state of adaptation to disease (McEwen, 2008; Meerlo et al., 2008). Therefore, future research in real-world environments should be undertaken to better understand the impact of sleep deprivation on cortisol in individuals such as shift workers who frequently experience periods of sleep deprivation. Such research would advance knowledge on the degree of disruption to the cortisol rhythm in real-world environments. Additionally, it may identify other factors that impact cortisol (e.g. food intake, workload). This may direct development of different targeted interventions for the health and wellbeing of individuals.

I am seeking, I am striving, I am in it
with all my heart.

- Vincent van Gogh



The impact of 62h sleep deprivation on the cortisol awakening response (CAR)

Chapter 7: The impact of 62h sleep deprivation on the cortisol awakening response

Abstract

The CAR is the sharp increase observed in cortisol levels that typically peaks ~30−45min post awakening. The CAR has been extensively explored in a range of laboratory and field studies using healthy and clinical populations with a variety of findings. Common among the results from these studies is that sleep, pre-sleep cortisol levels and an awakening process influence the CAR. However, there is limited exploration of how morning cortisol levels and the CAR is affected during and post TSD.

This study, conducted concurrently with Study 2 as reported in Chapter 6, aimed to examine the impact of sleep deprivation on the CAR. Participants (n = 21) completed 62h of TSD. Saliva was collected across five time points (07:00h, 07:15h, 07:30h and 07:45h) on two pre-study days, four study days and two post study days. Mixed-effects ANOVAs tested for fixed effects of day and time, their interactions on cortisol and cortisol AUC_i and AUC_g, with participants as a random effect, each with their own intercept.

There was a significant time-by-day interaction ($p < 0.001$) such that cortisol levels at +30 and +45min post awakening were greater on all days except TSD days. Salivary cortisol levels on awakening (0min) did not differ significantly between any of the study days. There was a significant effect of day on AUC_i and AUC_g ($p < 0.001$) such that AUC_i and AUC_g were lower on TSD days and greater on in-laboratory baseline and in-laboratory recovery days compared to all other study days.

This study explored morning cortisol levels and the CAR pre, during and post a TSD period. The CAR is absent when no awakening occurs and recovers well following a sleep opportunity. The literature demonstrates the CAR is associated with a variety of negative health outcomes, and is predictive of same day cognitive performance and response inhibition. As the CAR has been frequently used as a biomarker of HPA axis activity, it may play a role in physiology closely connected to circadian rhythms, and could therefore be used to detect negative health or cognitive performance outcomes. Future studies should examine the CAR longitudinally with populations who experience repeated episodes of sleep deprivation to better understand the impact of sleep deprivation on the CAR and its recovery.

7.1 Introduction

Pruessner et al. (1997) first described the CAR. It is a significant phenomenon of reference in a healthy cortisol circadian rhythm (Clow, Hucklebridge, & Thorn, 2010). This circadian rhythm is characterised by an increase in cortisol prior to awakening, the CAR and the gradual decline of cortisol levels across the remaining diurnal phase (Clow, Hucklebridge, & Thorn, 2010; Stalder et al., 2016). However, it is suggested that the CAR is a phenomenon independent of circadian influence (Clow, Hucklebridge, & Thorn, 2010) and instead is a reactive response of the HPA axis to awakening rather than a primary element of diurnal cortisol secretion (Wilhelm et al., 2007). The diurnal cortisol profile is separate to that of the CAR and is characterised by a gradual increase in levels prior to awakening and a steady decrease in cortisol levels across the day with lowest levels attained around midnight (Born et al., 1986).

The CAR is commonly characterised by a rapid increase in cortisol levels across the first 30−45min post awakening followed by a sharp decline following the peak and then a more gradual decline across the day (Pruessner et al., 1997). Factors such as sex, time of awakening, light, hippocampal functioning and glucocorticoid receptor feedback have been shown to influence the CAR (Clow, Hucklebridge, & Thorn, 2010). A typical CAR displays a 30−60% increase in cortisol relative to levels measured immediately upon awakening during the CAR period (Proulx et al., 2017; Pruessner et al., 1997; Stalder et al., 2016). Deviations from this typical pattern, for example, a flatter, less pronounced CAR or a steeper, enhanced CAR, have been associated with negative health outcomes. Individuals suffering from burnout display a blunted, less pronounced CAR (Pruessner et al., 1999). Healthy individuals self-reporting higher levels of depressive symptomology and individuals under chronic stress from either high workload or social stress display a steeper enhanced CAR compared to controls (M.

Pruessner et al., 2003; Wust et al., 2000). Chronic fatigue and post traumatic stress disorder patients display a significantly smaller CAR. Cortisol levels during the CAR period are small (Roberts et al., 2004; Wessa et al., 2006). Furthermore, literature suggests that approximately 23% of individuals do not show an increase in cortisol levels i.e., do not demonstrate the CAR, on awakening (Wust et al., 2000). The CAR is an important aspect of HPA axis function as it is regulated differently from the diurnal cortisol rhythm (Steptoe & Serwinski, 2016) and demonstrates a facility capable of revealing changes in HPA axis activity associated with poor health.

Awakening stimulates activation of the HPA axis in addition to numerous changes in the activity of other brain regions, for example, in the prefrontal cortex, hippocampus (Diorio et al., 1993; Herman et al., 2005) and neurotransmitter systems, many of which regulate HPA axis activity (Marino & Cudeiro, 2003; Wilhelm et al., 2007). The CAR, therefore, is not an isolated response to waking. Rather, it is a feature that is part of a series of functional interactions between brain regions closely tuned to circadian cycles essential for the reactivation of alertness and consciousness upon waking (Balkin et al., 2002) and maintenance of optimal human health.

Irregular sleep schedules, for example, sleep deprivation and circadian misalignment, have been associated with disruptions in the 24h diurnal cortisol rhythm, for example, increased cortisol levels at night during the circadian nadir (Chapotot et al., 2001; Guyon et al., 2014; Leproult et al., 1997; Wright et al., 2015). It is suggested that the CAR amplitude is partially determined by circadian factors, that is, the preceding nocturnal state of HPA axis activity prior to waking (Wilhelm et al., 2007).

Exploring the CAR in relation to the preceding sleep period, Wilhelm et al. (2007) found that the peak cortisol levels of the CAR were negatively correlated with average

cortisol concentrations during the night, that is, high nocturnal cortisol levels were significantly associated with attenuated incremental post awakening increases. Further there is evidence to suggest that changes in cortical and subcortical brain regions during the awakening process are associated with increased sensitivity to ACTH and posited to be responsible for the increase in cortisol levels post awakening (Clow, Hucklebridge, & Thorn, 2010). Some studies have reported on the CAR and shiftwork, (night shift vs. day shift) and found that the CAR is greater in day workers (with sleeping at night) and visible in night shiftworkers (with sleeping during the day), but it is significantly attenuated. Collectively, evidence from these studies suggests that sleep, pre-sleep cortisol levels and an awakening process influence the presence and magnitude of the CAR.

Numerous studies have explored the CAR and sleep duration (e.g., naps vs. night sleep) with mixed findings reported. Federenko et al., (2004) found individuals that napped for approximately 1h in the evening demonstrated no CAR on awakening. The authors concluded that waking up, by itself, was not sufficient to stimulate the CAR. Similarly, Devine and Wolf (2016) reported there was no CAR in participants waking from a 50min nap but, participants waking from a 90min nap (in the morning or afternoon) or a 6h night sleep displayed a CAR on awakening. However the CAR in participants waking from a 90min nap was blunted compared to participants waking from a 6h night sleep. Additionally, exploring forced/surprised awakenings during the night, Dettenborn et al., (2007) found no presentation of the CAR during these awakenings.

In contrast to this, the CAR has further been referenced as part of a healthy circadian process, and not solely an endocrinal response to waking (Clow et al., 2010b) Circadian rhythmicity largely controls the onset of the daily cortisol rise (Balbo et al., 2010). The circadian rhythm of the HPA axis is controlled by the body's master clock the SCN.

The SCN responds to light and darkness. Light stimulus regularly occurs with the sleep-wake transition providing excitant input to the SCN, that increases adrenal sensitivity to ACTH stimulating an increased release of cortisol (Clow et al., 2010b). Leproult et al., (2001) found, in participants exposed to 36h sleep deprivation, a stimulatory effect on cortisol levels in the absence of a sleep-wake transition. Participants exposed to bright light in the morning displayed significantly greater increases in cortisol levels compared to increases occurring at the same clock time relative to baseline.

The literature exploring the CAR and the impact of TSD is small. To our knowledge only one study has specifically explored the impact of TSD on the CAR. Vargas and Lopez-Duran (2017b) found participants exposed to 29h of TSD exhibited a 'blunted' CAR in the morning. This was characterised by a flatter initial acceleration and less acute deceleration versus participants in the control condition who had an 8h sleep opportunity and displayed significantly greater cortisol levels at +30, +45 and +60min post awakening. Interestingly the researchers suggested that if the CAR was a direct response to awakening, they would, in conditions of TSD, expect the CAR to be eliminated. The authors reported a blunting cortisol in the morning with no significant increases from baseline, that is, from the first sample collected at 08:00h. They further suggested waking may be necessary for the CAR to occur but offered no explanation for the flatter initial acceleration in cortisol levels observed in TSD participants. It is possible that the CAR was eliminated, and the observed cortisol levels in participants exposed to TSD were a continuation of the diurnal cortisol circadian rhythm.

These observations demonstrate that the CAR or the acute rise in cortisol in the morning does not necessarily require the awakening process to stimulate it. Further these studies highlight the importance of exploring TSD over a longer period of wake in controlled conditions with regular sampling. This may help determine if the CAR is a circadian

driven process or a response to awakening. Moreover, there have been no studies, exploring the CAR post a period of sleep deprivation after a recovery sleep and it is therefore unknown the time course of CAR recovery. Exploring recovery of the CAR post a period of sleep deprivation may help to further understand the functional significance of the CAR.

Therefore, this study aims to 1) examine if there is a sudden morning rise in salivary cortisol that mimics CAR when no awakening occurs, and 2) investigate recovery sleep CAR after a period of 62h TSD.

7.2 Ethical considerations

Approval was granted by UniSA's Human Research Ethics Committee (202112). Written consent was obtained prior to commencement and participants were aware they could withdraw at any time. Participants were compensated financially for their time. To ensure participant confidentiality, participant ID numbers were allocated to participants with all associated data coded accordingly.

7.3 Participants

A total of 21 healthy, non-shiftworking participants (n = 10 male; n = 11 female, mean age 22.8 ± 4.40 years) were recruited from the community via advertising flyers (Appendix 8) displayed on community and university notice boards, social media sites (Facebook) and classified posts on a free online marketplace (Gumtree Australia). A detailed screening process was conducted to determine eligibility. A confidential medical screening questionnaire, fasting blood analysis and the BDI (Beck et al., 1996) were used to determine good physical and mental health. Additionally, participants reported regular sleep patterns, with the absence of daytime napping and sleepiness or known sleep disorders, confirmed using the general health questionnaire, the PSQI

(Buysee et al., 1989) and the composite Morningness−Eveningness questionnaire (Horne & Östberg, 1976). Participants were excluded if they reported use of prescription or over-the-counter medications known to affect cortisol levels (e.g. steroid creams) or sleep, or they had engaged in transmeridian travel, smoking or illicit drug use, excessive alcohol (>2 standard drinks/day) or caffeine consumption (>2 cups/day) in the two months prior to the study.

7.4 Study protocol

This study was conducted in the sleep laboratory at the Body Brain Behaviour Research Centre at UniSA. The within group study design was conducted from October 2019–February 2020 and September 2020–October 2020. The laboratory has no clocks, is windowless, sound attenuated, room temperature maintained at a constant 22 (±1)°C and light intensity was set to 100 lux at head height (slightly dimmed light) during scheduled wake periods and approximately <0.03 lux (darkness) during all scheduled sleep periods. In the week prior to entering the laboratory participants completed a sleep–wake diary and wore a wrist activity monitor, GENEActiv (*Activinsights Ltd. GENEActiv: The Original, Wrist-Worn Raw Data Accelerometer*, 2021) to ensure a minimum of 8h sleep per night was obtained (23:00h–07:00h). Participants were instructed to use an alarm for waking if unable to wake by 07:00h without one, and instructed to abstain from napping, caffeine and alcohol in the week prior to the study.

7.4.1 At home pre and post study saliva sampling protocol

To obtain an accurate expression of participants' CAR prior to entering the laboratory and assess recovery of the CAR post TSD, on two consecutive mornings, prior to entering and post exiting the laboratory, participants were required to collect morning saliva samples at home. Samples were collected immediately upon awakening, +15, +30

and +45min post awakening. Participants were required to self-report wake times and sampling times of each sample (Appendix 16). To ensure validity of sample times, the required interval between sampling (15min) and reduce sampling inconsistencies, additional objective monitoring strategies were employed utilising the Medication Event Monitoring System (*Medication Event Monitoring System (MEMS®)*, 2021). MEMS is an electronic system that records the date/time of opening and closing of bottles containing the salivettes. Salivettes were stored in MEMS medication bottles. All participants were trained on how to collect their saliva sample using the salivette, instructed to not brush their teeth, eat or drink (water permitted) before and during the sampling protocol and requested to restrict the opening of bottles to the times of sample taking. All participants were provided with take-home written instructions. All participants were sent a text message the night prior to morning sampling reminding them to have all sampling equipment next to their bed ready for the morning protocol. Participants were required to respond to the text and acknowledge accordingly. Participants brought their samples with them to the laboratory on Day 1 of the study and returned post laboratory home recovery samples via express post on the day they completed their post laboratory home recovery Day 2 sampling.

7.4.2 In-laboratory study protocol

Participants entered the laboratory at 10:00h on Day 1 and were familiarised with the laboratory environment. All participants received an 8h TIB sleep opportunity from 23:00h to 07:00h. Participants then commenced 62h of sleep deprivation (07:00h Day 2–21:00h Day 4). This time period was chosen to allow for two full 24h circadian cycles of data collection. Participants remained in the sleep laboratory for the duration of the protocol and completed a number of neurobehavioural test batteries and a driving simulation task, the results of which have been reported in Marando et al., 2022 and

Volmer et al., 2022. Caffeine intake was prohibited, and staff monitored participants throughout the protocol to ensure protocol compliance and that participants did not fall asleep. On completion of 62h sleep deprivation participants received a 9.5h TIB recovery sleep opportunity (21:30h–07:00h). A detailed study protocol is shown in Figure 13.

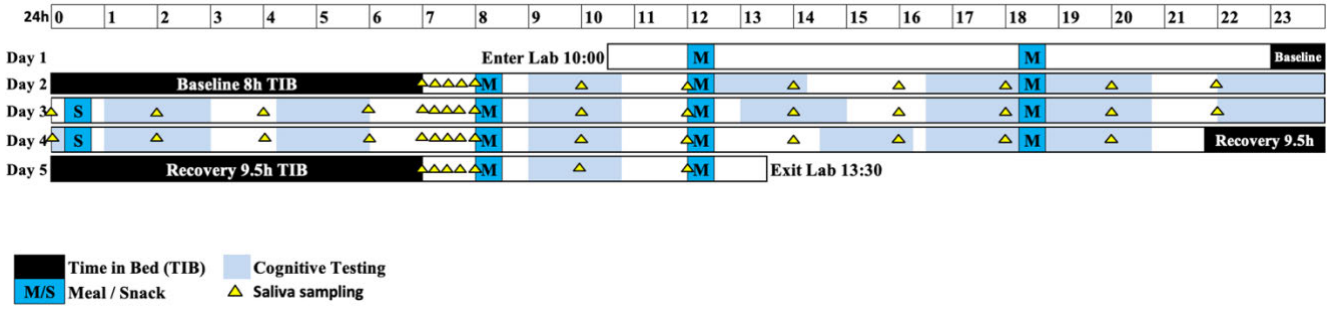


Figure 13.
Protocol schematic

Note: Black bars = scheduled sleep opportunities, white bars = wake periods, light blue bars = cognitive testings, yellow triangle = saliva samples, blue box with M = meals/snacks.

7.4.3 Saliva sampling

Saliva samples were collected using the Salivette® (Sarstedt, Numbrecht, Germany) with cotton swab inserts. Participants were instructed to place the cotton swab in their mouth without touching it and roll (not chew) it around in their mouth for 2min. After 2min participants were instructed to 'spit' the cotton swab back into the salivette, again without touching it. The Salivette® tubes were then stored at −80ºC prior to analysis.

Salivary sample collection commenced at 07:00h on Day 2 when participants awoke from their sleep and then at 07:00h on Day 3 and Day 4. Samples were collected on awakening (0min), +15, +30 and +45min post awakening. Participants were not permitted to brush their teeth, eat or drink (water permitted) before and during the

sampling protocol. Participants remained seated at the desk in their room during the sampling period.

7.4.4 Assay procedure

Saliva cortisol was determined at the Adelaide Research Assay Facility by ELISA (1-3002, Salimetrics, State College PA, USA). Samples were assayed directly as per the manufacturers' protocol. Saliva cortisol was determined by adding 25 µl of sample to a 96 well plate coated with anti cortisol monoclonal antibodies, and 25 µl of a 1:1600 dilution of cortisol conjugated to HRP. The plate was incubated for 60min, during which cortisol from the standards and unknowns competed for antibody binding sites with the HRP conjugated cortisol. The plate was then washed to remove unbound cortisol. Bound cortisol peroxidase was measured by the reaction of the HRP enzyme with the substrate TMB. This reaction was stopped by adding sulfuric acid and optical density was then read on a Biotek Synergy H1 plate reader (Winooski, Vermont, USA) at 450 nm. The amount of antibody bound cortisol-HRP detected, as measured by the intensity of colour, is inversely proportional to the amount of cortisol present. The amount of cortisol present in samples was determined by interpolation against the standard curve using the Biotek plate-reader analysis software package, Gen5. Standards and controls were assayed in duplicate, samples were assayed in single. The LLoQ for the assay was 0.012 ng/ml. The intra-assay coefficient of variation of the assays was 10.3%. The inter-assay coefficient of variation of the assays at the low-quality control level (0.108 ng/ml) was 10.5%. The inter-assay coefficient of variation of the assays at the high-quality control level (1.09 ng/ml) was 6.0%.

7.5 Statistical analyses

Analyses were completed using IBM SPSS Statistics for Macintosh, Version 28.0. (Armonk, NY: IBM Corp). To examine the effect of TSD on morning salivary cortisol levels during the same period CAR would be observed when waking occurs and recovery of the CAR following a sleep opportunity post TSD, cortisol values across five collection time points (0min, 15min, 30min and 45min) on pre-laboratory Day 1 (P1), pre-laboratory Day 2 (P2), in-laboratory baseline (LB), in-laboratory 24h TSD (L24), in laboratory 48h TSD (L48), in-laboratory recovery (LR), post laboratory home recovery Day 1 (R1) and post laboratory home recovery Day 2 (R2) were analysed. Mixed-effects ANOVA was conducted, specifying salivary cortisol (ng/mL) as a dependent variable with fixed effects of day (P1, P2, LB, L24, L48, LR, R1 and R2), time (0min, 15min, 30min and 45min), with day*time interaction and subject ID as a random effect on the intercept. Planned comparisons were conducted for differences at waking time points (0min) and for each 15min subsequent time point thereafter, relative to baseline for L24, L48 and LR.

AUC with respect to AUC_g and AUC_i was calculated using J.C Pruessner et al.'s (2003) formula (derived from the trapezoid formula). AUC_i reflects the CAR, that is, the dynamic change in cortisol levels that occur post awakening (Stalder et al., 2016). AUC_i measures the change in cortisol over repeated samples calculated with reference to the first value ignoring the distance from ground for all measurements (J.C Pruessner et al., 2003). AUC_g does not indicate the CAR but rather reflects the total post awakening cortisol concentrations combining the first sample on awakening (S1, the end point of the pre-awakening increase) and the CAR, that is, the changes in cortisol levels post awakening (Stalder et al., 2016). AUC_g measures total cortisol output encapsulating

intensity and sensitivity, that is, the difference between the single measurements from each other and the distance of these measures from the ground (Pruessner et al., 2003).

Data were assessed for normality and outliers. Data were normally distributed as assessed by the Shapiro-Wilk test ($p > 0.05$), and outliers were detected (via boxplots). Mixed-effects ANOVA with and without the outliers were run and results compared to determine whether including outliers returned sufficiently different results. Results were fundamentally equivalent; both returned a statistically significant result, and confidence intervals were not noticeably different. It was determined to therefore retain outliers in the data.

7.5.1 Missing data

Four participants omitted to collect saliva samples on Day 2 (R2) of the at-home recovery CAR sampling protocol. All data for these four participants were removed from analyses. All salivary cortisol analyses are for 17 participants (Figure 14).

7.6 Results

7.6.1 Participants

Twenty-three healthy, non-shiftworking participants were enrolled in the study. After commencement of the study, one participant withdrew due to illness, while another participant withdrew on training Day 1 as they were not confident they could complete the study requirements. A total of 21 participants completed the study ($n = 10$ male; $n = 11$ female, age 22.8 ± 4.40 years) (Figure 15.). All participants were intermediate chronotypes. The following ethnic backgrounds were included in this study; Asian (29%), Indian (29%) and Caucasian (42%).

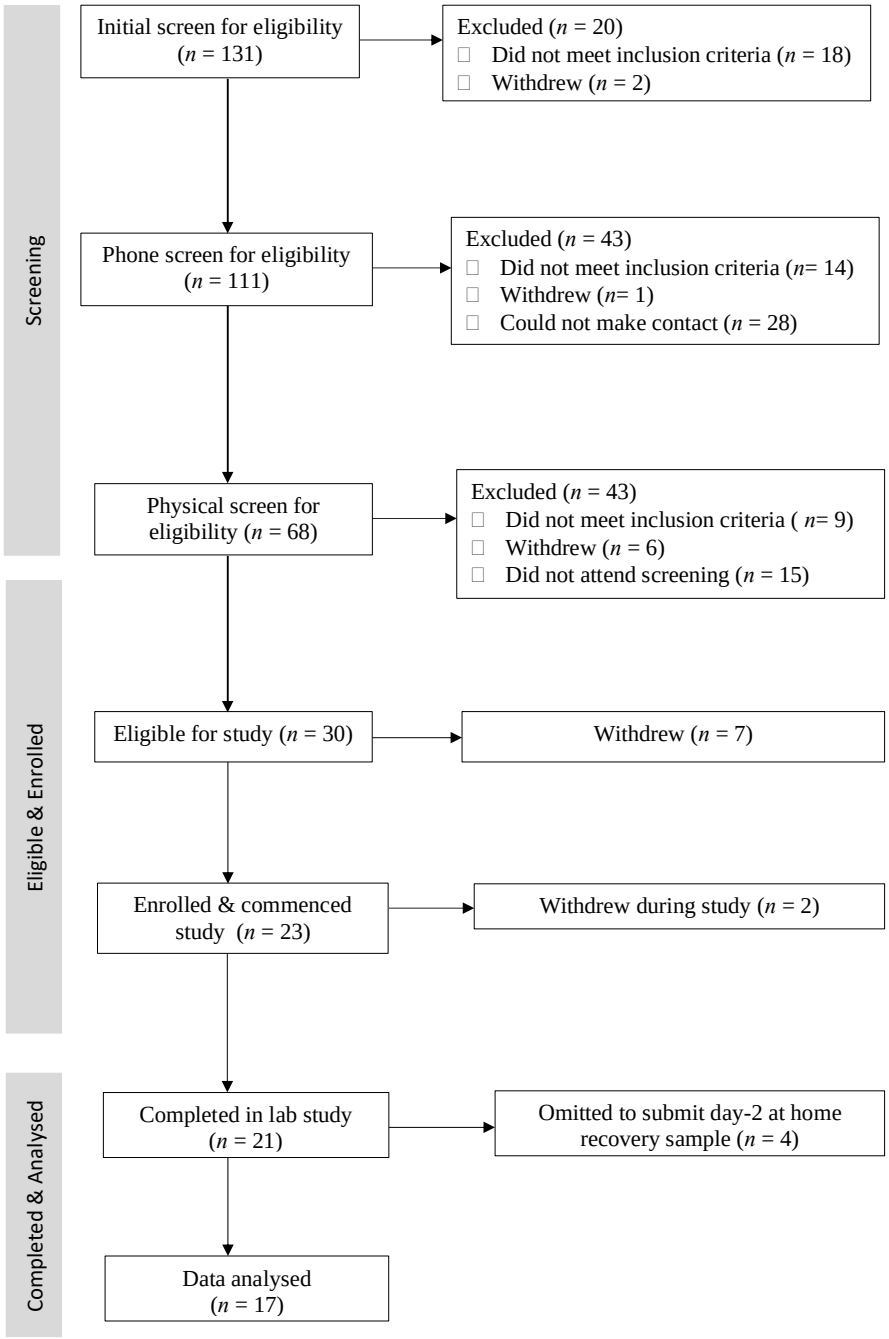


Figure 14.
Consort diagram of participants who were screened, enrolled and completed the laboratory protocol and data analysed

7.6.2 The effects of 62h total sleep deprivation on morning salivary cortisol levels

7.6.2.1 Analyses by timepoint

There was a significant effect of day ($F_{7,495} = 22.79$, $p < 0.001$), time ($F_{3, 495} = 46.41$, $p < 0.001$) and time*day interaction ($F_{21, 495} = 3.84$, $p < 0.001$). Salivary cortisol levels on waking (0min) did not significantly differ between any of the study days (Table 15). Compared to waking levels, cortisol levels at +30 and +45min post awakening were significantly greater ($p < 0.002$) on all days (P1, P2, LB, LR, R1, R2) except TSD days. In contrast, there were no significant differences across time points for L24 and L48 (Figure 15, Graph A).

7.6.2.2 Area under the curve analyses

There were significant effects of day on AUC_i ($F_{7,112} = 12.79$, $p < 0.001$) and AUC_g ($F_{7, 112} = 9.40$, $p < 0.001$) such that AUC_i and AUC_g were significantly lower on L24h and L48h during the TSD period compared to all other study days (Table 15, Figure 15 Graph B) and AUC_i and AUC_g were significantly greater on in-laboratory baseline (LB) day and in-laboratory recovery (LR) day compared to all other study days (Table 15, Figure 15 Graph B).

Table 15.
Salivary cortisol values (ng/ml) at 0 minutes across the study days for waking cortisol AUC_i and AUC_g

DAY	0 minutes (ng/ml)	AUC_i	AUC_g
	M (*SD*)	*M* (*SD*)	*M* (*SD*)
Pre lab Day 1 (P1)#	3.45(2.68)	5.65 (6.55)	15.99 (6.76)
Pre lab Day 2 (P2)#	3.45 (3.45)	4.90 (4.75)	14.58 (7.31)
In-lab baseline (LB)#	3.03 (1.71)	9.80 (5.74)	18.44 (6.54)
In-lab 24h TSD (L24)^	3.11 (2.09)	0.57 (3.07)	9.49 (6.06)
In-lab 48h TSD (L48)^	2.52 (2.24)	−0.20 (2.33)	7.09 (4.62)
In-lab recovery (LR)#	2.44(1.48)	11.44 (6.58)	18.42 (6.78)
Home recovery Day 1 (R1)#	2.82 (0.33)	5.41 (6.49)	13.96 (6.57)
Home recovery Day 2 (R2)#	2.4 (0.23)	6.39 (6.12)	13.85 (5.03)

Note: # = Cortisol value at 0 minutes on days when participants awoke from sleep denotes the endpoint of the pre-awakening cortisol increase; *M* and *SD* are raw cortisol values; ^ = indicates no awakening; 62h TSD from 07:00h on Day LB to 21:30h on Day L48; AUC_i = area under the curve with respect to the increase, emphasises intensity of changes over time reflecting the dynamic response to awakening i.e. the CAR; A negative value is regarded as an index of decrease rather than an area (Pruessner et al., 2003); TSD =total sleep deprivation;AUC_g = area under the curve with respect to ground, reflects total post awakening cortisol concentrations and not denoted as the cortisol awakening response (Stalder et al., 2016).

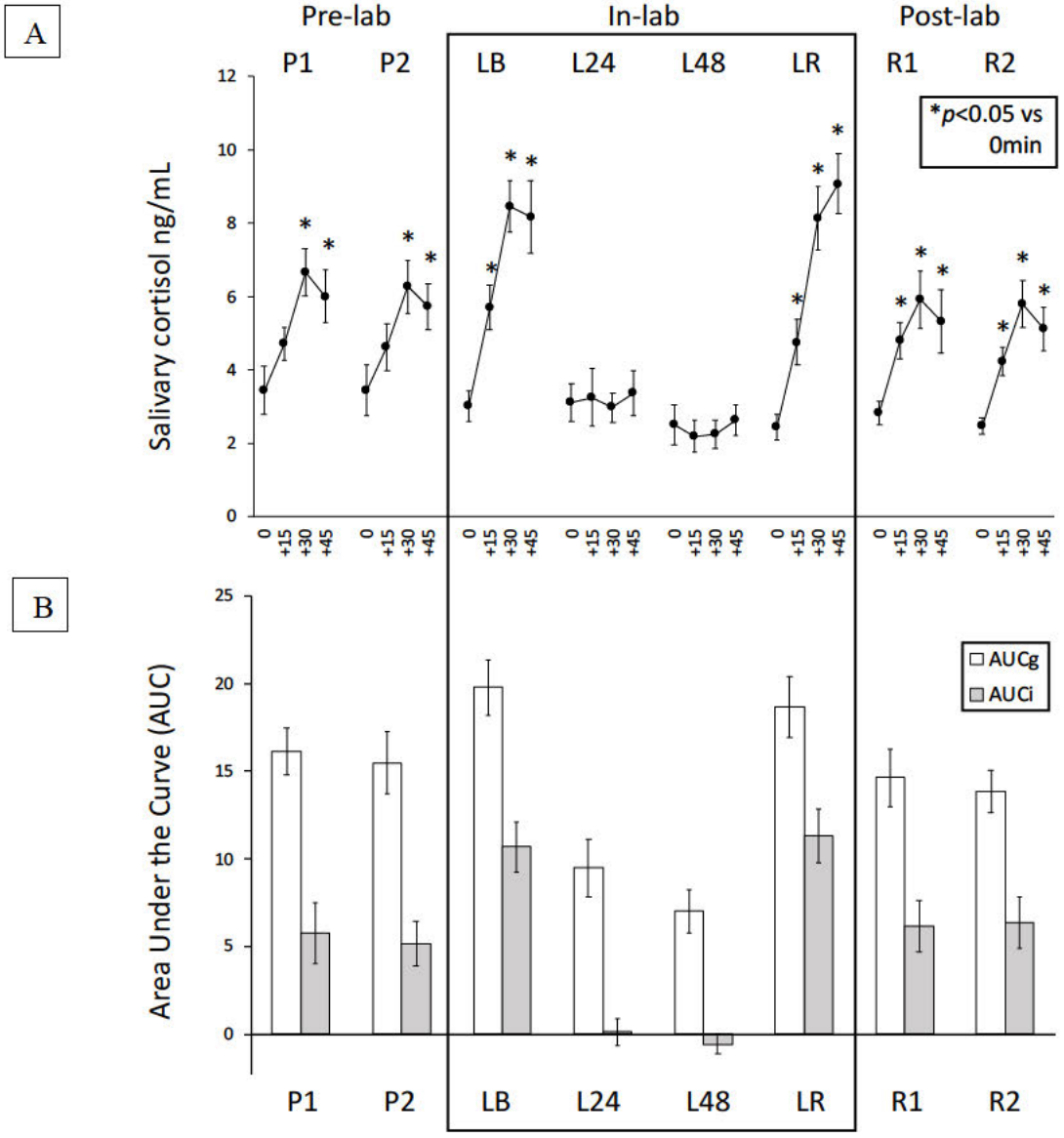


Figure 15.
Mean cortisol levels at 15min intervals across the salivary cortisol sampling period 0700h–0745h

Note: Graph A shows S1 salivary cortisol levels did not significantly differ between any of the study days. Average bedtime, at home, pre lab was 23:00h and post 62h TSD was 00:00h. Average wake time, at home, pre-labroratory was 07:00h and post 62h TSD was 08:30h as subjectively recorded by participants. Error bars represent *SE*. Graph B shows AUC_g and AUC_i for all study days. Error bars represent *SE*. *$p < 0.05$

7.7 Discussion

This study examined morning cortisol levels and the CAR pre,during and post sleep deprivation in healthy participants under controlled laboratory conditions. The findings demonstrate that the CAR is not present when waking does not occur. It was also found that following post-recovery sleep the CAR was similar to baseline CAR showing the same rapid increase in cortisol levels during the 30–45min post awakening as was observed prior to the TSD period. The increase in AUC was higher under laboratory conditions than when sampling occurred at home.

Within 15min post awakening from a baseline sleep of ~8h, cortisol levels on the in-laboratory baseline day demonstrated a rapid increase that continued with levels reaching a peak 30min post awakening. This acute increase was not observed during 48h of TSD. Additionally, there was no difference in mean salivary cortisol levels for the first samples (0min) collected across all study days. This would suggest the circadian pattern of cortisol secretion, specifically the early morning rise, is relatively independent of sleep and demonstrated good stability over the extended wake period.

Our data show that sleep and the process of waking from sleep elicits a rapid increase in cortisol levels that typify the phenomenon of the CAR. These results support those of Vargas et al. (2017b) and other research (Griefahn & Robens, 2010; Hall et al., 2019; Niu et al., 2015; Wilhelm et al., 2007) demonstrating the CAR is dependent on awakening and likely helps prepare for the day, but it is unclear which components of the awakening process modulate the CAR. Many unique changes occur in brain activity across the first 20min of wakefulness, most notably these changes are evident in the thalamus and pre-frontal cortices (PFC) (Balkin et al., 2002).

The PFC plays an underlying role in regulating HPA axis function and is vulnerable to sleep deprivation, with studies demonstrating deactivation of the PFC during periods of sleep deprivation (M. Thomas et al., 2000; M. Thomas et al., 2003). It might therefore be plausible to suggest that the physiological processes occurring in the PFC during the first 20min of wakefulness, specifically the integration of inputs from other brain regions occurring in the PFC, are responsible for modulating the CAR during the awakening process

No research to our knowledge has examined the CAR post a period of TSD. Harris et al. (2010) explored recovery of the CAR post a two-week night-shift schedule finding that after one week off the night-shift schedule the CAR had not returned to baseline (pre night shift) levels. Results from the present study indicate the CAR recovers to baseline levels on waking from a recovery sleep after 62h TSD, suggesting acute sleep deprivation does not impact recovery of the CAR. Cortisol levels across the first hour post awakening following the in-laboratory recovery sleep showed significant changes over time, as they did following an 8h baseline sleep. However, the CAR peaked at 45min post awakening compared to 30min for the peak following the in-laboratory baseline sleep. An observed peak at 45min post awakening is not considered unusual, as the CAR can reach its peak between 30 and 45min post awakening (Pruessner et al., 1997). However, as sleep duration has been suggested to influence the CAR, and the peak was greater following a longer sleep opportunity, it is possible the additional 1.5h sleep affected the CAR.

Findings to date on sleep duration and its relationship with the CAR are mixed; some suggest sleep duration has no influence (Pruessner et al., 1997; Wust et al., 2000) others suggest a positive relationship exists (Devine & Wolf, 2016; Kumari et al., 2009; Stalder et al., 2009). Possible explanations for the inconsistency of these findings may be the selected CAR measurement indices (e.g. awakening cortisol levels) and/or study design

methodology. Exploring self-reported sleep duration and morning cortisol rise, Kumari et al. (2009) found individuals reporting ~5h of sleep at night displayed a steeper morning rise while individuals who slept more than 5h at night displayed a progressively steeper morning rise in cortisol. Our data show participants displayed a steeper morning rise following an 8h sleep compared to the progressively steeper rise following 9.5h sleep. Similar to the current study, where sleep duration and saliva sampling were under controlled laboratory conditions, Devine and Wolf (2016) assessed the CAR in afternoon naps and night-time sleep finding an afternoon nap of 90min and a night-time sleep of 6h consistently resulted in a CAR, while shorter afternoon naps of 50min exhibited no CAR. Moreover, the CAR following the 6h night-time sleep was significantly greater than the CAR following the 90min nap. Collectively, these results and those from the current study provide support that a longer sleep duration of 9.5h possibly influences the magnitude of the CAR following a recovery sleep.

However, it is important to consider participants in the current study had experienced 62h of sleep deprivation prior to their sleep opportunity, whereas participants in previous sleep duration studies and other studies assessing the CAR had not. Considering no studies to date have assessed recovery of the CAR following a period of sustained wakefulness it is difficult to conclusively attribute the observed CAR post 62h TSD to sleep duration.

In addition to sleep duration, anticipation for the day ahead and prior day experiences have demonstrated an influence on the CAR. An increased CAR was associated with both negative prior day experiences (Fries et al., 2009; Steptoe & Serwinski, 2016) and successfully coping with the same day daily life stress (Powell & Schlotz, 2012). It has been suggested that the CAR provides sufficient resources (increased energy needs) to cope with the day ahead. It is therefore possible the increased magnitude reflects a

response that may be re-establishing energy needs from the TSD period and providing sufficient resources to cope with the day ahead, for example undertaking final cognitive testing measures before exiting the study. These conclusions, although plausible, are speculative.

The CAR on mornings following in-laboratory sleep opportunities (baseline and recovery) was greater in magnitude compared to the CAR measured in participants' homes. Additionally, the magnitude of the CAR on the first and second days at home post the TSD study (R1 and R2) followed a similar trajectory to that of the home pre-laboratory CARs. Previous studies have found that light in the early morning has a strong impact on morning cortisol levels (Leproult et al., 2001; Scheer & Buijs, 1999). Light intensity in the laboratory was set to <0.03 lux (darkness) during scheduled sleep periods and 100 lux during periods of wakefulness. It is possible the transition from complete darkness to bright light on the mornings following in-laboratory sleeps contributed to the increased magnitude of the CAR. Light intensity in participants' homes was not measured and, although participants were requested to sample with lights on, it is possible they did not, or the light intensity at home was significantly less than that in the laboratory and the magnitude of the CAR therefore attenuated. The effect of lighting was beyond the scope of this paper but could be a factor explored in future research when assessing recovery of the CAR following a period of sleep deprivation.

The present study controlled for as many extraneous factors as possible, specifically sleep, wake and saliva sampling times while participants were in the laboratory. During home sampling, sampling times were verified objectively with track caps (MEMs operating system), which reduced the impact of inaccurate measurement. However, as the study was conducted under controlled laboratory conditions and all participants were well rested prior to commencing the study, generalisability of the findings is limited.

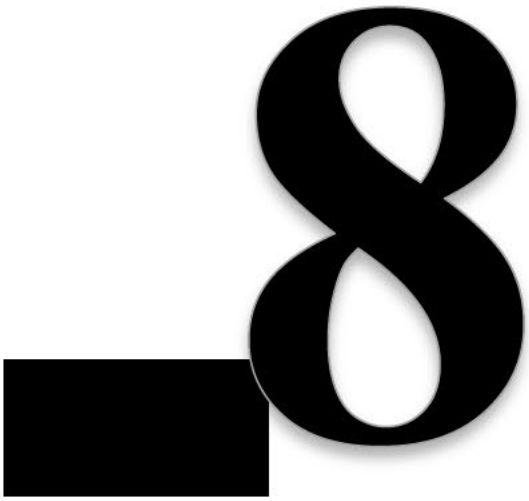
Individuals who experience repeated episodes of sleep deprivation may return different results.

7.8 Conclusion

In conclusion, results of this study demonstrate the CAR is not present during periods of TSD but recovers following a period of recovery sleep. While it is unclear why the magnitude of the CAR was greater following a recovery sleep, compared to baseline in the laboratory, these results suggest acute episodes of sleep deprivation do not adversely impact the CAR. However, a two-week night-shift schedule has demonstrated it takes longer than one week for the CAR return to baseline levels. Therefore, repetitive episodes of sleep deprivation may have implications on the CAR and its recovery. Future studies should examine the CAR longitudinally with populations who experience repeated episodes of sleep deprivation to better comprehend the impact of sleep deprivation on the CAR and its recovery. Observing such change may help to further understand the purpose of the CAR and potentially whether disruptions play a role in physiology closely connected to circadian rhythms.

Great things are done by a series of small things brought together.

- Vincent van Gogh



Discussion and conclusion

Chapter 8 Summary of research aims

This thesis examined the cumulative effects of four simulated night shifts and the concomitant behavioural changes that occur with altering sleep–wake patterns such as eating during the night on cortisol rhythms (Study 1, reported in Chapter 5). The impact of two nights of TSD on cortisol rhythms (reported in Chapter 6) and the CAR (reported in Chapter 7) was also examined (Study 2). The specific aims for each study are presented in Table 16, and Table 17 provides a summary of the findings.

This thesis found that:

1. While the cortisol rhythm was delayed with simulated shiftwork and was slightly elevated in the evening during sleep deprivation, the overall shape of the cortisol rhythm did not change (increase or flatten) with wakefulness through the night.

2. Behaviours that change with a night-shift schedule such as eating at night, temporarily increase cortisol for approximately 30 minutes after eating but rapidly return to premeal levels.

3. Sleep and wake are both required to generate the CAR.

This work contributes to the understanding of how cortisol rhythm changes during consecutive night shifts and sleep deprivation and how the CAR responds during and post a sleep deprivation period.

Table 16.
Overarching aims of the thesis

	Research Aim
1.	Determine the impact of four consecutive simulated night shifts on the cortisol rhythm
2.	Examine the impact of eating a meal, snack or not eating on cortisol during four consecutive simulated night shifts
3.	Investigate the impact of 62h of total sleep deprivation on cortisol rhythm
4.	Examine if there is a sudden morning rise in salivary cortisol that mimics CAR when no awakening occurs, and 2) investigate recovery sleep CAR after a period of 62h TSD.

Table 17.
Summary of thesis study results

Aim	Study	Chapter	Experimental manipulation	Outcome variable	Summary of main results
1	1	5	Four simulated night shifts	Cortisol levels	Cortisol levels at 20:00h significantly ↑ and cortisol levels at 05:30h significantly ↓ on Night 2 of the night-shift schedule. Levels continued to ↑ and ↓ at these times with each consecutive night shift
2	1	5	Eating a meal or snack vs. not eating on the night shift	Cortisol levels and AUC_i	Meal and snack conditions demonstrated significant ↑ in cortisol levels ~30min post food intake (00:30h) compared to No meal condition. Levels remained ↑ for ~1h AUC_i significantly greater on Night 2 and Night 3
3	2	6	62h TSD	Cortisol levels	Cortisol levels at 08:00h significantly ↓ on Days 2 and 3. Cortisol levels at 18:00h, 20:00h significantly ↑ on Days 2 and 3. Cortisol levels at 22:00h on Day 2 significantly ↑
4	2	7	62h TSD	Cortisol levels, the CAR	The CAR is absent during periods of TSD where there is no sleep or waking. The CAR recovered to baseline levels following a night of 9h recovery sleep post TSD

Note: ↑ = increase; ↓ = decrease; AUC_i = area under the curve with respect to increase; TSD = total sleep deprivation; CAR = cortisol awakening response

8.1 Cortisol displays a strong circadian rhythm and does not increase when awake during the night: Impact of night shift and total sleep deprivation

Both the shiftwork study (Chapter 5) and the sleep deprivation study (Chapters 6 and 7) found that cortisol had a strong circadian rhythm, with peak levels early in the morning, declining across the day with the lowest levels attained late at night, which is in line with previous research (Harris et al., 2010; Leproult et al., 1997; Wright et al., 2015). However, unlike some previous research (Touitou et al., 1990; von Treuer et al., 1996; Weibel et al., 1996) the shiftwork study (Chapter 5) and the sleep deprivation study (Chapter 6) did not find that cortisol was increased across the night. While the cortisol rhythm peak delayed with each night of simulated shiftwork, and cortisol was slightly elevated in the early evening during sleep deprivation, the overall shape of the cortisol rhythm did not change (increase or flatten) with wake at night. Sleep loss and altering the timing of sleep did not create a dramatic increase in cortisol or an acute stress response. Rather the impact on cortisol was small. These findings are similar to previous studies that also found an increase in early evening cortisol levels (Harris et al., 2015; Leproult et al., 1997; Wright et al., 2012).

Though an increase in cortisol levels was observed on two consecutive days in the early evening (18:00h and 20:00h) during the sleep deprivation study (Chapter 6), cortisol was only elevated for a short period. These observations would suggest a mild activation of the HPA axis.

Sleep loss appears to exert a mild influence on HPA axis activity (Balbo et al., 2010; Simpson et al., 2016). In a study by Simpson et al. (2016) exploring repeated periods of sleep loss and recovery over three weeks, it was found that repeated episodes of sleep loss and recovery were not stressful and did not cause the HPA axis to habituate (Grissom &

Bhatnagar, 2009). While habituation is considered to be an adaptive and/or protective process (Babb et al., 2014) habituation of the HPA axis to repeated episodes of sleep loss may be harmful rather than adaptive. Results from the current study demonstrate changes in HPA axis activity to be small, as prior sleep loss studies have also demonstrated. However, there is increasing evidence to suggest that the cumulative effect of small deviations in cortisol levels may play a role in the pathogenesis of health burdens such as cardiometabolic and cardiovascular disease (Jacobson, 2005; Puttonen et al., 2010; Ritonja et al., 2018; Spiegel et al., 1999), which provides further support for the importance of the small changes observed in the current study. Consequently, repeated increases in cortisol levels from rotating work schedules may lead to changes in physiological systems influenced by cortisol and eventually allostatic overload, increasing risk for disease (McEwen, 2006).

The findings from the shiftwork study (Chapter 5) and the sleep deprivation study (Chapter 6) are not without limitations. Participants in both studies were young, healthy, non-shiftworking individuals and the studies were conducted under controlled laboratory conditions. Additionally, participants experienced a single acute exposure to a night-shift schedule or sleep deprivation. Older shiftworking individuals who experience repeated night shifts, rotating work schedules or repeated periods of sleep loss may be impacted differently.

To address these limitations, future studies could examine real world stress in night shift workers as results from Study 1 and Study 2 may have been impacted by the fact general work and psychosocial stress was controlled. These stressors might cause different results. Future studies should endeavour to tease apart the impact of the shift, that is being awake and working at night , workload, psychosocial stress, on cortisol. A field study with real world shift workers could explore how reactive something like CAR is to

different sleep timing and the degree of workplace stress. For example, compare nurses working in a high tempo environment, like the emergency department, with nurses working in a low tempo ward like orthopaedics and assess if cortisol during the night stays high and whether eating or not eating stimulates further changes in cortisol levels. Future studies need to assess the effect of night shifts and exposure to regular episodes of sleep deprivation over a longer time frame, in healthy volunteers and those with varying shift schedules (individuals rotating onto and off night and shifts ≥24h multiple times). Such studies would provide an opportunity to assess and understand the relationship between repeated disruptions to the circadian pattern and signalling of cortisol, which over time may influence the functioning of peripheral systems. Future work could explore recovery of the HPA axis from repeated activations disrupting cortisol levels and determine how robust/resilient the system is over time. How quickly the system recovers would inform on the resiliency of the negative feedback system and potentially how long cortisol levels may remain elevated.

The current studies did not explore neurotransmitters such as norepinephrine or epinephrine to ascertain activation of the sympathetic-adreno-medullar axis. It would be important for future research to assess these and other mechanisms like heart rate/blood pressure, mood or stress ratings associated with a stress response. Such measures can contribute to the development of future strategies (e.g. sleep education) and interventions aimed at improving the health and wellbeing of individuals exposed to repeated episodes of sleep deprivation. Additionally, baseline and recovery data would have provided an opportunity to assess the degree of change in cortisol levels during exposure and rate of recovery post exposure. Such data may well provide a more comprehensive understanding of the impact of occupations and lifestyle factors that disrupt the sleep–

wake rhythm and cortisol, and subsequently physiological systems sensitive to cortisol. This would be an important addition to future literature.

8.2 Behavioural changes with night work and sleep deprivation: Impact of eating at night on cortisol

This thesis found eating during the night shift produced a temporary increase in cortisol levels approximately 30min post food intake, with cortisol levels remaining elevated for approximately 1h. In Study 1 (Chapter 5) the increase in cortisol levels post food consumption was temporary and did not significantly influence cortisol levels across the remainder of the night shift. Additionally, the cortisol response to food intake each night was the same. It did not increase with days of shiftwork and sleep loss. The findings also showed no increase in cortisol levels, at the same time of night, in participants who did not eat at night. In Study 2 (Chapter 6), participants received a snack at night (00:30h) similar in macronutrient content to the snack participants received in Study 1 (Chapter 5). While the frequency of sampling post meal was not as frequent as in Study 1, cortisol levels between 00:30h and 02:00h as depicted in Figure 12 (Chapter 6 results) suggest food consumption also impacted cortisol levels in Study 2. These results suggest that eating at night, irrespective of meal size, temporarily increases circulating levels of cortisol.

Small elevations in cortisol may have a compounding effect, and could play a role in the change in cortisol-sensitive peripheral tissue. Studies have demonstrated that food intake during the circadian rest phase leads to desynchronisation within the liver and a disturbed metabolism (Salgado-Delgado et al., 2013). Grant et al. (2017), exploring the impact of eating or not eating a meal at night on glucose metabolism, found that eating meals during the night impairs immediate postprandial glucose metabolism. Eating a meal during simulated night shift increased glucose (AUC) by ~30% following breakfast compared to

baseline. Additionally, there is some evidence to suggest that glucose and insulin responses to meals are modulated by circadian rhythmicity and that diurnal variation in meal tolerance may be facilitated by circulating (unbound) cortisol levels (Van Cauter et al., 1992). Exploring circadian modulation of glucose and insulin response to meals and relationship to cortisol rhythm, Van Cauter et al. (1992) found the magnitude of the morning to evening increase in insulin response to meals was correlated with the magnitude of the morning to evening decrease in cortisol levels.

The current thesis did not explore variations in metabolic response to eating across the night shift and the circadian rhythm of cortisol as these measures were outside the scope of this thesis. These would be important mechanisms to explore in the future to enhance understanding of the vulnerability of the metabolic system to circadian misalignment. Historically shift workers have an increased prevalence for type II diabetes, obesity and cardiometabolic syndrome (Knutsson, 2003). Future directions this research informs is the critical need to examine the potential mediating role of macronutrient content (carbohydrate vs. protein vs. fat) and frequency of meals across the night shift on cortisol levels. Additionally there is the need to explore the consumption of 'fresh' vs. 'processed' food/meals, the size and frequency of meals across the night shift and the impact these have on cortisol and associated cortisol-sensitive peripheral systems. Participants in the meal at night and snack at night conditions received a mixed nutrient meal or snack. Different dietary components – carbohydrate, protein, fats, and meal frequency – in previous studies have induced different effects on cortisol levels (Martens et al., 2010; Stimson et al., 2014) and should be explored in-laboratory controlled and real-world night-shift conditions in future studies.

Although the impact of food was not additive to the circadian changes observed in cortisol associated with night shift in this study, it may, however, be additive to circadian

changes observed in peripheral tissue/systems. Understanding the impact of eating at night on cortisol, cortisol-sensitive peripheral tissue/systems and the vulnerability of the metabolic system to circadian misalignment may help to inform dietary measures night workers can employ to aid in reducing disease risk.

Reporting food intake in future studies exploring sleep deprivation, night shift and cortisol levels will aid in characterising the degree of effect each factor – sleep deprivation, the night shift and the food – has on cortisol, and allow for enriched comparisons between future studies and advances in this area of study.

8.3 Sleep and wake are essential elements for the cortisol awakening response

This thesis has confirmed that a key process for the CAR is awakening from sleep. This sounds obvious, but as not been verified with multiple studies. This thesis, Study 2 (Chapter 7) clearly demonstrates that the CAR is initiated by, and present during, the awakening process from sleep but absent when waking does not occur. Further the CAR recovered following sleep after a period of sleep deprivation compared to baseline. Although not directly exploring the CAR, morning cortisol results post awakening in the study by Simpson et al. (2016) demonstrate a similar recovery pattern. Following repeated exposure of sleep restriction, the cortisol rise on the second day of recovery sleep was the same as the control. Acute periods of sleep deprivation do not appear to detrimentally impact the CAR. However, similar to the cortisol rhythm, repeated disruptions may have a compounding effect and play a role in health and/or performance. Harris et al. (2010) found the CAR had not recovered to baseline levels in workers one week after completing two weeks of night shift, suggesting recurring circadian misalignment has prolonged effects on the CAR. Circadian misalignment has been associated with negative health outcomes. The CAR may play a role in physiology

closely tuned to circadian rhythms. Although outside the scope of this study, unhealthy states may be detected by examining the CAR during periods of circadian misalignment and repeated exposure to sleep deprivation.

This study also assessed the CAR at home prior to commencing and post finishing the in-laboratory study. The magnitude of the CAR at home pre and post the study was the same; however, the profile was initially different. The initial increase in cortisol levels at 15min post awakening was greater in both post-study CARs compared with the pre-study CARs. This finding is novel; no prior studies have assessed the CAR post sleep deprivation. A suggested function of the CAR has been to increase energy needs as individuals transition from a rest to an active state to cope with the day ahead (Schulz et al., 1998). Therefore, it is reasonable to suggest this may be an adaptive response assisting with re-establishment and provision of energy needs diminished during the sleep deprivation period to ensure optimal functioning occurs. Future studies should assess energy expenditure, for example, food intake and physical activity, pre, during and post sleep deprivation to determine if energy levels/needs influence the early vigorous increase in the CAR post sleep deprivation.

The CAR is predictive of same day cognitive performance (Hodyl et al., 2016; Law et al., 2015; Shi et al., 2018). In spite of this, the impact of sleep deprivation on the CAR and cognitive processes the CAR is predictive of has received substantially limited attention within the literature. Results from this thesis demonstrate the CAR recovers well from an acute episode of sleep deprivation however, similar to the cortisol rhythm, there is a need to understand the impact of repeated disruptions on the CAR and cognitive performance. Future directions this research informs is a need to explore the effect of exposure to recurring episodes of sleep deprivation over a longer time frame, in healthy volunteers and those with varying shift schedules to ascertain associations between the CAR and

cognitive performance. Such research may help to understand the functional significance of the CAR in healthy people and the potential impact repeated periods of sleep deprivation may have on its role.

Additionally, it is important for future research to explore and understand what physiological inputs, beyond those of the HPA axis and hippocampus, contribute to the generation of the CAR. This would help to determine what constitutes a healthy and normal CAR or indicate dysfunction, particularly in individuals experiencing repeated periods of sleep deprivation. Without the waking process and the CAR it is possible there may be flow on effects that over time impact health and cognition. Over time, these implications may translate into higher economic and social costs for individuals, organisations and society.

8.4 Conclusion

This thesis examined the cumulative effects of simulated night shift and sleep deprivation, and the concomitant behavioural changes that occur with altering sleep–wake patterns on cortisol, such as eating during the night. It was found that cortisol displays a strong circadian rhythm. Results also suggest not eating at night maintains low cortisol levels, which may be important to reduce disease risk.While the role of the CAR remains unclear, findings of this thesis provide novel insight on the CAR post sleep deprivation. More importantly, it has identified factors that must be considered for future CAR research to takes steps towards understanding its purpose.

As modern civilisation increasingly operates on a 24-hour schedule, it is crucial to continue researching to further understanding how altered sleep and wake behaviours impact biological processes and health. This thesis showed it is important to examine altered sleep patterns and concomitant behaviours and health. Doing so can inform

workplace policy, for example reducing the duration and number of consecutive night shifts and, rostering for adequate time off between shifts that affords sufficient time for physiological systems like the metabolic system to recover from sleep loss and circadian misalignment. Further it can contribute to the development of future strategies (e.g. sleep education, dietary advice) and interventions aimed at improving the health and wellbeing of populations who experience recurring periods of sleep deprivation or night shift rostering.

References

Abbott, S. M., Malkani, R. G., & Zee, P. C. (2020). Circadian disruption and human health: A bidirectional relationship. *European Journal of Neuroscience*, *51*(1), 567–583. https://doi.org/10.1111/ejn.14298

Abrams, R. M. (2015). Sleep deprivation. *Obstetrics and Gynecology Clinics*, *42*(3), 493–506.

Activinsights Ltd. GENEActiv: The Original, Wrist-Worn Raw Data Accelerometer. (2021). Activinsights. https://activinsights.com/technology/geneactiv/

Adam, E. K., Quinn, M. E., Tavernier, R., McQuillan, M. T., Dahlke, K. A., & Gilbert, K. E. (2017). Diurnal cortisol slopes and mental and physical health outcomes: A systematic review and meta-analysis. *Psychoneuroendocrinology*, *83*, 25–41. https://doi.org/10.1016/j.psyneuen.2017.05.018

Adams, R. J., Appleton, S. L., Taylor, A. W., Gill, T. K., Lang, C., McEvoy, R. D., & Antic, N. A. (2017). Sleep health of Australian adults in 2016: Results of the 2016 Sleep Health Foundation national survey. *Sleep Health*, *3*(1), 35–42. https://doi.org/10.1016/j.sleh.2016.11.005

Åkerstedt, T., Kecklund, G., Ingre, M., Lekander, M., & Axelsson, J. (2009). *Sleep Homeostasis During Repeated Sleep Restriction and Recovery: Support from EEG Dynamics*. *32*(2), 6.

Åkerstedt, T., Palmblad, J., de la Torre, B., Marana, R., & Gillberg, M. (1980). Adrenocortical and Gonadal Steroids During Sleep Deprivation. *Sleep*, *3*(1), 23–30. https://doi.org/10.1093/sleep/3.1.23

Anagnostis, P., Athyros, V. G., Tziomalos, K., Karagiannis, A., & Mikhailidis, D. P. (2009). The pathogenetic role of cortisol in the metabolic syndrome: A

hypothesis. *The Journal of Clinical Endocrinology & Metabolism*, *94*(8), 2692–2701.

Anjum, B., Verma, N. S., Tiwari, S., Singh, R., Mahdi, A. A., Singh, R. B., & Singh, R. K. (2011). Association of salivary cortisol with chronomics of 24 hours ambulatory blood pressure/heart rate among night shift workers. *BioScience Trends*, 5(4) 182–188.

Arksey, H., & O'Malley, L. (2005). Scoping studies: Towards a methodological framework. *International Journal of Social Research Methodology*, *8*(1), 19–32. https://doi.org/10.1080/1364557032000119616

Australian Bureau of Statistics. (1997). *National Nutrition Survey Selected Highlights, Australia 1995: Summary of results* (ABS cat. no. 4802.0. Canberra: ABS). Australian Bureau of Statistics.

Babb, J. A., Masini, C. V., Day, H. E. W., & Campeau, S. (2014). Habituation of hypothalamic–pituitary–adrenocortical axis hormones to repeated homotypic stress and subsequent heterotypic stressor exposure in male and female rats. *Stress*, *17*(3), 224–234. https://doi.org/10.3109/10253890.2014.905534

Baker, F. C., & Driver, H. S. (2004). Self-reported sleep across the menstrual cycle in young, healthy women. *Journal of Psychosomatic Research*, *56*(2), 239–243. https://doi.org/10.1016/S0022-3999(03)00067-9

Balbo, M., Leproult, R., & Cauter, E. V. (2010). Impact of Sleep and Its Disturbances on Hypothalamo-Pituitary-Adrenal Axis Activity. *International Journal of Endocrinology*, 17.

Balkin, T. J., Braun, A. R., Wesensten, N. J., Jeffries, K., Varga, M., Baldwin, P., Belenky, G., & Herscovitch, P. (2002). The process of awakening: A PET study

of regional brain activity patterns mediating the re-establishment of alertness and consciousness. *Brain*, *125*(10), 2308–2319.

Balsalobre, A., Brown, S. A., Marcacci, L., Tronche, F., Kellendonk, C., Reichardt, H. M., Schutz, G., & Schibler, U. (2000). Resetting of circadian time in peripheral tissues by glucocorticoid signaling. *Science*, *289*(5488), 2344–2347.

Banks, S., & Dinges, D. F. (2011). Chapter 6—Chronic Sleep Deprivation. In M. H. Kryger, T. Roth, & W. C. Dement (Eds.), *Principles and Practice of Sleep Medicine (Fifth Edition)* (Fifth Edition, pp. 67–75). W.B. Saunders. https://doi.org/10.1016/B978-1-4160-6645-3.00006-2

Beck, A., Steer, R., & Brown, G. (1996). *Manual for the Beck Depression Inventory-II*. Psychological Corporation.

Bonham, M. P., Bonnell, E. K., & Huggins, C. E. (2016). Energy intake of shift workers compared to fixed day workers: A systematic review and meta-analysis. *Chronobiology International*, *33*(8), 1086–1100. https://doi.org/10.1080/07420528.2016.1192188

Borbely, A. A. (1982). A Two Process Model of Sleep Regulation. *Human Neurobiology*, *1*, 195–204.

Borbély, A. A., Daan, S., Wirz-Justice, A., & Deboer, T. (2016). The two-process model of sleep regulation: A reappraisal. *Journal of Sleep Research*, *25*(2), 131–143. https://doi.org/10.1111/jsr.12371

Born, J., Hansen, K., Marshall, L., Mölle, M., & Fehm, H. L. (1999). Timing the end of nocturnal sleep. *Nature*, *397*(6714), 29–30.

Born, J., Kern, W., Bieber, K., Fehm-Wolfsdorf, G., Schiebe, M., & Fehm, H. L. (1986). Night-time plasma cortisol secretion is associated with specific sleep stages.

Biological Psychiatry, *21*(14), 1415–1424. https://doi.org/10.1016/0006-3223(86)90333-1

Bostock, S., & Steptoe, A. (2013). Influences of early shift work on the diurnal cortisol rhythm, mood and sleep: Within-subject variation in male airline pilots. *Psychoneuroendocrinology*, *38*(4), 533–541. https://doi.org/10.1016/j.psyneuen.2012.07.012

Bozovic, D., Racic, M., & Ivkovic, N. (2013). Salivary Cortisol Levels as a Biological Marker of Stress Reaction. *Medical Archives*, *67*, 374. https://doi.org/10.5455/medarh.2013.67.374-377

Buchanan, T. W., Kern, S., Allen, J. S., Tranel, D., & Kirschbaum, C. (2004). Circadian regulation of cortisol after hippocampal damage in humans. *Biological Psychiatry*, *56*(9), 651–656. https://doi.org/10.1016/j.biopsych.2004.08.014

Buckingham, J. C. (2006). Glucocorticoids: Exemplars of multi-tasking. *British Journal of Pharmacology*, *147*(Suppl 1), S258–S268. https://doi.org/10.1038/sj.bjp.0706456

Buckley, T. M., & Schatzberg, A. F. (2005). On the Interactions of the Hypothalamic-Pituitary-Adrenal (HPA) Axis and Sleep: Normal HPA Axis Activity and Circadian Rhythm, Exemplary Sleep Disorders. *The Journal of Clinical Endocrinology & Metabolism*, *90*(5), 3106–3114. https://doi.org/10.1210/jc.2004-1056

Buysse, D. J., Reynolds, C. F., Monk, T. H., Berman, S. R., & Kupfer, D. J. (1989). The Pittsburgh sleep quality index: A new instrument for psychiatric practice and research. *Psychiatry Research*, *28*(2), 193–213. https://doi.org/10.1016/0165-1781(89)90047-4

Cain, D. W., & Cidlowski, J. A. (2017). Immune regulation by glucocorticoids. *Nature Reviews Immunology*, *17*(4), 233–247. https://doi.org/10.1038/nri.2017.1

Cannizzaro, E., Cirrincione, L., Mazzucco, W., Scorciapino, A., Catalano, C., Ramaci, T., Ledda, C., & Plescia, F. (2020). Night-Time Shift Work and Related Stress Responses: A Study on Security Guards. *International Journal of Environmental Research and Public Health*, *17*(2), 562. https://doi.org/10.3390/ijerph17020562

Cannizzaro, E., Plescia, F., Cirrincione, L., Lo Pinto, E., & Plescia, F. (2018). Sport for job. Differences in cortisol levels in a water polo team at different times of workout. *Euromediterranean Biomed. J*, *13*, 181–184.

Carev, M., Karanovi, N., Bagatin, J., Berovi, N., Pecoti, R., Vali, M., Marinovi, I., & Karanovi, S. (2011). Blood pressure dipping and salivary cortisol as markers of fatigue and sleep deprivation in staff anesthesiologists. *Collegium Antropologicum*, 35(1), 133–138.

Casement, M. D., Broussard, J. L., Mullington, J. M., & Press, D. Z. (2006). The contribution of sleep to improvements in working memory scanning speed: A study of prolonged sleep restriction. *Biological Psychology*, *72*(2), 208–212. https://doi.org/10.1016/j.biopsycho.2005.11.002

Chang, W. P. (2018). Influence of shift type on sleep quality of female nurses working monthly rotating shifts with cortisol awakening response as mediating variable. *Chronobiology International*, *35*(11), 1503–1512. https://doi.org/10.1080/07420528.2018.1493491

Chapotot, F., Buguet, A., Gronfier, C., & Brandenberger, G. (2001). Hypothalamo-pituitary-adrenal axis activity is related to the level of central arousal: Effect of sleep deprivation on the association of high-frequency waking electroencephalogram with cortisol release. *Neuroendocrinology*, *73*(5), 312–321.

Charles, L. E., Fekedulegn, D., Burchfiel, C. M., Hartley, T. A., Andrew, M. E., Violanti, J. M., & Miller, D. B. (2016). Shiftwork and diurnal salivary cortisol patterns among police officers. *Journal of Occupational and Environmental Medicine / American College of Occupational and Environmental Medicine*, *58*(6), 542–549. https://doi.org/10.1097/JOM.0000000000000729

Charmandari, E., Tsigos, C., & Chrousos, G. (2005). Endocrinology of the stress response. *Annual Review of Physiology*, *67*(1), 259–284. https://doi.org/10.1146/annurev.physiol.67.040403.120816

Chellappa, S. L., Morris, C. J., & Scheer, F. A. J. L. (2018). Daily circadian misalignment impairs human cognitive performance task-dependently. *Scientific Reports*, *8*(1), 3041. https://doi.org/10.1038/s41598-018-20707-4

Chida, Y., & Steptoe, A. (2009). Cortisol awakening response and psychosocial factors: A systematic review and meta-analysis. *Biological Psychology*, *80*(3), 265–278. https://doi.org/10.1016/j.biopsycho.2008.10.004

Choshen-Hillel, S., Ishqer, A., Mahameed, F., Reiter, J., Gozal, D., Gileles-Hillel, A., & Berger, I. (2021). Acute and chronic sleep deprivation in residents: Cognition and stress biomarkers. *Medical Education*, *55*(2), 174–184. https://doi.org/10.1111/medu.14296

Chu, B., Marwaha, K., Sanvictores, T., & Ayers, D. (2021). Physiology, stress reaction. In *StatPearls [Internet]*. StatPearls Publishing.

Chung, S., Son, G. H., & Kim, K. (2011). Circadian rhythm of adrenal glucocorticoid: Its regulation and clinical implications. *Biochimica et Biophysica Acta (BBA) – Molecular Basis of Disease*, *1812*(5), 581–591. https://doi.org/10.1016/j.bbadis.2011.02.003

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*(1), 97–103. https://doi.org/10.1016/j.neubiorev.2009.12.011

Clow, A., Hucklebridge, F., & Thorn, L. (2010). The cortisol awakening response in context. In *International review of neurobiology* (Vol. 93, pp. 153–175). Elsevier.

Clow, A., Law, R., Evans, P., Vallence, A. M., Hodyl, N. A., Goldsworthy, M. R., Rothwell, J. R., & Ridding, M. C. (2014). Day differences in the cortisol awakening response predict day differences in synaptic plasticity in the brain. *Stress*, *17*(3), 219–223. https://doi.org/10.3109/10253890.2014.905533

Clow, A., Thorn, L., Evans, P., & Hucklebridge, F. (2004). The awakening cortisol response: Methodological issues and significance. *Stress*, *7*(1), 29–37. https://doi.org/10.1080/10253890410001667205

Costa, G. (2003). Factors influencing health of workers and tolerance to shift work. *Theoretical Issues in Ergonomics Science*, *4*(3–4), 263–288. https://doi.org/10.1080/14639220210158880

Costa, G., & Di Milia, L. (2008). Aging and shift work: A complex problem to face. *Chronobiology International*, *25*(2–3), 165–181. https://doi.org/10.1080/07420520802103410

Cuesta, M., Cermakian, N., & Boivin, D. B. (2015). Glucocorticoids entrain molecular clock components in human peripheral cells. *The FASEB Journal*, *29*(4), 1360–1370.

Curtis, V., Henry, C. J. K., Birch, E., & Ghusain-Choueiri, A. (1996). Intraindividual variation in the basal metabolic rate of women: Effect of the menstrual cycle. *American Journal of Human Biology*, *8*(5), 631–639.

https://doi.org/10.1002/(SICI)1520-6300(1996)8:5<631::AID-AJHB8>3.0.CO;2-Y

Czeisler, C. A., & Gooley, J. J. (2007). Sleep and circadian rhythms in humans. *Cold Spring Harbor Symposia on Quantitative Biology*, *72*(1), 579–597. https://doi.org/10.1101/sqb.2007.72.064

Damiola, F., Le Minh, N., Preitner, N., Kornmann, B., Fleury-Olela, F., & Schibler, U. (2000). Restricted feeding uncouples circadian oscillators in peripheral tissues from the central pacemaker in the suprachiasmatic nucleus. *Genes & Development*, *14*(23), 2950–2961.

de Assis, M. A. A., Kupek, E., Nahas, M. V., & Bellisle, F. (2003). Food intake and circadian rhythms in shift workers with a high workload. *Appetite*, *40*(2), 175–183.

Dedovic, K., Duchesne, A., Andrews, J., Engert, V., & Pruessner, J. C. (2009). The brain and the stress axis: The neural correlates of cortisol regulation in response to stress. *Neuroimage*, *47*(3), 864–871.

Dettenborn, L., Rosenloecher, F., & Kirschbaum, C. (2007). No effects of repeated forced wakings during three consecutive nights on morning cortisol awakening responses (CAR): A preliminary study. *Psychoneuroendocrinology*, *32*(8–10), 915–921. https://doi.org/10.1016/j.psyneuen.2007.06.011

Devine, J. K., & Wolf, J. M. (2016). Determinants of cortisol awakening responses to naps and nighttime sleep. *Psychoneuroendocrinology*, *63*, 128–134. https://doi.org/10.1016/j.psyneuen.2015.09.016

Dinges, D. F., Douglas, S. D., Zaugg, L., Campbell, D. E., McMann, J. M., Whitehouse, W. G., Orne, E. C., Kapoor, S. C., Icaza, E., & Orne, M. T. (1994). Leukocytosis and natural killer cell function parallel neurobehavioral fatigue induced by 64

hours of sleep deprivation. *Journal of Clinical Investigation*, *93*(5), 1930–1939. https://doi.org/10.1172/JCI117184

Dinneen, S., Alzaid, A., Miles, J., & Rizza, R. (1993). Metabolic effects of the nocturnal rise in cortisol on carbohydrate metabolism in normal humans. *Journal of Clinical Investigation*, *92*(5), 2283–2290.

Diorio, D., Viau, V., & Meaney, M. J. (1993). The role of the medial prefrontal cortex (cingulate gyrus) in the regulation of hypothalamic-pituitary-adrenal responses to stress. *Journal of Neuroscience*, *13*(9), 3839–3847.

Driver, H. S., Werth, E., Dijk, D. J., & Borbély, A. A. (2008). The menstrual cycle effects on sleep. *Sleep Medicine Clinics*, *3*(1), 1–11.

Edwards, S., Evans, P., Hucklebridge, F., & Clow, A. (2001). Association between time of awakening and diurnal cortisol secretory activity. *Psychoneuroendocrinology*, *26*(6), 613–622. https://doi.org/10.1016/S0306-4530(01)00015-4

Elder, G. J., Wetherell, M. A., Barclay, N. L., & Ellis, J. G. (2014). The cortisol awakening response–applications and implications for sleep medicine. *Sleep Medicine Reviews*, *18*(3), 215–224.

Ernst, F., Rauchenzauner, M., Zoller, H., Griesmacher, A., Hammerer-Lercher, A., Carpenter, R., Schuessler, G., & Joannidis, M. (2014). Effects of 24h working on-call on psychoneuroendocrine and oculomotor function: A randomized cross-over trial. *Psychoneuroendocrinology*, *47*, 221–231. https://doi.org/10.1016/j.psyneuen.2014.05.019

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*(2), 174–184. https://doi.org/10.1016/S0306-4530(03)00021-0

Fekedulegn, D., Burchfiel, C. M., Violanti, J. M., Hartley, T. A., Charles, L. E., Andrew, M. E., & Miller, D. B. (2012). Associations of long-term shift work with waking salivary cortisol concentration and patterns among police officers. *Industrial Health*, *50*(6), 476–486.

Ferrara, M., & De Gennaro, L. (2001). How much sleep do we need? *Sleep Medicine Reviews*, *5*(2), 155–179. https://doi.org/10.1053/smrv.2000.0138

Frankenfield, D. C., Rowe, W. A., Smith, J. S., & Cooney, R. (2003). Validation of several established equations for resting metabolic rate in obese and nonobese people. *Journal of the American Dietetic Association*, *103*(9), 1152–1159.

Fries, E., Dettenborn, L., & Kirschbaum, C. (2009). The cortisol awakening response (CAR): Facts and future directions. *International Journal of Psychophysiology*, *72*(1), 67–73. https://doi.org/10.1016/j.ijpsycho.2008.03.014

Fujiwara, S., Shinkai, S., Kurokawa, Y., & Watanabe, T. (1992). The acute effects of experimental short-term evening and night shifts on human circadian rhythm: The oral temperature, heart rate, serum cortisol and urinary catecholamines levels. *International Archives of Occupational and Environmental Health*, *63*(6), 409–418. https://doi.org/10.1007/BF00386937

Gaillard, A., & Wientjes, C. (1994). Mental load and work stress as two types of energy mobilization. *Work & Stress*, *8*(2), 141–152.

Grant, C. L., Coates, A. M., Dorrian, J., Kennaway, D. J., Wittert, G. A., Heilbronn, L. K., Pajcin, M., Della Vedova, C., Gupta, C. C., & Banks, S. (2017). Timing of food intake during simulated night shift impacts glucose metabolism: A controlled study. *Chronobiology International*, *34*(8), 1003–1013. https://doi.org/10.1080/07420528.2017.1335318

Griefahn, B., & Robens, S. (2008). The cortisol awakening response: A pilot study on the effects of shift work, morningness and sleep duration. *Psychoneuroendocrinology*, *33*(7), 981–988. https://doi.org/10.1016/j.psyneuen.2008.04.004

Griefahn, B., & Robens, S. (2010). The normalization of the cortisol awakening response and of the cortisol shift profile across consecutive night shifts—An experimental study. *Psychoneuroendocrinology*, *35*(10), 1501–1509. https://doi.org/10.1016/j.psyneuen.2010.05.002

Grissom, N., & Bhatnagar, S. (2009). Habituation to repeated stress: Get used to it. *Neurobiology of Learning and Memory*, *92*(2), 215–224. https://doi.org/10.1016/j.nlm.2008.07.001

Gross, K. L., & Cidlowski, J. A. (2008). Tissue-specific glucocorticoid action: A family affair. *Trends in Endocrinology & Metabolism*, *19*(9), 331–339. https://doi.org/10.1016/j.tem.2008.07.009

Grosser, L., Knayfati, S., Yates, C., Dorrian, J., & Banks, S. (2022). Cortisol and shiftwork: A scoping review. *Sleep Medicine Reviews*, *64*, 101581. https://doi.org/10.1016/j.smrv.2021.101581

Gu, C., Brereton, N., Schweitzer, A., Cotter, M., Duan, D., Børsheim, E., Wolfe, R. R., Pham, L. V., Polotsky, V. Y., & Jun, J. C. (2020). Metabolic effects of late dinner in healthy volunteers—A randomized crossover clinical trial. *The Journal of Clinical Endocrinology & Metabolism*, *105*(8), 2789–2802. https://doi.org/10.1210/clinem/dgaa354

Gupta, C. C., Dorrian, J., Grant, C. L., Pajcin, M., Coates, A. M., Kennaway, D. J., Wittert, G. A., Heilbronn, L. K., Della Vedova, C. B., & Banks, S. (2017). It's not just what you eat but when: The impact of eating a meal during simulated shift

work on driving performance. *Chronobiology International*, *34*(1), 66–77. https://doi.org/10.1080/07420528.2016.1237520

Gur, C., Boz, M., Muderrisoglu, C., & Polat, H. (2014). The relationship between insulin resistance and cortisol levels. *Istanbul Medical Journal*, *16*. https://doi.org/10.5152/imj.00377

Guyon, A., Balbo, M., Morselli, L. L., Tasali, E., Leproult, R., L'Hermite-Balériaux, M., Van Cauter, E., & Spiegel, K. (2014). Adverse Effects of Two Nights of Sleep Restriction on the Hypothalamic-Pituitary-Adrenal Axis in Healthy Men. *The Journal of Clinical Endocrinology & Metabolism*, *99*(8), 2861–2868. https://doi.org/10.1210/jc.2013-4254

Hall, S. J., Aisbett, B., Tait, J. L., Turner, A. I., Ferguson, S. A., & Main, L. C. (2016). The acute physiological stress response to an emergency alarm and mobilization during the day and at night. *Noise & Health*, *18*(82), 150.

Hall, S. J., Turner, A. I., Robertson, S. J., Ferguson, S. A., & Aisbett, B. (2019). Salivary cortisol profiles of on-call from home fire and emergency service personnel. *Stress*, *22*(4), 436–445. https://doi.org/10.1080/10253890.2019.1584178

Hamidovic, A., Karapetyan, K., Serdarevic, F., Choi, S. H., Eisenlohr-Moul, T., & Pinna, G. (2020). Higher circulating cortisol in the follicular vs. Luteal phase of the menstrual cycle: A meta-analysis. *Frontiers in Endocrinology*, *11*, 311.

Hannibal, K. E., & Bishop, M. D. (2014). Chronic Stress, Cortisol Dysfunction, and Pain: A Psychoneuroendocrine Rationale for Stress Management in Pain Rehabilitation. *Physical Therapy*, *94*(12), 1816–1825. https://doi.org/10.2522/ptj.20130597

Harris, A., Waage, S., Ursin, H., Hansen, Å. M., Bjorvatn, B., & Eriksen, H. R. (2010). Cortisol, reaction time test and health among offshore shift workers.

Psychoneuroendocrinology, *35*(9), 1339–1347. https://doi.org/10.1016/j.psyneuen.2010.03.006

Harris, J. A., & Benedict, F. G. (1919). *A biometric study of basal metabolism in man* (Issue 279). Carnegie institution of Washington.

Harris, R., Sims, S., Parr, J., & Davies, N. (2015). Impact of 12h shift patterns in nursing: A scoping review. *International Journal of Nursing Studies*, *52*(2), 605–634. https://doi.org/10.1016/j.ijnurstu.2014.10.014

Hastings, M. H., Maywood, E. S., & Brancaccio, M. (2018). Generation of circadian rhythms in the suprachiasmatic nucleus. *Nature Reviews Neuroscience*, *19*(8), 453–469. https://doi.org/10.1038/s41583-018-0026-z

Haus, E., & Smolensky, M. (2006). Biological clocks and shift work: Circadian dysregulation and potential long-term effects. *Cancer Causes & Control*, *17*(4), 489–500.

Heaney, J. (2013). Hypothalamic-pituitary-adrenal axis. In M. D. Gellman & J. R. Turner (Eds.), *Encyclopedia of Behavioral Medicine* (pp. 1017–1018). Springer New York. https://doi.org/10.1007/978-1-4419-1005-9_460

Hellhammer, D. H., Wüst, S., & Kudielka, B. M. (2009). Salivary cortisol as a biomarker in stress research. *Psychoneuroendocrinology*, *34*(2), 163–171. https://doi.org/10.1016/j.psyneuen.2008.10.026

Hellhammer, J., Fries, E., Schweisthal, O. W., Schlotz, W., Stone, A. A., & Hagemann, D. (2007). Several daily measurements are necessary to reliably assess the cortisol rise after awakening: State- and trait components. *Psychoneuroendocrinology*, *32*(1), 80–86. https://doi.org/10.1016/j.psyneuen.2006.10.005

Hennig, J., Kieferdorf, P., Moritz, C., Huwe, S., & Netter, P. (1998). Changes in cortisol secretion during shiftwork: Implications for tolerance to shiftwork? *Ergonomics*, *41*(5), 610–621. https://doi.org/10.1080/001401398186784

Herichova, I. (2013). Changes of physiological functions induced by shift work. *Endocrine Regulations*, *47*, 159–170. https://doi.org/10.4149/endo_2013_03_159

Herman, J. P., Ostrander, M. M., Mueller, N. K., & Figueiredo, H. (2005). Limbic system mechanisms of stress regulation: Hypothalamo-pituitary-adrenocortical axis. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *29*(8), 1201–1213.

Hill, E. E., Zack, E., Battaglini, C., Viru, M., Viru, A., & Hackney, A. C. (2008). Exercise and circulating cortisol levels: The intensity threshold effect. *Journal of Endocrinological Investigation*, *31*(7), 587–591. https://doi.org/10.1007/BF03345606

Hiller-Sturmhöfel, S., & Bartke, A. (1998). The endocrine system. *Alcohol Health and Research World*, *22*(3), 153–164.

Hindmarsh, P. C., & Geertsma, K. (2017). *Congenital Adrenal Hyperplasia: A Comprehensive Guide*. Academic Press.

Hodyl, N. A., Schneider, L., Vallence, A.-M., Clow, A., Ridding, M. C., & Pitcher, J. B. (2016). The cortisol awakening response is associated with performance of a serial sequence reaction time task. *International Journal of Psychophysiology*, *100*, 12–18. https://doi.org/10.1016/j.ijpsycho.2015.12.007

Holmbäck, U., Forslund, A., Forslund, J., Hambraeus, L., Lennernäs, M., Lowden, A., Stridsberg, M., & Åkerstedt, T. (2002). Metabolic Responses to Nocturnal Eating in Men Are Affected by Sources of Dietary Energy. *The Journal of Nutrition*, *132*(7), 1892–1899. https://doi.org/10.1093/jn/132.7.1892

Horne, J. A., & Östberg, O. (1976). A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *International Journal of Chronobiology*.

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*(7), 900–904. https://doi.org/10.1016/j.psyneuen.2006.03.005

Hucklebridge, F. H., Clow, A., Abeyguneratne, T., Huezo-Diaz, P., & Evans, P. (1999). The awakening cortisol response and blood glucose levels. *Life Sciences*, *64*(11), 931–937. https://doi.org/10.1016/S0024-3205(99)00019-3

Iob, E., & Steptoe, A. (2019). Cardiovascular disease and hair cortisol: A novel biomarker of chronic stress. *Current Cardiology Reports*, *21*(10), 116. https://doi.org/10.1007/s11886-019-1208-7

Ishizuka, B., Quigley, M. E., & Yen, S. S. C. (1983). Pituitary hormone release in response to food ingestion: Evidence for neuroendocrine signals from gut to brain*. *The Journal of Clinical Endocrinology & Metabolism*, *57*(6), 1111–1116. https://doi.org/10.1210/jcem-57-6-1111

Jacobson, L. (2005). Hypothalamic–pituitary–adrenocortical axis regulation. *Endocrinology and Metabolism Clinics*, *34*(2), 271–292.

James, S. M., Honn, K. A., Gaddameedhi, S., & Van Dongen, H. P. A. (2017). Shift Work: Disrupted Circadian Rhythms and Sleep—Implications for Health and Well-being. *Current Sleep Medicine Reports*, *3*(2), 104–112. https://doi.org/10.1007/s40675-017-0071-6

Jenkins, D. J., Wolever, T. M., Vuksan, V., Brighenti, F., Cunnane, S. C., Rao, A. V., Jenkins, A. L., Buckley, G., Patten, R., & Singer, W. (1989). Nibbling versus

gorging: Metabolic advantages of increased meal frequency. *New England Journal of Medicine*, *321*(14), 929–934.

Jensen, M. A., Hansen, Å. M., Kristiansen, J., Nabe-Nielsen, K., & Garde, A. H. (2016). Changes in the diurnal rhythms of cortisol, melatonin, and testosterone after 2, 4, and 7 consecutive night shifts in male police officers. *Chronobiology International*, *33*(9), 1280–1292. https://doi.org/10.1080/07420528.2016.1212869

Jessop, D. S., & Turner-Cobb, J. M. (2008). Measurement and meaning of salivary cortisol: A focus on health and disease in children: Review. *Stress*, *11*(1), 1–14. https://doi.org/10.1080/10253890701365527

Kadmiel, M., & Cidlowski, J. A. (2013). Glucocorticoid receptor signaling in health and disease. *Trends in Pharmacological Sciences*, *34*(9), 518–530. https://doi.org/10.1016/j.tips.2013.07.003

Kagias, K., Nehammer, C., & Pocock, R. (2012). Neuronal responses to physiological stress. *Frontiers in Genetics*, *3*. https://doi.org/10.3389/fgene.2012.00222

Kant, G. J., Genser, S. G., Thorne, D. R., Pfalser, H. L., & Mougey, E. H. (1984). Effects of 72 hour sleep deprivation on urinary cortisol and indices of metabolism. *Sleep*, *7*(2), 142–146. https://doi.org/10.1093/sleep/7.2.142

Karatsoreos, I. N., & McEwen, B. S. (2011). Psychobiological allostasis: Resistance, resilience and vulnerability. *Trends in Cognitive Sciences*, *15*(12), 576–584.

Karlson, B., Carlsson Eek, F., Hansen, A. M., Garde, A. H., Osterberg, K., & Orbaek, P. (2006). Diurnal cortisol pattern of shift workers on a workday and a day off. *Scandinavian Journal of Work Environment and Health*, *32*, 27.

Katsu, Y., & Baker, M. E. (2021). Cortisol. In *Handbook of hormones* (pp. 947–949). Elsevier.

Khani, S., & Tayek, J. A. (2001). Cortisol increases gluconeogenesis in humans: Its role in the metabolic syndrome. *Clinical Science*, *101*(6), 739–747.

Khoury, J. E., Gonzalez, A., Levitan, R. D., Pruessner, J. C., Chopra, K., Basile, V. S., Masellis, M., Goodwill, A., & Atkinson, L. (2015). Summary cortisol reactivity indicators: Interrelations and meaning. *Neurobiology of Stress*, *2*, 34–43. https://doi.org/10.1016/j.ynstr.2015.04.002

Kirschbaum, C., & Hellhammer, D. H. (1994). Salivary cortisol in psychoneuroendocrine research: Recent developments and applications. *Psychoneuroendocrinology*, *19*(4), 313–333.

Knutson, K. L., Spiegel, K., Penev, P., & Van Cauter, E. (2007). The metabolic consequences of sleep deprivation. *Sleep Medicine Reviews*, *11*(3), 163–178. https://doi.org/10.1016/j.smrv.2007.01.002

Knutsson, A. (2003). Health disorders of shift workers. *Occupational Medicine*, *53*(2), 103–108. https://doi.org/10.1093/occmed/kqg048

Koch, C. E., Leinweber, B., Drengberg, B. C., Blaum, C., & Oster, H. (2016). Interaction between circadian rhythms and stress. *Neurobiology of Stress*, *6*, 57–67. https://doi.org/10.1016/j.ynstr.2016.09.001

Koren, L., Whiteside, D., Fahlman, Å., Ruckstuhl, K., Kutz, S., Checkley, S., Dumond, M., & Wynne-Edwards, K. (2012). Cortisol and corticosterone independence in cortisol-dominant wildlife. *General and Comparative Endocrinology*, *177*(1), 113–119.

Korompeli, A., Sourtzi, P., Tzavara, C., & Velonakis, E. (2009). Rotating shift-related changes in hormone levels in intensive care unit nurses. *Journal of Advanced Nursing*, *65*(6), 1274–1282. https://doi.org/10.1111/j.1365-2648.2009.04987.x

Kudielka, B. M., Buchtal, J., Uhde, A., & Wüst, S. (2007). Circadian cortisol profiles and psychological self-reports in shift workers with and without recent change in the shift rotation system. *Biological Psychology*, *74*(1), 92–103. https://doi.org/10.1016/j.biopsycho.2006.08.008

Kudielka, B. M., Gierens, A., Hellhammer, D. H., Wüst, S., & Schlotz, W. (2012). Salivary cortisol in ambulatory assessment—Some do's, some don'ts, and some open questions: *Psychosomatic Medicine*, *74*(4), 418–431. https://doi.org/10.1097/PSY.0b013e31825434c7

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*(1), 35–47. https://doi.org/10.1016/S0306-4530(02)00008-2

Kumari, M., Badrick, E., Ferrie, J., Perski, A., Marmot, M., & Chandola, T. (2009). Self-reported sleep duration and sleep disturbance are independently associated with cortisol secretion in the Whitehall II Study. *The Journal of Clinical Endocrinology & Metabolism*, *94*(12), 4801–4809. https://doi.org/10.1210/jc.2009-0555

Lac, G., & Chamoux, A. (2004). Biological and psychological responses to two rapid shiftwork schedules. *Ergonomics*, *47*(12), 1339–1349. https://doi.org/10.1080/00140130410001724237

Lammers-van der Holst, H. M., & Kerkhof, G. A. (2015). Individual differences in the cortisol-awakening response during the first two years of shift work: A longitudinal study in novice police officers. *Chronobiology International*, *32*(8), 1162–1167. https://doi.org/10.3109/07420528.2015.1064130

Law, R., Evans, P., Thorn, L., Hucklebridge, F., & Clow, A. (2015). The cortisol awakening response predicts same morning executive function: Results from a 50-

day case study. *Stress*, *18*(6), 616–621. https://doi.org/10.3109/10253890.2015.1076789

Law, R., Hucklebridge, F., Thorn, L., Evans, P., & Clow, A. (2013). State variation in the cortisol awakening response. *Stress*, *16*(5), 483–492. https://doi.org/10.3109/10253890.2013.817552

Legler, M., Brandenberger, G., Hietter, B., Siméoni, M., & Reinhardt, B. (1982). Diurnal cortisol peaks and their relationships to meals. *The Journal of Clinical Endocrinology & Metabolism*, *55*(4), 757–761. https://doi.org/10.1210/jcem-55-4-757

Leproult, R., Colecchia, E. F., Riaux, M. L.-B., & Cauter, E. V. (2001). Transition from dim to bright light in the morning induces an immediate elevation of cortisol levels. *The Journal of Clinical Endocrinology & Metabolism*, *86*(1), 151–157.

Leproult, R., Copinschi, G., Buxton, O., & Van Cauter, E. (1997). Sleep loss results in an elevation of cortisol levels the next evening. *Sleep*. https://doi.org/10.1093/sleep/20.10.865

Leproult, R., & Van Cauter, E. (2010). Role of sleep and sleep loss in hormonal release and metabolism. *Endocrine Development*, *17*, 11–21. https://doi.org/10.1159/000262524

Leung, G. K. W., Davis, R., Huggins, C. E., Ware, R. S., & Bonham, M. P. (2021). Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers. *Nutrition, Metabolism and Cardiovascular Diseases*, *31*(6), 1890–1902. https://doi.org/10.1016/j.numecd.2021.03.008

Levac, D., Colquhoun, H., & O'Brien, K. K. (2010). Scoping studies: Advancing the methodology. *Implementation Science, 5*(1), 69. https://doi.org/10.1186/1748-5908-5-69

Levine, A., Zagoory-Sharon, O., Feldman, R., Lewis, J. G., & Weller, A. (2007). Measuring cortisol in human psychobiological studies. *Physiology & Behavior*, *90*(1), 43–53. https://doi.org/10.1016/j.physbeh.2006.08.025

Li, J., Bidlingmaier, M., Petru, R., Gil, F. P., Loerbroks, A., & Angerer, P. (2018). Impact of shift work on the diurnal cortisol rhythm: A one-year longitudinal study in junior physicians. *Journal of Occupational Medicine and Toxicology*, *13*(1), 1–9.

Liyanarachchi, K., Ross, R., & Debono, M. (2017). Human studies on hypothalamo-pituitary-adrenal (HPA) axis. *Best Practice & Research Clinical Endocrinology & Metabolism, 31*(5), 459–473. https://doi.org/10.1016/j.beem.2017.10.011

Lundberg, U. (1999). Coping with stress: Neuroendocrine reactions and implications for health. *Noise & Health*, *1*(4), 67–74.

Macfarlane, D. P., Forbes, S., & Walker, B. R. (2008). Glucocorticoids and fatty acid metabolism in humans: Fuelling fat redistribution in the metabolic syndrome. *Journal of Endocrinology, 197*(2), 189–204.

Manenschijn, L., Schaap, L., van Schoor, N. M., van der Pas, S., Peeters, G. M. E. E., Lips, P., Koper, J. W., & van Rossum, E. F. C. (2013a). High long-term cortisol levels, measured in scalp hair, are associated with a history of cardiovascular disease. *The Journal of Clinical Endocrinology & Metabolism*, *98*(5), 2078–2083. https://doi.org/10.1210/jc.2012-3663

Marando, I., Matthews, R. W., Grosser, L., Yates, C., & Banks, S. (2022). The effect of time on task, sleep deprivation, and time of day on simulated driving performance. *Sleep*, *45*(9), zsac167.

Marino, J., & Cudeiro, J. (2003). Nitric oxide-mediated cortical activation: A diffuse wake-up system. *Journal of Neuroscience*, *23*(10), 4299–4307.

Martens, M. J., Rutters, F., Lemmens, S. G., Born, J. M., & Westerterp-Plantenga, M. S. (2010). Effects of single macronutrients on serum cortisol concentrations in normal weight men. *Physiology & Behavior*, *101*(5), 563–567.

Mason, I. C., Qian, J., Adler, G. K., & Scheer, F. A. J. L. (2020). Impact of circadian disruption on glucose metabolism: Implications for type 2 diabetes. *Diabetologia*, *63*(3), 462–472. https://doi.org/10.1007/s00125-019-05059-6

McEwen, B. S. (2005). Stressed or stressed out: What is the difference? *Journal of Psychiatry and Neuroscience*, *30*(5), 315–318.

McEwen, B. S. (2006). Sleep deprivation as a neurobiologic and physiologic stressor: Allostasis and allostatic load. *Metabolism*, *55*, S20–S23. https://doi.org/10.1016/j.metabol.2006.07.008

McEwen, B. S., & Karatsoreos, I. N. (2015). Sleep deprivation and circadian disruption. *Sleep Medicine Clinics*, *10*(1), 1–10. https://doi.org/10.1016/j.jsmc.2014.11.007

Medication Event Monitoring System®. (2021). http://www.AARDEXgroup.com

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*(3), 197–210.

Merkus, S. L., Holte, K. A., Huysmans, M. A., Hansen, Å. M., van de Ven, P. M., van Mechelen, W., & van der Beek, A. J. (2015). Neuroendocrine recovery after 2-week 12-h day and night shifts: An 11-day follow-up. *International Archives of Occupational and Environmental Health*, *88*(2), 247–257.

Morales, J., Yáñez, A., Fernández-González, L., Montesinos-Magraner, L., Marco-Ahulló, A., Solana-Tramunt, M., & Calvete, E. (2019). Stress and autonomic

response to sleep deprivation in medical residents: A comparative cross-sectional study. *PLOS ONE*, *14*(4), e0214858. https://doi.org/10.1371/journal.pone.0214858

Morris, C. J., Yang, J. N., Garcia, J. I., Myers, S., Bozzi, I., Wang, W., Buxton, O. M., Shea, S. A., & Scheer, F. A. J. L. (2015). Endogenous circadian system and circadian misalignment impact glucose tolerance via separate mechanisms in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(17), E2225–E2234.

Motohashi, Y. (1992). Alteration of circadian rhythm in shift-working ambulance personnel. Monitoring of salivary cortisol rhythm. *Ergonomics*, *35*(11), 1331–1340. https://doi.org/10.1080/00140139208967396

Munck, A., & Náray-Fejes-Tóth, A. (1992). The ups and downs of glucocorticoid physiology Permissive and suppressive effects revisited. *Molecular and Cellular Endocrinology*, *90*(1), C1–C4. https://doi.org/10.1016/0303-7207(92)90091-J

Niu, S. F., Chung, M. H., Chu, H., Tsai, J. C., Lin, C. C., Liao, Y. M., Ou, K. L., O'Brien, A. P., & Chou, K. R. (2015). Differences in cortisol profiles and circadian adjustment time between nurses working night shifts and regular day shifts: A prospective longitudinal study. *International Journal of Nursing Studies*, *52*(7), 1193–1201. https://doi.org/10.1016/j.ijnurstu.2015.04.001

Oster, H., Challet, E., Ott, V., Arvat, E., de Kloet, E. R., Dijk, D.-J., Lightman, S., Vgontzas, A., & Van Cauter, E. (2017b). The Functional and Clinical Significance of the 24-Hour Rhythm of Circulating Glucocorticoids. *Endocrine Reviews*, *38*(1), 3–45. https://doi.org/10.1210/er.2015-1080

Peters, M., Godfrey, C., McInerney, P., Munn, Z., Tricco, A., & Khalil, H. (2020). Chapter 11: Scoping Reviews (2020 version). In: Aromataris E, Munn Z (Editors).

JBI Manual for Evidence Synthesis, JBI, 2020. In *JBI Manual For Evidence Synthesis: Chapter 11: Scoping Reviews (2020 version).* https://synthesismanual.jbi.global. https://doi.org/10.46658/JBIMES-20-12

Plat, L., Byrne, M. M., Sturis, J., Polonsky, K. S., Mockel, J., Fery, F., & Van Cauter, E. (1996). Effects of morning cortisol elevation on insulin secretion and glucose regulation in humans. *American Journal of Physiology-Endocrinology and Metabolism*, *270*(1), E36–E42. https://doi.org/10.1152/ajpendo.1996.270.1.E36

Powell, D. J., & Schlotz, W. (2012). Daily life stress and the cortisol awakening response: Testing the anticipation hypothesis. *PLoS ONE*, *7*(12). https://doi.org/10.1371/journal.pone.0052067

Proulx, J., Klee, D., & Oken, B. S. (2017). Do psychosocial predictors affect the following days' cortisol awakening response? Expanding the temporal frame with which to explore morning cortisol. *Stress*, *20*(4), 398–403. https://doi.org/10.1080/10253890.2017.1346076

Pruessner, J. C., Hellhammer, D. H., & Kirschbaum, C. (1999). Burnout, perceived stress, and cortisol responses to awakening. *Psychosomatic Medicine*, *61*(2), 197–204.

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*(7), 916–931. https://doi.org/10.1016/S0306-4530(02)00108-7

Pruessner, J. C., Wolf, O. T., Hellhammer, D. H., & Buske-Kirschbaum, A. (1997). *Free cortisol levels after awakening: A reliabile biological marker for the assessment of adrenocortical activity*. *61*(26), 11.

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*(1), 92–99. https://doi.org/10.1097/01.PSY.0000040950.22044.10

Puttonen, S., Härmä, M., & Hublin, C. (2010). Shift work and cardiovascular disease—Pathways from circadian stress to morbidity. *Scandinavian Journal of Work, Environment & Health*, *36*(2), 96–108.

Qian, J., Dalla Man, C., Morris, C. J., Cobelli, C., & Scheer, F. A. J. L. (2018). Differential effects of the circadian system and circadian misalignment on insulin sensitivity and insulin secretion in humans. *Diabetes, Obesity and Metabolism*, *20*(10), 2481–2485. https://doi.org/10.1111/dom.13391

Quigley, M., & Yen, S. (1979). A mid-day surge in cortisol levels. *The Journal of Clinical Endocrinology & Metabolism*, *49*(6), 945–947.

Ramamoorthy, S., & Cidlowski, J. A. (2016). Corticosteroids-mechanisms of action in health and disease. *Rheumatic Diseases Clinics of North America*, *42*(1), 15–31. https://doi.org/10.1016/j.rdc.2015.08.002

Reeves, S. L., Newling-Ward, E., & Gissane, C. (2004). The effect of shift-work on food intake and eating habits. *Nutrition & Food Science*, *34*(5), 216–221. https://doi.org/10.1108/00346650410560398

Reppert, S. M., & Weaver, D. R. (2002). Coordination of circadian timing in mammals. *Nature*, *418*(6901), 935–941.

Ritonja, J., Aronson, K. J., Day, A. G., Korsiak, J., & Tranmer, J. (2018). Investigating cortisol production and pattern as mediators in the relationship between shift work and cardiometabolic risk. *Canadian Journal of Cardiology*, *34*(5), 683–689. https://doi.org/10.1016/j.cjca.2018.02.006

Rizza, R. A., Mandarino, L. J., & Gerich, J. E. (1982). Cortisol-induced insulin resistance in man: impaired suppression of glucose production and stimulation of glucose utilization due to a postreceptor defect of insulin action*. *The Journal of Clinical Endocrinology & Metabolism*, *54*(1), 131–138. https://doi.org/10.1210/jcem-54-1-131

Roberts, A. D., 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*(2), 136–141.

Roden, M., Koller, M., Pirich, K., Vierhapper, H., & Waldhauser, F. (1993). The circadian melatonin and cortisol secretion pattern in permanent night shift workers. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *265*(1), R261–R267. https://doi.org/10.1152/ajpregu.1993.265.1.R261

Rogers, M., Coates, A. M., & Banks, S. (2021). Meal timing, sleep and cardiometabolic outcomes. *Current Opinion in Endocrine and Metabolic Research*, *18*, p.128-132.

Rosmond, R., Holm, G., & Björntorp, P. (2000). Food-induced cortisol secretion in relation to anthropometric, metabolic and haemodynamic variables in men. *International Journal of Obesity*, *24*(4), 416–422. https://doi.org/10.1038/sj.ijo.0801173

Ross, K. M., Murphy, M. L., Adam, E. K., Chen, E., & Miller, G. E. (2014). How stable are diurnal cortisol activity indices in healthy individuals? Evidence from three multi-wave studies. *Psychoneuroendocrinology*, *39*, 184–193.

Russell, G., & Lightman, S. (2019). The human stress response. *Nature Reviews Endocrinology*, *15*(9), 525–534. https://doi.org/10.1038/s41574-019-0228-0

Sagmeister, M., Harper, L., & Hardy, R. (2022). Cortisol excess in chronic kidney disease–a review of changes and impact on mortality. *Frontiers in Endocrinology*.

Salgado-Delgado, R., Angeles-Castellanos, M., Saderi, N., Buijs, R. M., & Escobar, C. (2010). Food intake during the normal activity phase prevents obesity and circadian desynchrony in a rat model of night work. *Endocrinology*, *151*(3), 1019–1029. https://doi.org/10.1210/en.2009-0864

Salgado-Delgado, R., Saderi, N., Basualdo, M. del C., Guerrero-Vargas, N. N., Escobar, C., & Buijs, R. M. (2013). Shift work or food intake during the rest phase promotes metabolic disruption and desynchrony of liver genes in male rats. *PLoS ONE*, *8*(4), e60052. https://doi.org/10.1371/journal.pone.0060052

Salín-Pascual, R. J., Ortega-Soto, H., Huerto-Delgadillo, L., Camacho-Arroyo, I., Roldán-Roldán, G., & Tamarkin, L. (1988). The effect of total sleep deprivation on plasma melatonin and cortisol in healthy human volunteers. *Sleep*, *11*(4), 362–369. https://doi.org/10.1093/sleep/11.4.362

Scheer, F. A. J. L., & Buijs, R. M. (1999). Light affects morning salivary cortisol in humans. *The Journal of Clinical Endocrinology & Metabolism*, *84*(9), 3395–3398. https://doi.org/10.1210/jcem.84.9.6102

Schulz, P., Kirschbaum, C., Prüßner, J., & Hellhammer, D. (1998). Increased free cortisol secretion after awakening in chronically stressed individuals due to work overload. *Stress Medicine*, *14*(2), 91–97.

Schüssler, P., Uhr, M., Ising, M., Weikel, J., Schmid, D., Held, K., Mathias, S., & Steiger, A. (2006). Nocturnal ghrelin, ACTH, GH and cortisol secretion after sleep deprivation in humans. *Psychoneuroendocrinology*, *31*(8), 915–923.

Selmaoui, B., & Touitou, Y. (2003). Reproducibility of the circadian rhythms of serum cortisol and melatonin in healthy subjects: A study of three different 24-h cycles

over six weeks. *Life Sciences*, *73*(26), 3339–3349. https://doi.org/10.1016/j.lfs.2003.05.007

Shi, X., Sun, X., Yao, Z., Yuan, Y., Wu, J., & Clow, A. (2018). The cortisol awakening response predicts response inhibition in the afternoon of the same day. *Psychoneuroendocrinology*, *89*, 23–29. https://doi.org/10.1016/j.psyneuen.2017.12.016

Simpson, N. S., Diolombi, M., Scott-Sutherland, J., Yang, H., Bhatt, V., Gautam, S., Mullington, J., & Haack, M. (2016). Repeating patterns of sleep restriction and recovery: Do we get used to it? *Brain, Behavior, and Immunity*, *58*, 142–151. https://doi.org/10.1016/j.bbi.2016.06.001

Slag, M. F., Ahmed, M., Gannon, M. C., & Nuttall, F. Q. (1981). Meal stimulation of cortisol secretion: A protein induced effect. *Metabolism*, *30*(11), 1104–1108. https://doi.org/10.1016/0026-0495(81)90055-X

Sletten, T. L., Cappuccio, F. P., Davidson, A. J., Van Cauter, E., Rajaratnam, S. M. W., & Scheer, F. A. J. L. (2020). Health consequences of circadian disruption. *Sleep*, *43*(1). https://doi.org/10.1093/sleep/zsz194

Smyth, J., Ockenfels, M. C., Porter, L., Kirschbaum, C., Hellhammer, D. H., & Stone, A. A. (1998). Stressors and mood measured on a momentary basis are associated with salivary cortisol secretion. *Psychoneuroendocrinology*, *23*(4), 353–370.

Smyth, N., Hucklebridge, F., Thorn, L., Evans, P., & Clow, A. (2013). Salivary cortisol as a biomarker in social science research. *Social and Personality Psychology Compass*, *7*(9), 605–625.

Song, H., Sun, X., Yang, T., Zhang, L., Yang, J., & Bai, J. (2015). Effects of sleep deprivation on serum cortisol level and mental health in servicemen. *International Journal of Psychophysiology*, *96*(3), 169–175.

Spencer, R. L., & Deak, T. (2017). A users guide to HPA axis research. *Physiology & Behavior*, *178*, 43–65. https://doi.org/10.1016/j.physbeh.2016.11.014

Spiegel, K., Leproult, R., & Van Cauter, E. (1999). Impact of sleep debt on metabolic and endocrine function. *The Lancet*, *354*(9188), 1435–1439.

Stalder, T., Bäumler, D., Miller, R., Alexander, N., Kliegel, M., & Kirschbaum, C. (2013). The cortisol awakening response in infants: Ontogeny and associations with development-related variables. *Psychoneuroendocrinology*, *38*(4), 552–559. https://doi.org/10.1016/j.psyneuen.2012.07.015

Stalder, T., Hucklebridge, F., Evans, P., & Clow, A. (2009). Use of a single case study design to examine state variation in the cortisol awakening response: Relationship with time of awakening. *Psychoneuroendocrinology*, *34*(4), 607–614.

Stalder, T., & Kirschbaum, C. (2013). Cortisol. In M. D. Gellman & J. R. Turner (Eds.), *Encyclopedia of Behavioral Medicine* (pp. 507–512). Springer. https://doi.org/10.1007/978-1-4419-1005-9_171

Stalder, T., Kirschbaum, C., Kudielka, B. M., Adam, E. K., Pruessner, J. C., Wüst, S., Dockray, S., Smyth, N., Evans, P., Hellhammer, D. H., Miller, R., Wetherell, M. A., Lupien, S. J., & Clow, A. (2016). Assessment of the cortisol awakening response: Expert consensus guidelines. *Psychoneuroendocrinology*, *63*, 414–432. https://doi.org/10.1016/j.psyneuen.2015.10.010

Steptoe, A., & Serwinski, B. (2016). Cortisol Awakening Response. In *Stress: Concepts, Cognition, Emotion, and Behavior* (pp. 277–283). Elsevier. https://doi.org/10.1016/B978-0-12-800951-2.00034-0

Stimson, R. H., Mohd-Shukri, N. A., Bolton, J. L., Andrew, R., Reynolds, R. M., & Walker, B. R. (2014). The postprandial rise in plasma cortisol in men is mediated by macronutrient-specific stimulation of adrenal and extra-adrenal cortisol

production. *The Journal of Clinical Endocrinology & Metabolism*, *99*(1), 160–168. https://doi.org/10.1210/jc.2013-2307

Stone, A. A., Schwartz, J. E., Smyth, J., Kirschbaum, C., Cohen, S., Hellhammer, D., & Grossman, S. (2001). Individual differences in the diurnal cycle of salivary free cortisol: A replication of flattened cycles for some individuals. *Psychoneuroendocrinology*, *26*(3), 295–306.

Thau, L., Gandhi, J., & Sharma, S. (2021). Physiology, cortisol. In *StatPearls [Internet]*. StatPearls Publishing.

Thomas, C., Hertzman, C., & Power, C. (2009). Night work, long working hours, psychosocial work stress and cortisol secretion in mid-life: Evidence from a British birth cohort. *Occupational and Environmental Medicine*, *66*(12), 824–831.

Thomas, M., Sing, H., Belenky, G., Holcomb, H., Mayberg, H., Dannals, R., Wagner, H., Thorne, D., & Popp, K. (2000). Neural basis of alertness and cognitive performance impairments during sleepiness. I. Effects of 24 h of sleep deprivation on waking human regional brain activity. *Journal of Sleep Research*, *9*(4), 335-352.

Thomas, M., Sing, H., Belenky, G., Holcomb, H. H., Mayberg, H. S., Dannals, R. F., Wagner Jr, H. N., Thorne, D. R., Popp, K. A., & Rowland, L. M. (2003). Neural basis of alertness and cognitive performance impairments during sleepiness II. Effects of 48 and 72 h of sleep deprivation on waking human regional brain activity. *Thalamus & Related Systems*, *2*(3), 199–229.

Thorn, L., Hucklebridge, F., Evans, P., & Clow, A. (2009). The cortisol awakening response, seasonality, stress and arousal: A study of trait and state influences. *Psychoneuroendocrinology*, *34*(3), 299–306. https://doi.org/10.1016/j.psyneuen.2008.11.005

Touitou, Y., Motohashi, Y., Reinberg, A., Touitou, C., Bourdeleau, P., Bogdan, A., & Auzéby, A. (1990). Effect of shift work on the night-time secretory patterns of melatonin, prolactin, cortisol and testosterone. *European Journal of Applied Physiology and Occupational Physiology*, *60*(4), 288–292. https://doi.org/10.1007/BF00379398

Tsigos, C., & Chrousos, G. P. (2002). Hypothalamic–pituitary–adrenal axis, neuroendocrine factors and stress. *Journal of Psychosomatic Research*, *53*(4), 865–871. https://doi.org/10.1016/S0022-3999(02)00429-4

Ulhôa, M. A., Marqueze, E. C., Kantermann, T., Skene, D., & Moreno, C. (2011). When Does Stress End? Evidence of a Prolonged Stress Reaction in Shiftworking Truck Drivers. *Chronobiology International*, *28*(9), 810–818. https://doi.org/10.3109/07420528.2011.613136

Van Cauter, E., Shapiro, E. T., Tillil, H., & Polonsky, K. S. (1992). Circadian modulation of glucose and insulin responses to meals: Relationship to cortisol rhythm. *American Journal of Physiology-Endocrinology and Metabolism*, *262*(4), E467–E475. https://doi.org/10.1152/ajpendo.1992.262.4.E467

Van Dycke, K. C. G., Pennings, J. L. A., van Oostrom, C. T. M., van Kerkhof, L. W. M., van Steeg, H., van der Horst, G. T. J., & Rodenburg, W. (2015). Biomarkers for circadian rhythm disruption independent of time of day. *PLoS ONE*, *10*(5). https://doi.org/10.1371/journal.pone.0127075

Vangelova, K. (2008). The effect of shift rotation on variations of cortisol, fatigue and sleep in sound engineers. *Industrial Health*, *46*(5), 490–493. https://doi.org/10.2486/indhealth.46.490

Vargas, I., & Lopez-Duran, N. (2017a). Investigating the effect of acute sleep deprivation on hypothalamic-pituitary-adrenal-axis response to a psychosocial stressor.

Psychoneuroendocrinology, *79*, 1–8. https://doi.org/10.1016/j.psyneuen.2017.01.030

Vargas, I., & Lopez-Duran, N. (2017b). The cortisol awakening response after sleep deprivation: Is the cortisol awakening response a "response" to awakening or a circadian process? *Journal of Health Psychology*, 135910531773832. https://doi.org/10.1177/1359105317738323

Vincent, G. E., Jay, S. M., Preece, H., Hall, S. J., Aisbett, B., Baumert, M., Sprajcer, M., Lack, L., & Ferguson, S. A. (2019). Overnight heart rate variability and next day cortisol response during simulated on-call conditions. *Psychoneuroendocrinology*, *109*, 104406. https://doi.org/10.1016/j.psyneuen.2019.104406

Vining, R. F., McGinley, R. A., & Symons, R. G. (1983). Hormones in saliva: Mode of entry and consequent implications for clinical interpretation. Clinical Chemistry, 29(10), 1752–1756.

Volmer, B., Baumeister, J., Matthews, R., Grosser, L., Von Itzstein, S., Banks, S., & Thomas, B. H. (2022). A comparison of spatial augmented reality predictive cues and their effects on sleep deprived users. In *2022 IEEE Conference on Vuirtual Reality and 3D User Interfaces (VR)* (pp. 589–598). IEEE

von Treuer, K., Norman, T. R., & Armstrong, S. M. (1996). Overnight human plasma melatonin, cortisol, prolactin, TSH, under conditions of normal sleep, sleep deprivation, and sleep recovery. *Journal of Pineal Research*, *20*(1), 7–14. https://doi.org/10.1111/j.1600-079X.1996.tb00232.x

Wajchenberg, B. L. O. (2000). Subcutaneous and visceral adipose tissue: Their relation to the metabolic syndrome. *Endocrine Reviews*, *21*(6), 697–738.

Walker, B. R. (2006). Cortisol—Cause and cure for metabolic syndrome? *Diabetic Medicine*, *23*(12), 1281–1288. https://doi.org/10.1111/j.1464-5491.2006.01998.x

Walker, B. R. (2007). Glucocorticoids and cardiovascular disease. *European Journal of Endocrinology*, *157*(5), 545–559.

Watson, N. F., Badr, M. S., Belenky, G., Bliwise, D. L., Buxton, O. M., Buysse, D., Dinges, D. F., Gangwisch, J., Grandner, M. A., Kushida, C., Malhotra, R. K., Martin, J. L., Patel, S. R., Quan, S., & Tasali, E. (2015). Recommended amount of sleep for a healthy adult: A joint consensus statement of the American Academy of Sleep Medicine and Sleep Research Society. *SLEEP*. https://doi.org/10.5665/sleep.4716

Wehrens, S. M. T., Christou, S., Isherwood, C., Middleton, B., Gibbs, M. A., Archer, S. N., Skene, D. J., & Johnston, J. D. (2017). Meal timing regulates the human circadian system. *Current Biology*, *27*(12), 1768-1775.e3. https://doi.org/10.1016/j.cub.2017.04.059

Weibel, L., & Brandenberger, G. (1998). Disturbances in hormonal profiles of night workers during their usual sleep and work times. *Journal of Biological Rhythms*, *13*(3), 202–208. https://doi.org/10.1177/074873098129000048

Weibel, L., Follenius, M., Spiegel, K., Ehrhart, J., & Brandenberger, G. (1995). Comparative effect of night and daytime sleep on the 24-hour cortisol secretory profile. *Sleep*. https://doi.org/10.1093/sleep/18.7.549

Weibel, L., Spiegel, K., Follenius, M., Ehrhart, J., & Brandenberger, G. (1996). Internal dissociation of the circadian markers of the cortisol rhythm in night workers. *American Journal of Physiology-Endocrinology and Metabolism*, *270*(4), E608–E613. https://doi.org/10.1152/ajpendo.1996.270.4.E608

Weitzman, E. D., Fukushima, D., Nogeire, C., Roffwarg, H., Gallagher, T. F., & Hellman, L. (1971). Twenty-four hour pattern of the episodic secretion of cortisol

in normal subjects. *The Journal of Clinical Endocrinology & Metabolism*, *33*(1), 14–22. https://doi.org/10.1210/jcem-33-1-14

Weitzman, E. D., Zimmerman, J. C., Czeisler, C. C., & Ronda, J. (1983). Cortisol secretion is inhibited during sleep in normal man. *The Journal of Clinical Endocrinology & Metabolism*, *56*(2), 352–358.

Wessa, M., Rohleder, N., Kirschbaum, C., & Flor, H. (2006). Altered cortisol awakening response in posttraumatic stress disorder. *Psychoneuroendocrinology*, *31*(2), 209–215. https://doi.org/10.1016/j.psyneuen.2005.06.010

Wilhelm, I., Born, J., Kudielka, B. M., Schlotz, W., & Wüst, S. (2007). Is the cortisol awakening rise a response to awakening? *Psychoneuroendocrinology*, *32*(4), 358–366. https://doi.org/10.1016/j.psyneuen.2007.01.008

Wirth, M., Burch, J., Violanti, J., Burchfiel, C., Fekedulegn, D., Andrew, M., Zhang, H., Miller, D. B., Hébert, J. R., & Vena, J. E. (2011). Shiftwork duration and the awakening cortisol response among police officers. *Chronobiology International*, *28*(5), 446–457. https://doi.org/10.3109/07420528.2011.573112

Wolf, O. T. (2009). Stress and memory in humans: Twelve years of progress? *Brain Research*, *1293*, 142–154.

Wolkow, A., Aisbett, B., Reynolds, J., Ferguson, S. A., & Main, L. C. (2016a). Acute psychophysiological relationships between mood, inflammatory and cortisol changes in response to simulated physical firefighting work and sleep restriction. *Applied Psychophysiology and Biofeedback*, *41*(2), 165–180.

Wolkow A., Aisbett B., Reynolds J., Ferguson S.A., & Main L.C. (2016b). The impact of sleep restriction while performing simulated physical firefighting work on cortisol and heart rate responses. *International Archives of Occupational and*

Environmental Health, *89*(3), 461–475. https://doi.org/10.1007/s00420-015-1085-3

Wright, K. P., Drake, A. L., Frey, D. J., Fleshner, M., Desouza, C. A., Gronfier, C., & Czeisler, C. A. (2015). Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. *Brain, Behavior, and Immunity*, *47*, 24–34. https://doi.org/10.1016/j.bbi.2015.01.004

Wright, K. P., Lowry, C. A., & Lebourgeois, M. K. (2012). Circadian and wakefulness-sleep modulation of cognition in humans. *Frontiers in Molecular Neuroscience*, *5*, 50. https://doi.org/10.3389/fnmol.2012.00050

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*(7), 79.

Xyris Pty Ltd. (2015). *FoodWorks 8 Professional* (v8.0) [Computer software].

Yeager, M. P., Pioli, P. A., & Guyre, P. M. (2011). Cortisol exerts bi-phasic regulation of inflammation in humans. *Dose-Response*, *9*(3), dose-response.

Appendix 1: Permission to reproduce article

Tuesday, March 14, 2023 at 10:38:52 Australian Central Daylight Time
Subject: Re: Seeking permission to use authored article in a PhD thesis [230305-004972]
Date: Monday, 6 March 2023 at 6:46:48 pm Australian Central Daylight Time
From: Permissions Helpdesk
To: Grosser, Linda Rachel - grolr001
Category: Blue category

Dear Linda Grosser,
Hope you are doing good.

As an Elsevier journal author, you retain the right to Include the article in a thesis or dissertation (provided that this is not to be published commercially) whether in full or in part, subject to proper acknowledgment; see https://www.elsevier.com/about/policies/copyright for more information. As this is a retained right, no written permission from Elsevier is necessary.

As outlined in our permissions licenses, this extends to the posting to your university's digital repository of the thesis provided that if you include the published journal article (PJA) version, it is embedded in your thesis only and not separately downloadable.

Thank you.
Kind regards,

Thomas Rexson Yesudoss
Copyrights Coordinator
ELSEVIER | HCM - Health Content Management
Visit Elsevier Permissions

Appendix 2: Written permission of co-authors

Linda Grosser *Sleep and Chronobiology Laboratory, University of South Australia*

- Contribution to study conception and design
- Substantial contribution to acquisition of data
- Substantial contribution to analysis and interpretation of data
- Writing and drafting of manuscript
- Critical revision of manuscript for important intellectual content
- Completed revisions suggested in the publication process
- Final approval of manuscript

… [redacted] ……..

Sara Knayfati *Sleep and Chronobiology Laboratory, University of South Australia*

- Substantial contribution to acquisition of data
- Substantial contribution to analysis and interpretation of data
- Drafting of manuscript
- Critical revision of manuscript for important intellectual content

…[redacted]……..

Crystal Grant *Sleep and Chronobiology Laboratory, University of South Australia*

- Substantial contribution to acquisition of data
- Substantial contribution to analysis and interpretation of data
- Drafting of manuscript
- Critical revision of manuscript for important intellectual content

[redacted] ……..

Jillian Dorrian *Sleep and Chronobiology Laboratory, University of South Australia*

- Substantial contribution to study conception and design
- Substantial contribution to analysis and interpretation of data
- Drafting of manuscript
- Critical revision of manuscript for important intellectual content

…[redacted]……..

Siobhan Banks *Sleep and Chronobiology Laboratory, University of South Australia*

- Substantial contribution to study conception and design
- Substantial contribution to interpretation of data
- Drafting of manuscript
- Critical revision of manuscript for important intellectual content

[redacted]……..

Appendix 3: Participant information sheet − Study 1

University of South Australia

Participant Information Sheet

Sleep and Chronobiology Laboratory
School of Psychology
Division of Arts, Education, Social Sciences

The impact of sleeping during the day on metabolism, performance and mood

Prof Siobhan Banks, A/Prof Jill Dorrian, A/Prof Alison Coates, Dr. Stephanie Centofanti, Charlotte Gupta, Jackie Stepien-Hulleman, Crystal Grant, Dr Raymond Matthews, Dr Amy Hutchison, A/Prof Leonie Heilbronn, Prof David Kennaway & Prof Gary Wittert

---

You have been invited to participate in a research study. Before agreeing to participate in the study, it is important that you read and understand the following explanation of the study and the procedures. This form describes the purpose, procedures, benefits, risks, discomforts and alternative procedures that are available to you. If you choose to participate, you have the right to withdraw from the study at any time.

What is the study about?

The main objective of this study is to determine the impact of sleeping during the day (simulated shiftwork) on performance, metabolism, sleep structure and mood. The results we obtain from this study will help us to better understand the impact of night shift on health.

Who can participate and how does the study work?

Healthy, non-smokers who are aged between 18 to 50 years old, taking no medication and have a regular sleep pattern are eligible to participate in this study. Before commencing the study, potential participants will be required to complete a telephone screening interview, and attend two screening sessions. One session will be at the UniSA City East campus and one session will be at the Sleep and Chronobiology Laboratory at the UniSA Magill campus). Participants will be required to complete a 7-night sleep/wake diary and wear a wrist activity monitor (small device the same size as a wrist watch). These two measures will allow us to assess your sleep pattern. A simple blood test to assess general health and metabolic function, and urine sample to test for illicit drugs will also be done during the screening process. Participants will also be asked to complete a handedness questionnaire and a four day weighed food diary two weeks prior to entering the lab to determine their normal energy intake.

What happens during the study?

All investigations will be carried out at the Sleep and Chronobiology Laboratory at the University of South Australia. If you are eligible to participate, you will be asked to attend the laboratory for six consecutive nights/ seven days: one baseline night and day, followed by four nights of being awake/ sleeping during the day (simulated night work), and one recovery night and day. You will be randomised to one of three conditions: 1) eating a meal at night during the night work phase of the 2

study, 2) eating a snack at night during the night work phase of the study, or 3) not eating at night during the night work phase of the study. You will be given the same amount of food each day just at different times of day.
On the first day, you will be required to arrive at the laboratory no later than 10am. At this time, you will undergo training on a variety of tests (metabolic) and performance tasks (cognitive performance assessments). For the baseline night you will be given an 8 hour sleep, your bedtime will be scheduled at 10:00pm and you will be woken at 6:00am the following morning. Following the baseline period, you will then undergo four nights of simulated night shift with a 7 hour sleep opportunity each day. Sleep laboratory staff will instruct you when it is time to go to sleep and when it is time to wake up. Because of these changes to your sleep and wake schedule, you may experience a range of symptoms, including increased sleepiness, fatigue, irritability, and mild stomach upset.
During all wake periods (baseline, simulated shift work and recovery day), you will also be asked to complete a series of performance tasks at set times. Tasks will include: questionnaires, computer-based cognitive tasks. During wake periods, when you are not completing these tasks, you may read, watch TV (DVDs), play boardgames, talk, or listen to music etc.
During certain sleep periods (baseline, day time sleep post simulated night shift and recovery sleep) your sleep will be monitored using standard sleep and respiratory polysomnography, which involves placing electrodes on your head, face (taped to the skin) and chest. During sleep periods you will be recorded using small, visible cameras that are located in the ceiling of each bedroom. These cameras are not located in any of the bathrooms. The Research Staff and Students conducting this study will be the only group that will have access to these data to ensure your confidentiality.
In order to measure your blood glucose levels, you will also be wearing a subcutaneous glucose monitor. The monitor has a small, flexible sensor which inserts in to the fatty layer of skin on your stomach. A small transmitter, slightly larger than the size of a 10 cent piece, is attached to the sensor which sends readings to a pager clipped on to the waistband on your pants. To calibrate the devices, your finger will be pricked with a lancet approximately every 4-6 hours to produce a droplet of blood. We will also be taking samples of your blood regularly throughout the study to measure biomarkers and genetic differences between individuals.
At baseline (day 2) and after recovery sleep (day 7) you will undergo a standard oral glucose tolerance test. This involves a cannula (a thin, flexible plastic tube) inserted into a vein of your forearm to enable blood samples to be collected after drinking a sugary drink. Blood will be drawn before consuming the sugary drink (i.e., fasting) and 30, 60, 90, 120 and 150 minutes after. On days 2 and 5 a cannula will be inserted prior to dinner in order for blood to be taken at 4 time points across the night. In the morning, blood will be taken prior to breakfast (i.e., fasting), as well as 30, 60, 90, 120 and 150 minutes after breakfast. The cannula will be removed at the end of the test i.e. after the 150 minute blood draw). You will not sleep with the cannula in your arm, nor will you be able to shower when the cannula is in place. In total, no more than 650ml of blood will be drawn during the seven day lab phase of the study. 3

The blood collected will be used to measure the levels of a number of physiological markers and will also be used for genetic testing. The genes that we are interested in are related to sleep quantity and quality. They are not associated with any genetic disease nor are they sufficient to identify you genetically. Saliva samples will also be collected at regular intervals during the waking period of the protocol.

All of your meals will be supplied during the study, and you will not be allowed to bring any food with you. Meals will be provided at set times and no food is to be consumed outside of these meal times. It is important to remember that throughout the study, and in the week prior to the first baseline night, you will be asked to refrain from consuming any alcohol, caffeine or medication.

Are there any risks?

We will be monitoring you closely at all times during the study, and it is important for you to let Research or nursing staff know if you are experiencing any untoward symptoms so that the appropriate action and treatment can be initiated. We have procedures in place for monitoring and responding to possible risks to you during the study, as outlined below, and every effort is taken to minimise these risks.

Physical injury, pain or discomfort

There is a low risk of bacterial infection associated with venous blood sampling. We will do everything possible to minimise this risk and you will be monitored throughout the protocol for any indications of a problem. Any unusual pain should be reported to the research staff, who will notify the nursing staff.

The insertion of intravenous cannulas may cause some pain and swelling at the insertion site and the cannula itself my cause some discomfort and irritation. It is possible one or more attempts to insert a cannula are not successful, and another stick to insert it will be necessary. This may cause some additional pain, swelling or discomfort. There is a low risk of bacterial infection associated with cannulation. We do everything possible to minimise this risk and you will be monitored throughout the protocol for any indications of a problem associated with the cannula. Any unusual pain should be reported to the research staff, who will notify the nursing staff.

Similarly, the insertion of the small, flexible plastic tube of the glucose monitor may cause some mild pain and swelling at the insertion site and the bandage may cause some discomfort and irritation to the skin. Any unusual pain should be reported to the staff, who will notify the study nurse.

Over the course of the study, we will draw no more than a total of 650ml of blood. This is close to the amount given in a single donation at the blood bank. The blood will be drawn in small amounts over the duration of the laboratory phase of the study. At times during the study you may feel dizzy or light-headed. If these feelings persist, the study nurse will assess your condition.

You may experience minor skin irritation from the small electrodes that will be used to monitor your sleep. These are placed on your temples (next to your eyes), your chin, your forehead and your collarbone and are taped to your skin. Attempts will be made to minimize any discomfort by alternating the position of these leads. 4

Psychological or emotional stress

The changes to your normal sleep/wake cycle may also cause a range of symptoms, including sleepiness, fatigue and changes to your mood. Further, since participants stay in our laboratory, away from family and friends during the study, we are aware that this can cause changes in emotional state.

Potential civil, criminal or other proceedings

During the initial screening phase of the study, participants will be asked questions about history of drug use. Disclosure of information regarding illicit substances and illegal activities will remain confidential, except in the event of legal proceedings where a court ordered subpoena is issued.

How does my family get in touch with me if there is an emergency?

An important part of the study protocol is that while you are living in the sleep laboratory, you are unaware of the time of day. This means you will not have access to your mobile phone. Should your family need to get in touch with you at any time, they can contact the sleep laboratory (staff will be present 24hrs a day) on the following numbers**: (08) 8302 1235 or (08) 8302 1712**

What do I need to bring?

You will be staying in the sleep laboratory for 7 days and 6 nights, so you will need to bring enough clothes and toiletries to cover this time. It is also advised you bring a warm jumper with loose fitting sleeves to ensure the cannula is not disrupted. We will provide you with all of your linen (sheets, blankets, towels, pillows etc.), but you may wish to bring your own pillow from home. You will have your own room for the duration of the study but you will be sharing a living area, shower and toilet facilities with up to three other participants in the study. Because there will be periods of spare time throughout the study, please feel free to bring along your own music, books or movies.

What happens at the end of the study?

We recommend that you avoid the following activities [driving a car, riding a bike, and/or operating heavy machinery] until you have obtained at least one full night's sleep (uninterrupted for 7 hours or more) following the study. Therefore, it is advised that you organise someone to pick you up at the completion of the study or take a taxi home. Your reimbursement for participating in the study also covers these travel costs. In the 24h following completion of the study, or if you have withdrawn from the study early, staff at the Sleep and Chronobiology Laboratory will contact you via telephone for follow-up. Following completion of the study, should you need to get in contact with study staff at any time, they can be reached on **(08) 8302 1235 or (08) 8302 1712.**

What happens to the results?

All data from this study will be stored on computers at the Sleep and Chronobiology Laboratory, with hard drives as backups in lockable filing cabinets. All information collected as part of the study will be destroyed at the end of five years. Summarised results will be stored as hard copies at the Sleep and Chronobiology Laboratory and may be used in theses, journal publications and conferences. Data collected in the study may be used for other projects but all records containing personal information will remain STRICTLY CONFIDENTIAL **-** no information that would lead to the identification of any individual will be released. 5

What will I get out of the study?
While you will not directly benefit from this study, you will be compensated for the inconvenience and loss of time associated with your participation. If you complete the study, you will receive $1050 ($125 participation payment per night + $175 study completion payment). This reimbursement also covers travel costs from the Sleep and Chronobiology Laboratory. If for some reason you do not complete the whole study, you will receive a pro rata participation payment based on the number of hours that you spent in the sleep laboratory. Upon request, you will be provided with a copy of the final research report.

Voluntary participation - what happens if I say no?
Before deciding whether or not to take part in this study, you may wish to discuss the matter with a relative, friend or your local doctor. You should feel free to do this. It is important that you understand that your participation in this study is voluntary, as is the case with all research projects at the Sleep and Chronobiology Laboratory. If you do not wish to take part, you are under no obligation to do so. If you decide to take part but later change your mind, you are free to withdraw from the study at any stage. Your decision to take part, not to take part, or to withdraw, will not affect your routine medical treatment or your relationship with those treating you or your relationship with the Sleep and Chronobiology Laboratory.

How do I become a participant?
If you are interested in participating or have any questions, please email shiftworkstudy@unisa.edu.au

UniSA Human Research Ethics Committee Contact Details
This study has been approved by the University of South Australia's Human Research Ethics Committee. Should you wish to speak to a person not directly involved in the study, particularly in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer the University of South Australia's Human Research Ethics Committee on (08) 8302 3118 or by email Vicki.Allen@unisa.edu.au.

Appendix 4: Participant information Sheet – Study 2

University of South Australia

Participant Information Sheet

Sleep and Chronobiology Laboratory
School of Psychology
Division of Arts, Education, Social Sciences

The impact of sleeping during the day on metabolism, performance and mood

Prof Siobhan Banks, A/Prof Jill Dorrian, A/Prof Alison Coates, Dr. Stephanie Centofanti, Charlotte Gupta, Jackie Stepien-Hulleman, Crystal Grant, Dr Raymond Matthews, Dr Amy Hutchison, A/Prof Leonie Heilbronn, Prof David Kennaway & Prof Gary Wittert

---

You have been invited to participate in a research study. Before agreeing to participate in the study, it is important that you read and understand the following explanation of the study and the procedures. This form describes the purpose, procedures, benefits, risks, discomforts and alternative procedures that are available to you. If you choose to participate, you have the right to withdraw from the study at any time.

What is the study about?

The main objective of this study is to determine the impact of sleeping during the day (simulated shiftwork) on performance, metabolism, sleep structure and mood. The results we obtain from this study will help us to better understand the impact of night shift on health.

Who can participate and how does the study work?

Healthy, non-smokers who are aged between 18 to 50 years old, taking no medication and have a regular sleep pattern are eligible to participate in this study. Before commencing the study, potential participants will be required to complete a telephone screening interview, and attend two screening sessions. One session will be at the UniSA City East campus and one session will be at the Sleep and Chronobiology Laboratory at the UniSA Magill campus). Participants will be required to complete a 7-night sleep/wake diary and wear a wrist activity monitor (small device the same size as a wrist watch). These two measures will allow us to assess your sleep pattern. A simple blood test to assess general health and metabolic function, and urine sample to test for illicit drugs will also be done during the screening process. Participants will also be asked to complete a handedness questionnaire and a four day weighed food diary two weeks prior to entering the lab to determine their normal energy intake.

What happens during the study?

All investigations will be carried out at the Sleep and Chronobiology Laboratory at the University of South Australia. If you are eligible to participate, you will be asked to attend the laboratory for six consecutive nights/ seven days: one baseline night and day, followed by four nights of being awake/ sleeping during the day (simulated night work), and one recovery night and day. You will be randomised to one of three conditions: 1) eating a meal at night during the night work phase of the 2

study, 2) eating a snack at night during the night work phase of the study, or 3) not eating at night during the night work phase of the study. You will be given the same amount of food each day just at different times of day.
On the first day, you will be required to arrive at the laboratory no later than 10am. At this time, you will undergo training on a variety of tests (metabolic) and performance tasks (cognitive performance assessments). For the baseline night you will be given an 8 hour sleep, your bedtime will be scheduled at 10:00pm and you will be woken at 6:00am the following morning. Following the baseline period, you will then undergo four nights of simulated night shift with a 7 hour sleep opportunity each day. Sleep laboratory staff will instruct you when it is time to go to sleep and when it is time to wake up. Because of these changes to your sleep and wake schedule, you may experience a range of symptoms, including increased sleepiness, fatigue, irritability, and mild stomach upset.
During all wake periods (baseline, simulated shift work and recovery day), you will also be asked to complete a series of performance tasks at set times. Tasks will include: questionnaires, computer-based cognitive tasks. During wake periods, when you are not completing these tasks, you may read, watch TV (DVDs), play boardgames, talk, or listen to music etc.
During certain sleep periods (baseline, day time sleep post simulated night shift and recovery sleep) your sleep will be monitored using standard sleep and respiratory polysomnography, which involves placing electrodes on your head, face (taped to the skin) and chest. During sleep periods you will be recorded using small, visible cameras that are located in the ceiling of each bedroom. These cameras are not located in any of the bathrooms. The Research Staff and Students conducting this study will be the only group that will have access to these data to ensure your confidentiality.
In order to measure your blood glucose levels, you will also be wearing a subcutaneous glucose monitor. The monitor has a small, flexible sensor which inserts in to the fatty layer of skin on your stomach. A small transmitter, slightly larger than the size of a 10 cent piece, is attached to the sensor which sends readings to a pager clipped on to the waistband on your pants. To calibrate the devices, your finger will be pricked with a lancet approximately every 4-6 hours to produce a droplet of blood. We will also be taking samples of your blood regularly throughout the study to measure biomarkers and genetic differences between individuals.
At baseline (day 2) and after recovery sleep (day 7) you will undergo a standard oral glucose tolerance test. This involves a cannula (a thin, flexible plastic tube) inserted into a vein of your forearm to enable blood samples to be collected after drinking a sugary drink. Blood will be drawn before consuming the sugary drink (i.e., fasting) and 30, 60, 90, 120 and 150 minutes after. On days 2 and 5 a cannula will be inserted prior to dinner in order for blood to be taken at 4 time points across the night. In the morning, blood will be taken prior to breakfast (i.e., fasting), as well as 30, 60, 90, 120 and 150 minutes after breakfast. The cannula will be removed at the end of the test i.e. after the 150 minute blood draw). You will not sleep with the cannula in your arm, nor will you be able to shower when the cannula is in place. In total, no more than 650ml of blood will be drawn during the seven day lab phase of the study. 3

The blood collected will be used to measure the levels of a number of physiological markers and will also be used for genetic testing. The genes that we are interested in are related to sleep quantity and quality. They are not associated with any genetic disease nor are they sufficient to identify you genetically. Saliva samples will also be collected at regular intervals during the waking period of the protocol.

All of your meals will be supplied during the study, and you will not be allowed to bring any food with you. Meals will be provided at set times and no food is to be consumed outside of these meal times. It is important to remember that throughout the study, and in the week prior to the first baseline night, you will be asked to refrain from consuming any alcohol, caffeine or medication.

Are there any risks?

We will be monitoring you closely at all times during the study, and it is important for you to let Research or nursing staff know if you are experiencing any untoward symptoms so that the appropriate action and treatment can be initiated. We have procedures in place for monitoring and responding to possible risks to you during the study, as outlined below, and every effort is taken to minimise these risks.

Physical injury, pain or discomfort

There is a low risk of bacterial infection associated with venous blood sampling. We will do everything possible to minimise this risk and you will be monitored throughout the protocol for any indications of a problem. Any unusual pain should be reported to the research staff, who will notify the nursing staff.

The insertion of intravenous cannulas may cause some pain and swelling at the insertion site and the cannula itself my cause some discomfort and irritation. It is possible one or more attempts to insert a cannula are not successful, and another stick to insert it will be necessary. This may cause some additional pain, swelling or discomfort. There is a low risk of bacterial infection associated with cannulation. We do everything possible to minimise this risk and you will be monitored throughout the protocol for any indications of a problem associated with the cannula. Any unusual pain should be reported to the research staff, who will notify the nursing staff.

Similarly, the insertion of the small, flexible plastic tube of the glucose monitor may cause some mild pain and swelling at the insertion site and the bandage may cause some discomfort and irritation to the skin. Any unusual pain should be reported to the staff, who will notify the study nurse.

Over the course of the study, we will draw no more than a total of 650ml of blood. This is close to the amount given in a single donation at the blood bank. The blood will be drawn in small amounts over the duration of the laboratory phase of the study. At times during the study you may feel dizzy or light-headed. If these feelings persist, the study nurse will assess your condition.

You may experience minor skin irritation from the small electrodes that will be used to monitor your sleep. These are placed on your temples (next to your eyes), your chin, your forehead and your collarbone and are taped to your skin. Attempts will be made to minimize any discomfort by alternating the position of these leads. 4

Psychological or emotional stress

The changes to your normal sleep/wake cycle may also cause a range of symptoms, including sleepiness, fatigue and changes to your mood. Further, since participants stay in our laboratory, away from family and friends during the study, we are aware that this can cause changes in emotional state.

Potential civil, criminal or other proceedings

During the initial screening phase of the study, participants will be asked questions about history of drug use. Disclosure of information regarding illicit substances and illegal activities will remain confidential, except in the event of legal proceedings where a court ordered subpoena is issued.

How does my family get in touch with me if there is an emergency?

An important part of the study protocol is that while you are living in the sleep laboratory, you are unaware of the time of day. This means you will not have access to your mobile phone. Should your family need to get in touch with you at any time, they can contact the sleep laboratory (staff will be present 24hrs a day) on the following numbers**: (08) 8302 1235 or (08) 8302 1712**

What do I need to bring?

You will be staying in the sleep laboratory for 7 days and 6 nights, so you will need to bring enough clothes and toiletries to cover this time. It is also advised you bring a warm jumper with loose fitting sleeves to ensure the cannula is not disrupted. We will provide you with all of your linen (sheets, blankets, towels, pillows etc.), but you may wish to bring your own pillow from home. You will have your own room for the duration of the study but you will be sharing a living area, shower and toilet facilities with up to three other participants in the study. Because there will be periods of spare time throughout the study, please feel free to bring along your own music, books or movies.

What happens at the end of the study?

We recommend that you avoid the following activities [driving a car, riding a bike, and/or operating heavy machinery] until you have obtained at least one full night's sleep (uninterrupted for 7 hours or more) following the study. Therefore, it is advised that you organise someone to pick you up at the completion of the study or take a taxi home. Your reimbursement for participating in the study also covers these travel costs. In the 24h following completion of the study, or if you have withdrawn from the study early, staff at the Sleep and Chronobiology Laboratory will contact you via telephone for follow-up. Following completion of the study, should you need to get in contact with study staff at any time, they can be reached on **(08) 8302 1235 or (08) 8302 1712.**

What happens to the results?

All data from this study will be stored on computers at the Sleep and Chronobiology Laboratory, with hard drives as backups in lockable filing cabinets. All information collected as part of the study will be destroyed at the end of five years. Summarised results will be stored as hard copies at the Sleep and Chronobiology Laboratory and may be used in theses, journal publications and conferences. Data collected in the study may be used for other projects but all records containing personal information will remain STRICTLY CONFIDENTIAL **-** no information that would lead to the identification of any individual will be released. 5

What will I get out of the study?
While you will not directly benefit from this study, you will be compensated for the inconvenience and loss of time associated with your participation. If you complete the study, you will receive $1050 ($125 participation payment per night + $175 study completion payment). This reimbursement also covers travel costs from the Sleep and Chronobiology Laboratory. If for some reason you do not complete the whole study, you will receive a pro rata participation payment based on the number of hours that you spent in the sleep laboratory. Upon request, you will be provided with a copy of the final research report.

Voluntary participation - what happens if I say no?
Before deciding whether or not to take part in this study, you may wish to discuss the matter with a relative, friend or your local doctor. You should feel free to do this. It is important that you understand that your participation in this study is voluntary, as is the case with all research projects at the Sleep and Chronobiology Laboratory. If you do not wish to take part, you are under no obligation to do so. If you decide to take part but later change your mind, you are free to withdraw from the study at any stage. Your decision to take part, not to take part, or to withdraw, will not affect your routine medical treatment or your relationship with those treating you or your relationship with the Sleep and Chronobiology Laboratory.

How do I become a participant?
If you are interested in participating or have any questions, please email shiftworkstudy@unisa.edu.au

UniSA Human Research Ethics Committee Contact Details
This study has been approved by the University of South Australia's Human Research Ethics Committee. Should you wish to speak to a person not directly involved in the study, particularly in relation to matters concerning policies, information about the conduct of the study or your rights as a participant, or should you wish to make a confidential complaint, you may contact the Executive Officer the University of South Australia's Human Research Ethics Committee on (08) 8302 3118 or by email Vicki.Allen@unisa.edu.au.

Appendix 5: Particpant consent form – Study 1

University of South Australia

Participant Consent Form

Sleep and Chronobiology Laboratory
School of Psychology Division of Arts, Education, Social Sciences

Project Title:
The impact of sleeping during the day on metabolism, performance and mood

Principal Researchers' names:
Prof Siobhan Banks, A/Prof Jill Dorrian, A/Prof Alison Coates, Dr. Stephanie Centofanti, Charlotte Gupta, Jackie Stepien-Hulleman, Crystal Grant, Dr Raymond Matthews, Dr Amy Hutchison, A/Prof Leonie Heilbronn, Prof David Kennaway & Prof Gary Wittert

---

I have read the Information Sheet, and the nature and the purpose of the research project has been explained to me. I understand and agree to take part.

I understand that I may not directly benefit from taking part in the study.

I understand that while information gained during the study may be published, I will not be identified, and my personal results will remain confidential.

I understand that participation in the study is voluntary and that I can withdraw from the study at any stage.

I understand that information gathered during the study may be used for other purposes by the researchers. In this circumstance, I understand that I will not be identified and my personal results will remain confidential.

I confirm that I am over 18 years of age.

I understand that I will be videotaped during the study. I understand that the tape will be stored in digital format for 5 years in a lockable filing cabinet at the Sleep and Chronobiology Laboratory. The named researchers and sleep scorers will have access to this tape during this period.

I understand the statement concerning compensation for taking part in the study that is contained in the Information Sheet.

I understand that there is a risk of physical injury, pain or discomfort during the study, particularly related to the blood collection procedures.

I understand that there is a low risk of bacterial infection related to insertion of the cannula.

I understand that blood collected will be used for limited genetic testing.

I acknowledge that the results of the study are unknown and I may suffer from a range of symptoms including sleepiness, fatigue and changes to mood. This list of symptoms is not exhaustive.

I acknowledge that I have been advised that I should avoid the following activities: driving a car, riding a bike, and/or operating heavy machinery, following the completion of the study, until I have obtained at least one full night's sleep (uninterrupted for 7 hours or more).

I understand that disclosure of information regarding illicit substances and illegal activities will remain confidential, except in the event of legal proceedings where a court ordered subpoena is issued.
I release and indemnify the University, its employees, students and agents against liability in respect of all claims, costs and expenses and for all loss, damage, injury or death to persons or property caused or contributed by me in connection with my failure to follow the after-study instructions.

I **give/ do not give** (please circle one) the researchers permission to use the data collected for future research purposes. I give permission to be contacted after the study to give my consent to use the data for future related research purposes in de-identified form only. In this circumstance, I understand that I will not be identified and my personal results will remain confidential.

Name of Participant

……

Signed

……

Dated

……

I have explained the study to the participant and consider that he/she understands what is involved.

Researcher

……

Signed

……

Dated

……

Appendix 6: Participant consent form – Study 2

This project has been approved by the University of South Australia's Human Research Ethics Committee. If you have any ethical concerns about the project or questions about your rights as a participant please contact the Executive Officer of this Committee, Tel: +61 8 8302 3118; Email: humanethics@unisa.edu.au

SECTION 1: CONTACT AND PROJECT DETAILS

Researcher's Full Name:	A/Prof Siobhan Banks
Contact Details:	University of South Australia, C Building, Magill Campus, St Bernards Road, Adelaide, Australia 5072 Ph: +61 88302 1712, Email: siobhan.banks@unisa.edu.au
Supervisor's Full Name:	
Contact Details:	
Protocol Number:	202112
Project Title:	Optimising Human Performance using AI

SECTION 2: CERTIFICATION

Participant Certification

In signing this form, I confirm that:

- I have read the Participant Information Sheet and the nature and purpose of the research project has been explained to me. I understand and agree to take part.
- I understand the purpose of the research project and my involvement in it.
- I understand that I will be informed if any blood tests taken during the screening process come back showing abnormal results. I will then follow up with my GP.
- I understand that I may withdraw from the research project at any stage and that this will not affect my status now or in the future.
- I understand that while information gained during the study may be published, I will not be identified and my personal results will remain confidential, unless required by law.
- I understand that I will be videotaped during the study.
- I understand that data collected in the study may be used for other projects but all records containing personal information will remain STRICTLY CONFIDENTIAL – no information that would lead to the identification of any individual will be released.
- I understand that data from this study (including video footage) will be stored on password protected computers at the Sleep and Chronobiology Laboratory, with hard drives as backups in lockable filing cabinet. Only researchers directly involved with the research will have access to my data. All information collected as part of the study will be destroyed at the end of five years.
- I understand the statement in the information sheet concerning payment to me for taking part in the study.

Participant Signature	*Printed Name*	*Date*

Researcher Certification

I have explained the study to subject and consider that he/she understands what is involved.

Researcher Signature	*Printed Name*	*Date*

Appendix 7: Advertising flyer – Study 1

VOLUNTEER RESEARCH PARTICIPANTS NEEDED

University of South Australia

The *Sleep and Chronobiology Laboratory* at UniSA is seeking volunteers to participate in a **SLEEP STUDY**.

We are looking for healthy **males and females**
who are non-smokers,
aged 18-45 years,
willing to stay in the sleep laboratory for **7 consecutive days**.
The study includes blood, saliva and urine collection, and performance testing

All meals provided and participants will be financially compensated up to $1050 for their participation in the study.

Study dates available:

26th Feb – 4th March

For more information, please email: shiftworkstudy@unisa.edu.au

This project has been approved by the University of South Australia's Human Research Ethics Committee (Approved Ethics Protocol # 0000033621)

Sleep Study email: shiftworkstudy@unisa.edu.au

Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au
Sleep Study email: shiftworkstudy@unisa.edu.au

Appendix 8: Advertising flyer – Study 2

VOLUNTEER RESEARCH PARTICIPANTS NEEDED

University of South Australia

The Sleep and Chronobiology Laboratory at UniSA Magill campus is seeking volunteers to participate in a SLEEP DEPRIVATION STUDY.

We are looking for healthy young adults who are non-smokers, aged 18-40 years, and willing to stay in the Sleep and Chronobiology Laboratory for 5 consecutive days. The study will involve a period of sleep deprivation (no sleep for 62 hours). The study includes performance testing, saliva collections and a blood sample.

All meals provided and participants will be financially compensated up to $600 for their participation in the study.

Study Dates:

4th February – 8th February 2020

25th February – 29th February 2020

11th April – 15th April 2020

5th May – 9th May 2020

For more information, please email: shiftworkstudy@unisa.edu.au

This project has been approved by the University of South Australia's Human Research Ethics Committee (Approved Ethics Protocol # 202112)

