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Male shift workers and cardio-metabolic function: the role of
homeostatic desynchronisation and exercise as an
intervention

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Declaration of Authorship

I, Blake Collins, hereby declare that this submission is my own work and to the best of my knowledge and belief, understand that it contains no material previously published or written by another person nor material which, to a substantial extent, has been accepted for the award of any other degree or diploma at Charles Sturt University or any other educational institutions, except where due acknowledgements is made in the thesis "Male shift workers and cardio-metabolic function: the role of homeostatic desynchronisation and exercise as an intervention". Any contribution made to the research by colleagues with whom I have worked with at Charles Sturt University or elsewhere during my candidature is fully acknowledged.

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Signature: Blake Collins

Date: 17/06/2021

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'The fight is won or lost far away from witnesses – behind the lines, in the gym and out there on the road, long before I dance under those lights.' – Muhammad Ali

List of Publications

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Collins, B. E. G., Marino, F. E., Hartmann, T. E., & Skein, M. (*in preparation*). A comparison of acute high- and moderate-intensity exercise on cardio-metabolic function and sleep among shift workers.

Collins, B. E. G., Marino, F. E., Hartmann, T. E., & Skein, M. (*in preparation*). Shift work and exercise adherence: the effect of a 12-week mixed-modality training intervention on cardio-metabolic health.

List of Abbreviations

A	Afternoon	FFT	Fast Fourier transform
ACTH	Adrenocorticotrophic hormone	FGIR	Fasting glucose/insulin ratio
ADHERE	Attended \geq 24 sessions	FM	Fat mass
AMPK	Adenosine monophosphate-activated protein kinase	GABA	Gamma-aminobutyric acid
ANOVA	Analysis of variance	GH	Growth Hormone
ANS	Autonomic nervous system	GLUT	Glucose transport
APSS	Adult Pre-exercise Screening Tool	GXT	Graded exercise test
AR	Autoregressive	HDL	High-density lipoproteins
AT	Anaerobic threshold	HDL-C	High-density lipoproteins cholesterol
ATP	Adenosine triphosphate	HIT	High intensity interval training
AUC	Area under the curve	HOMA	Homeostasis Model Assessment
BGL	Blood glucose levels	HOMA-IR	Homeostasis Model Assessment indexed insulin resistance
BMAL-1	Brain and muscle Arnt-like protein-1	HPA	Hypothalamic-pituitary-adrenal
BMI	Body mass index	HR	Heart rate
BP	Blood pressure	HRV	Heart rate variability
CHD	Coronary heart disease	ICAM-1	Intracellular adhesion molecule-1
CLOCK	Clock genes	IFN- α	Interferon- α
CNS	Central nervous system	IFN- γ	Interferon- γ
CPAP	Continuous positive airway pressure	IHD	Ischemic heart disease
CRH	Corticotrophin releasing hormone	IL-1	Interleukin-1
CRP	C-reactive protein	IL-1 β	Interleukin-1 β
<i>Cry</i>	Cryptochrome	IL-1Ra	Interleukin-1 receptor agonist
CVD	Cardiovascular disease	IL-6	Interleukin-6
DASS21	Depression anxiety and stress scale	LC	Locus coeruleus
DEXA	Dual x-ray absorptiometry	LC-NE	Locus coeruleus-norepinephrine
DMH	Dorsomedial nucleus of the hypothalamus	LDE	Long-duration exercise
E	Evening	LDL	Low density lipoprotein
ECG	Electrocardiogram	LDT	Laterodorsal tegmental nuclei
EDTA	Ethylenediaminetetraacetic acid	LH	Lateral hypothalamus
EEG	Electroencephalogram	LM	Lean mass
ELISA	Enzyme-linked immunosorbent assays	M	Morning
EMG	Electromyogram	MANOVA	Multivariate analysis of variance
EOG	Electro-oculogram	MCP-1	Monocyte chemoattractant protein 1
ESS	Epworth Sleepiness Scale	MetS	Metabolic syndrome
FFM	Fat free mass	MPO	Medial preoptic area

N	Night	RMSSD	Mean square root differences of the standard deviation
NADHERE	Attended < 24 sessions	ROS	Reactive oxygen species
NE	Norepinephrine	RR	Relative risk
NN	Normal-to-normal	SCN	Suprachiasmatic nucleus
NO	Nitric Oxide	SD	Standard deviation
NREM	Non-rapid eye movement	SDNN	Standard deviation of normal-to-normal intervals
NSHIFT	Non-shift worker	SEE	Standard error estimates
OGTT	Oral glucose tolerance test	SHIFT	Shift worker
ORX	Orexin	SNS	Sympathetic nervous system
PA	Physical activity	SPZ	Sub-paraventricular zone
<i>Per</i>	Canonical clock genes period	SST	Serum separator tubes
PGC-1 α	Proliferator-activated receptor γ	SW	Shift work
pNN50	percentage of normal beats more than 50 millisecond deviation from the previous beat	TIB	Time in bed
PNS	Parasympathetic nervous system	TMN	Tuberomammillary nucleus
PPT	Pedunculo pontine	TNF- α	Tumor necrosis factor- α
PRC	Phase-response curves	TRH	Thyrotropin-releasing hormone
PSD	Power spectral density	TSH	Thyroid stimulating hormone
PSG	Polysomnography	TST	Total sleep time
PSQI	Pittsburgh Sleep Quality Index	T2DM	Type 11 diabetes mellitus
PVN	Paraventricular nucleus	VCAM-1	Vascular cell adhesion molecule-1
QRS	QRS complex in a standard electrocardiogram	VLF	Very low frequency
QUICKI	Quantitative insulin sensitivity check index	VLPO	Ventrolateral preoptic nucleus
RHR	Resting heart rate	WASO	Wake after sleep onset
RHT	Retinohypothalamic tract	WHO	World Health Organisation
REM	Rapid eye movement	WHR	Waist-to-hip ratio

List of Symbols and Units

α	Alpha	$\mu\text{IU}\cdot\text{mL}^{-1}$	Microunits per millilitre
\sim	Approximately	ml	millilitre
bpm	Beats per minute	$\text{ml}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$	Millilitres per kilogram per minute
β	Beta	$\text{ml}\cdot\text{min}^{-1}$	Millilitres per minute
r	Correlation coefficient	mm	Millimetre
d	Day	mmHg	Millimetres of mercury
$^{\circ}\text{C}$	Degrees Celsius	$\text{mm}\cdot\text{sec}^{-1}$	Millimetres per second
Δ	Delta change	$\text{mmol}\cdot\text{L}^{-1}$	Millimoles per litre
\$	Dollar	$\text{mmol}\cdot\text{L}\cdot\text{min}^{-1}$	Millimoles per litre per minute
=	Equals	-	Minus
γ	Gamma	min	Minute
g	Grams	$\text{min}\cdot\text{wk}^{-1}$	Minutes per week
>	Greater than	$\text{ng}\cdot\text{ml}^{-1}$	Nanograms per millilitre
\geq	Greater than or equal to	n.u.	Normative units
HR_{max}	Heart rate maximum	n	Number of participants
Hz	Hertz	$\text{VO}_{2\text{peak}}$	Peak measured oxygen uptake
h	Hour	%	Percent
kg	Kilogram	$\text{pg}\cdot\text{ml}^{-1}$	Pictograms per millilitre
$\text{Kg}\cdot\text{m}^2$	Kilograms per metre squared	+	Plus
$\text{km}\cdot\text{h}$	Kilometres per hour	\pm	Plus-minus sign
<	Less than	s	Second
\leq	Less than or equal to	W	Watt
L	Litre	$\text{W}\cdot\text{min}^{-1}$	Watts per minute
$\text{VO}_{2\text{max}}$	Maximum oxygen consumption	y	Year
μl	Microlitre		

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Abstract

Shift work is a work structure designed to increase total labour opportunity and meet the growing demands of modern society. However, the extended and rotating labour periods are associated with adverse health conditions including an increased risk of developing cardio-metabolic disorders. Given the integrated nature of cardio-metabolic regulation, the biologically disruptive labour characteristics of shift work, including night and rotating shifts, may facilitate disease progression. Additionally, given the central role of shift work within the modern workforce, developing interventions to improve health outcomes have become a research focus. Exercise is a thoroughly researched and validated intervention to improve the general health of a variety of population groups. However, limited research currently substantiates these observations among shift workers, a population group with established barriers to exercise participation. Therefore, the aims of the current thesis are to i) examine the effect of employment in rotational shift work on markers of cardio-metabolic function and ii) examine the effect of exercise, including variations in mode, intensity, and chronicity (acute vs training), on markers of cardio-metabolic function among rotational shift workers.

The first study in this thesis aimed to investigate the effect of rotational shift work on measures of cardio-metabolic function including sleep, inflammatory status, glucose metabolism and insulin sensitivity, body composition and autonomic regulation. Eighty-seven sedentary but healthy men, matched for lifestyle behaviours including smoking status, average sleep quality and physical activity (PA) status were categorised via occupation; shift workers ($n = 44$) and non-shift workers ($n = 43$). The shift worker group reported significantly reduced aerobic capacity (VO_{2peak}), increased response time to an oral glucose tolerance test, higher body fat, and higher resting values of interleukin (IL)-6. Conclusively, shift work is associated with an increase in independent risk factors for cardio-metabolic disorders, suggesting a detrimental effect of shift on the health and well-being of male employees.

The second study in this thesis explored the acute effect of moderate continuous (MICT) and high intensity interval training (HIT) on markers of cardio-metabolic function and sleep among rotational shift workers. Twenty-six sedentary men currently employed in rotational shift work underwent baseline laboratory testing (including 7 days sleep assessment) before being randomly allocated a 30 min cycling intervention of either HIT: 1:4 ratio of 60 s at 100 % and 240 s at 50 % VO_{2peak} , or MICT; continuous cycling at 60 % VO_{2peak} . Laboratory testing was recorded post intervention (immediately, 30- and 60-minutes post) before subsequent sleep opportunity was assessed. A significant increase in IL-1 receptor agonist (IL-Ra) was observed immediately and 30 minutes post HIT but not MICT. Alternatively, MICT significantly reduced wake after sleep onset (WASO) in the subsequent sleep opportunity, a result not observed within the HIT group. Collectively both HIT and MICT acutely improved markers and modulators of cardio-metabolic function and warrant further investigation into the potential intensity dependant adaptations.

The final study in this thesis investigated the effect of a 12-week training intervention of MICT or resistance training (RT) among thirty-eight sedentary male shift workers. Randomly assigned a control, MICT or RT group, participants underwent baseline laboratory testing before completing 12 weeks of training, prescribed 3 days a week. Mean sessional attendance across the intervention was 25 (\pm 7) of a possible 36 sessions despite the provision of free, personalised and semi-supervised training. A significant effect was observed among the MICT group, reducing c-reactive protein (CRP) levels post intervention. Both the MICT and RT increased total sleep time (TST) following a night shift post-intervention. Finally, data was redistributed based on sessional attendance to investigate a potential dose-response effect. A significant reduction in body fat was observed when 24 or more total sessions were attended during the intervention. The results suggest exercise is a valid intervention to improve the cardio-metabolic function of male rotational shift workers however several barriers to exercise adherence need to be investigated.

Collectively, the thesis contributes to the body of research investigating the increased risk of cardio-metabolic disorders among male rotational shift workers by exploring the effect of homeostatic desynchronisation on markers of cardio-metabolic function. Further, this research contributes to the currently limited body of exercise-based intervention research among rotational shift workers. Both acute and chronic training interventions improved measures commonly associated with poor cardio-metabolic function and future outcomes. However, further research is required to investigate intensity and mode-based adaptations as well as adherence factors.

Chapter One:

Introduction

1.1 Overview

With global expansion, advancement in technologies, and changes in cultural expectations, society may be considered a '24/7' environment. In response, employers across a range of industries utilise specific schedules to extend total labour opportunity beyond the 'typical 9 to 5' workday (Schilperoort, Rensen, & Kooijman, 2020). The extension is achieved through division of total labour into operational shifts, with employees following each other in a rotational manner. Consequently, shift work is considered an essential labour structure in vital sectors including health, emergency services, manufacturing, and hospitality. However, incorporating rotational shifts, or fixed shifts in opposition to pre-determined biological rhythms, may induce adverse health conditions (Kecklund & Axelsson, 2016; Kervezee, Kosmadopoulos, & Boivin, 2018). Specifically, cardio-metabolic function is under the integrated control of several biological mediators, including circadian rhythms, the sleep-wake cycle, and immune system, to provide predictive function in response to a dynamic environment. However, such integration allows misaligned cues to propagate across multiple systems, resulting in homeostatic desynchronisation, which under chronic conditions, may facilitate pathogenesis (Kervezee et al., 2018). The observed increase in cardio-metabolic conditions among shift workers supports the theory of a pathological relationship; however, the exact mechanisms, including the role of homeostatic desynchronisation, are yet to be fully explored. Further, the necessity of shift work across multiple industries justifies the need for interventions aimed at improving employee health and well-being, with exercise a viable suggestion. While a number of pharmacological and structural interventions have been implemented, this thesis will aim to investigate the validity of exercise, which to date, has had somewhat limited exploration within current literature (Flahr, Brown, & Kolbe-Alexander, 2018). The *Introduction Chapter* will therefore provide an overview of shift work as a labour structure, the potential pathogenic effect of employment and rationalisation for exercise as intervention method.

1.2 Shift work and potential health effects

1.2.1 Shift work

With evolving societal expectations and increased workforce demands, it is not uncommon to know a shift worker, or to have been personally employed as one. Conventionally utilised in health and emergency services such as nursing or policing, shift work has multiple variations in structure to meet the growing demands of modern society, and includes any work completed outside of the 'normal' daytime hours (Flahr et al., 2018; Sallinen & Kecklund, 2010). Varying in shift length, subsequent breaks, order, and direction of rotation, shift work collectively aims to extend total labour opportunity beyond what would be achievable within a conventional workday. Therefore, mining, manufacturing and hospitality additionally employ shift work to achieve the day-to-day requirements of their respective industries. Employment that translate to an estimated 16 % of the Australian workforce, equating to 1.5 million people (ABS, 2020; Deloitte Access Economics, 2019). Conclusively, regardless of the industry, shift work is a vital labour structure with increasing participation within our modern society.

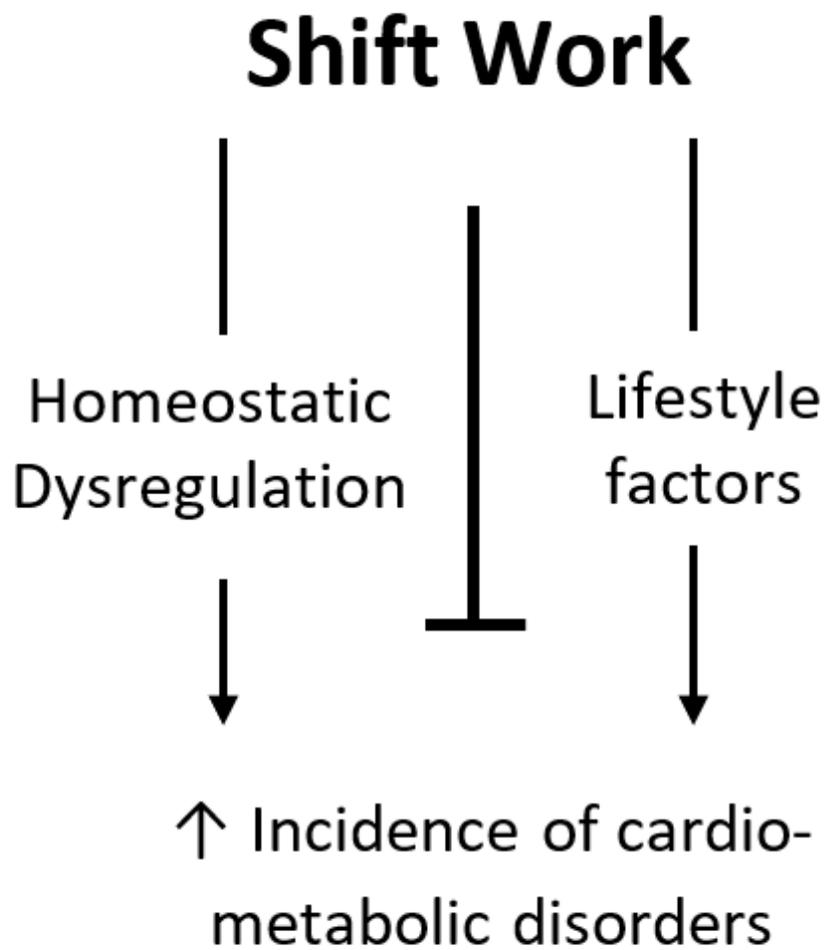
1.2.2 Proposed health effects of shift work

Shift workers commonly experience changes in their health and behaviour, reporting withdrawal from physical activity (PA), reduced or poor sleep, and weight gain over the course of their employment (Faraut, Boudjeltia, Vanhamme, & Kerkhofs, 2012; Reutrakul & Knutson, 2015). Observational research also associates shift work with pathological lifestyle factors and health conditions including reduced engagement in PA and increased incidence of cardio-metabolic conditions (Blake, Stanulewicz, & McGill, 2017; Puttonen, Härmä, & Hublin, 2010). However, these observations have not been conclusive, with criticism including inconsistent measures, independent effect of differing shift structures and a dependence on indirect measures including health questionnaires, disputing an direct

pathogenic role (Kecklund & Axelsson, 2016). Consequently, the health effect of shift work is an area of ongoing research and a central theme of the current thesis. A theoretical model has been included (Figure 1.1) to frame the thesis approach, which will be updated throughout the chapter as further exploration of topics and justification are provided.

Despite criticisms, comparative research assessing occupation and cardio-metabolic function have concluded that rotational shift workers are at an increased risk of developing adverse conditions. The relative risk of developing the metabolic syndrome (MetS) can range from 1.5 (Sookoian et al., 2007) to 1.77 (De Bacquer et al., 2009) times more likely among shift workers compared to their fixed-day counterparts. Similarly, a strong association between shift work and cardiovascular disease (CVD) has been demonstrated with the relative risk as high as 1.4 when comparing shift and non-shift workers (Frost, Kolstad, & Bonde, 2009). As shown in *Figure 1.1*, shift work does appear to adversely affect the health of employees, however the primary cause is still debated with both lifestyle and physiological risk factors plausibly responsible. Lifestyle factors, including higher rates of smoking, drinking excessive amounts of alcohol, availability of non-nutritious foods during night shifts or established cardio-metabolic risks including excessive body fat and decreased PA have all been proposed as aetiological causes (Dorrian, Heath, Sargent, Banks, & Coates, 2017; García-Díaz, Fernández-Feito, Arias, & Lana, 2015; Schilperoort et al., 2020). Alternatively, given the integrated nature of the cardio-metabolic system and the influential role of sleep, circadian rhythms and inflammatory responses in healthy function, shift work disorders may be facilitated by labour specific manipulations in biological rhythms (Flahr et al., 2018; Schilperoort et al., 2020).

Figure 1.1 A fundamental thesis question: *Is shift work associated with increased incidence of cardio-metabolic disorders, and if so, is the pathology related to lifestyle behaviours or the physiological effect of the labour structure?*



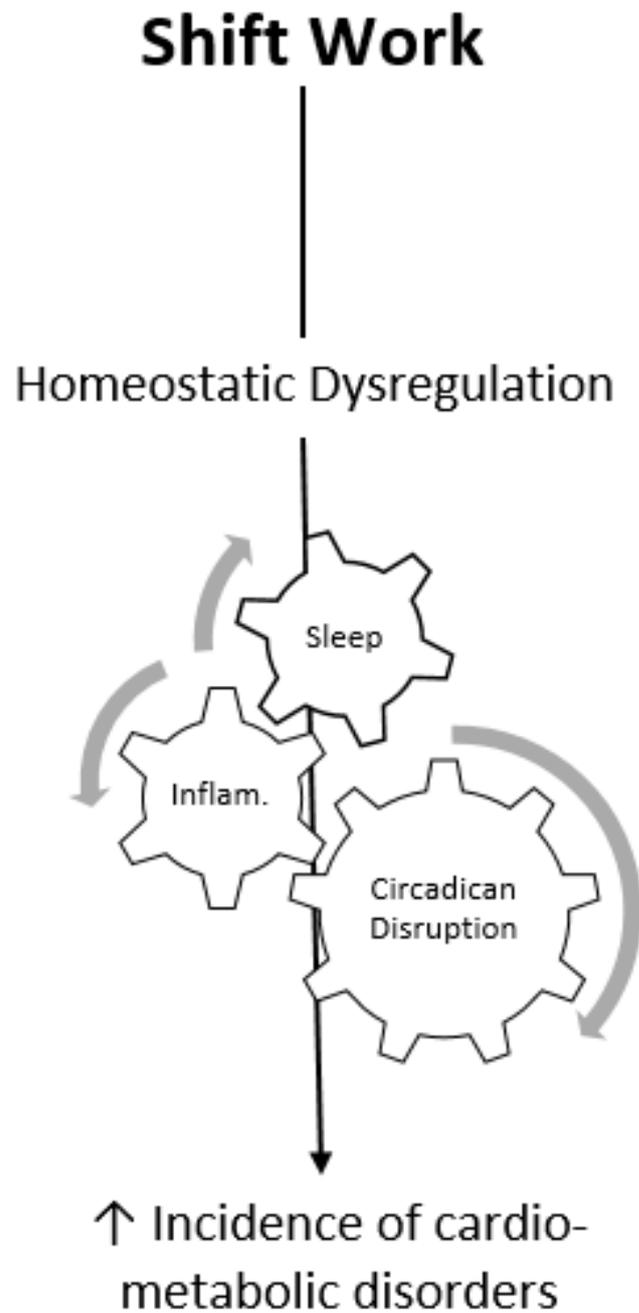
1.2.3 Disturbed regulatory systems and pathogenesis

Shift work is associated with several co-factors plausibly responsible for the development of non-communicable disease states (Liu et al., 2018; O'Brien et al., 2020). However, the structural arrangements of shift work systematically disrupt homeostatic regulation of the cardio-metabolic system. The chronic effect of which may play a direct etiological role in adverse cardiometabolic function and indirectly contribute to cardio-metabolic disorders through altered lifestyle co-factors such as reduced PA and obesity (Kecklund & Axelsson, 2016; Kervezee et al., 2018).

Homeostatic regulation of cardio-metabolic function involves the integrations of several regulatory behaviours and systems including sleep, circadian rhythmicity and the immune system (Fuller, Gooley, & Saper, 2006; Lange, Dimitrov, & Born, 2010). These mechanisms are often discussed separately, however their regulatory role is achieved through shared anatomical structures, neuronal projections and integrated humoral factors to provide predictive rather than reactive responses (Lange et al., 2010). For example, in fulfilling its role in maintaining normal tissue function, the immune system acts as an autocrine, paracrine, and endocrine messenger capable of interacting with sleep and circadian regulatory centres as well as partitioning metabolic resources (Del Giudice & Gangestad, 2018; Libby, 2007; Swirski & Nahrendorf, 2018). Sleep regulatory centres including the hypothalamus have both immune-creative neurons and signalling receptors for cytokines demonstrated to influence sleep behaviour (Imeri & Opp, 2009). Peripheral immune cells contain molecular components which mimic transcription-translation feedback loops found in the circadian control centre of the brain, and demonstrate cyclic peaks and nadirs in immune cell function (Morris, Aeschbach, & Scheer, 2012). Finally, due to the high metabolic cost of a functioning immune system, immune cells are capable of impairing insulin signalling to redirect resources from organs with high glucose demand such as skeletal muscle (Lackey & Olefsky, 2016).

However, the trade-off of an integrated and predictive system is the dependence on external and internal cues to synchronise function. Consequently, misaligned or conflicting regulatory cues including sleeping, eating or being physically active in opposition to the predictive biological timing can disrupt the regulatory systems and result in homeostatic desynchronisation (Irwin & Cole, 2011). Under chronic conditions homeostatic dysregulation is associated with the development and progression of non-communicable disease states. As highlighted in *Figure 1.2*, disturbed circadian rhythms and inverted sleep-wake cycle effect immune, cardiovascular and metabolic function (Puttonen et al., 2010). For example, immune cell count and cardiac tone display temporal variations associated with both circadian and sleep-wake cycles (Scheiermann, Kunisaki, & Frenette, 2013; Schilperoort et al., 2020) with disruptions impacting immune cell proliferation, autonomic cardiac tone, and insulin sensitivity (Puttonen et al., 2010; Schilperoort et al., 2020). Further, chronic disruptions in circadian and sleep rhythmicity prolong inflammatory responses, themselves capable of facilitating cellular damage and pro-inflammatory cascades associated with cardio-metabolic disorders including diabetes and atherosclerosis (Castanon-Cervantes et al., 2010; Libby, 2007). Shift work, with variations in shift length, direction and speed of rotation provides conflicting regulatory cues capable of facilitating homeostatic desynchronisation and subsequent cardio-metabolic disorders. A primary aim of this thesis will be to explore the physiological effect of rotational shift work on cardio-metabolic function to investigate potential pathogenic mechanisms.

Figure 1.2 Cardio-metabolic function is mediated by integrated regulatory systems including circadian rhythms, sleep-wake cycles, and the immune system. Consequently, homeostatic dysregulation may facilitate cardio-metabolic disorders among shift workers

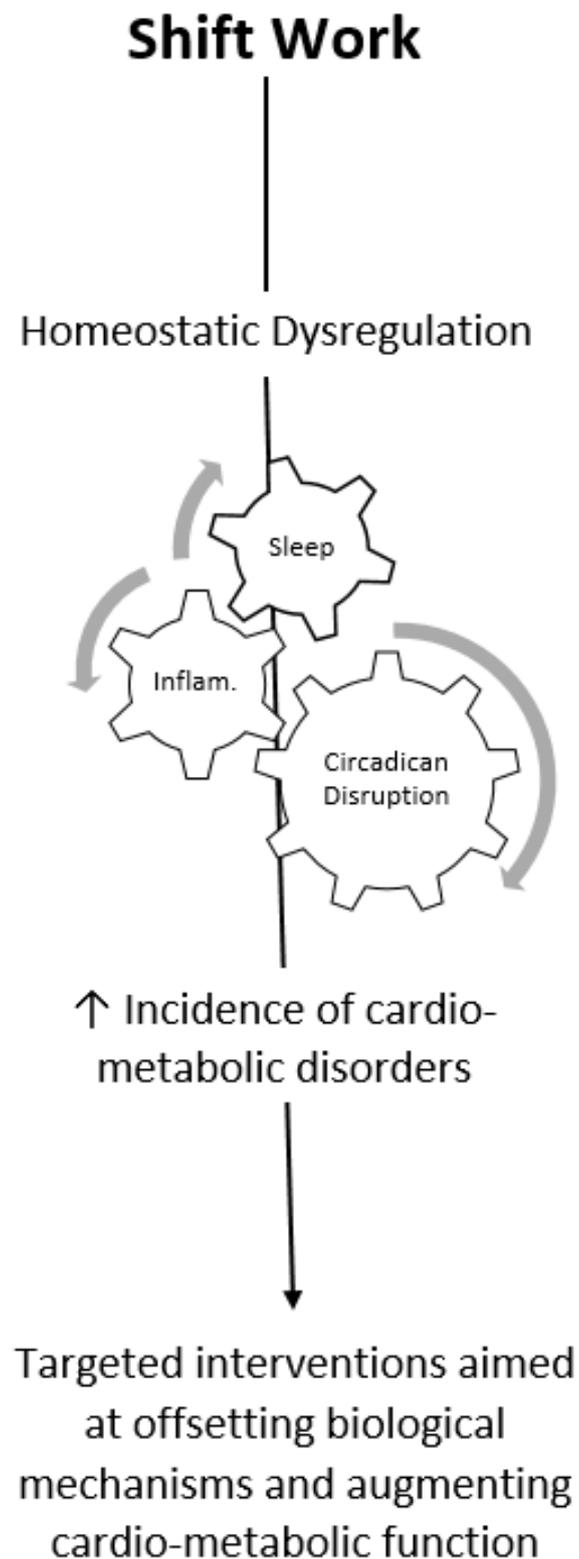


1.3 Shift work Health Interventions

The necessity of shift work, combined with identified labour induced health effects, have resulted in the promotion of interventional strategies aimed at improving employee health. Given the potential pathogenic role of homeostatic desynchronisation, interventions have been developed to improve cardio-metabolic function via minimising biological disruptions and augmenting cardio-metabolic pathways (*Figure 1.3*).

The interventions aimed at improving the health of shift workers conventionally fall into four categories; 1) controlled light and dark exposure, 2) shift schedule changes, 3) behavioural or lifestyle interventions and 4) pharmacological aids to promote sleep (Neil-Sztramko, Pahwa, Demers, & Gotay, 2014). For example, as light is the primary entraining signal for circadian rhythms, appropriately-timed and controlled bright light exposure may be used to realign circadian rhythm to a specific shift pattern (Neil-Sztramko et al., 2014). Shift scheduling is an additional method in which the characteristics of specific shifts including rotation, length, speed of transition and start time, are manipulated to minimise the potential disruptive effect (Neil-Sztramko et al., 2014). Lifestyle interventions mainly include educational programs aimed at improving lifestyle behaviour affected by shift work such as diet, PA levels and maximising sleep hygiene (Atkinson, Fullick, Grindey, & Maclaren, 2008; Crispim et al., 2011; James, Honn, Gaddameedhi, & Van Dongen, 2017). Finally, pharmacological interventions include the use of sleeping pills, melatonin and appropriate use of caffeine (before shifts rather than after) are collectively advocated to improve sleep (Neil-Sztramko et al., 2014). The methodology may vary, however the interventions are designed to minimise or offset the disruptive effect of shift work and improve biological regulation (*Figure 1.3*).

Figure 1.3 Given the necessity of shift work to meet the current demands of our modern society, several intervention strategies have been investigated to improve employee health.



1.3.1 Exercise as an intervention

Exercise exerts a broad range of positive health affects among healthy and clinical populations (Warburton, Nicol, & Bredin, 2006). Associated with improvements in sleep, circadian entrainment (Driver & Taylor, 2000), inflammatory status and body composition (Golbidi, Mesdaghinia, & Laher, 2012), exercise is capable of both offsetting the effect of homeostatic desynchronisation and independently improving cardio-metabolic function (Flahr et al., 2018). Consequently, exercise is hypothesised to be an effective tool to address some of the risk factors hypothesised to induce disease development among shift workers.

However, several variables are yet to be fully explored regarding the prescription of exercise among shift workers. Differences in specific adaptations to mode, intensity, duration and regularity of exercise may potentially modulate the effectiveness of exercise as a health intervention (Yao & Basner, 2019). For example, high intensity interval training (HIT) may acutely induce a pro or anti-inflammatory effect based on the individuals' conditioning or exercise tolerance (Brown, Davison, McClean, & Murphy, 2015; Woods, Wilund, Martin, & Kistler, 2012). Consequently, prescribing HIT among sedentary shift workers with low exercise tolerance may exacerbate an already increased pro-inflammatory state. Further, given the labour specific barriers to exercise including a perceived lack of time (Nea et al., 2017), differences in mode, intensity, and regularity of exercise may influence exercise prescription as researchers attempt to identify the most effective interventions.

Despite both the justification and potential considerations for exercise, a limited number of research projects have been conducted among shift workers. An observation that may, in part, be due to the complex interplay of various biological mechanisms coupled with variations in workforce demands and labour structures. A recent meta-analysis by Flahr and colleagues (2018) assessing shift work and exercise-based interventions identified only seven studies between 1988 and 2017 (Flahr et al., 2018).

Of the research projects identified, prescribed PA (commonly walking or aerobic activity) significantly improved outcome measures including body composition, aerobic fitness and subjective sleep. However, several issues exist, including inconsistent exercise interventions and a lack of information regarding shift rostering. From the Flahr review and previous work, it is evident that further research is needed to assess the effectiveness of exercise as an intervention among shift workers to improve health outcomes.

1.3.2 Barriers to Exercise

A potential reason for the limited exercise interventions among shift workers may be barriers to exercise embedded within the labour structure. The conventional approach to exercise training includes structured aerobic (cycling or running) and resistance-based exercise programs to elicit physiological benefits specific to inflammation, glucose regulation and body composition, to name a few (Gebel et al., 2015). However, shift workers experience a number of barriers to exercise participation, including a lack of time between consecutive shifts to achieve the recommended PA guidelines (Atkinson et al., 2008). Further, the effect of fatigue following consecutive shifts may impact the motivation to exercise within the limited time available (Kline, 2014). Cumulatively, exercise may be a feasible intervention strategy to improve the health of rotational shift workers, however several barriers may impact exercise adherence. Therefore, a key consideration of interventions-based research conducted among shift workers is how to increase exercise adherence and facilitate the proposed health benefits.

1.4 Summary

A conservative assessment of shift work participation estimates over 1.5 million Australians are employed in shift work across a broad range of industries. While an effective labour structure, the

characteristics of shift work are associated with increased incidence of adverse health conditions. Specifically, homeostatic desynchronisation, facilitated by shift work through rotational labour periods and misaligned biological cues, is associated with increased risk of developing cardio-metabolic disorders. However, further investigation is required to determine the specific causal mechanisms to understand pathogenesis, and design intervention strategies to improve shift worker health. Exercise is a valid intervention strategy, previously demonstrated to improve homeostatic regulation and cardio-metabolic function. However, employment in shift work exacerbates several barriers to exercise participation and to-date, a limited amount of research has substantiated the proposed health effects among shift workers.

1.5 Statement of the Problem

Shift work is utilised to meet the current demands of society and employs millions of people across a variety of industries. However, the labour structure is associated with an increased relative risk of developing cardio-metabolic disorders. Homeostatic desynchronisation is implicated in the pathogenesis, however the specific mechanisms including the aetiological role of circadian disruption, disturbed sleep and systemic inflammation requires further investigation. Further, exercise is a plausible intervention method with a myriad of research supporting the general health benefits and role in augmenting cardio-metabolic function. However, there is limited research examining not only the effect of exercise as a health intervention, but the adherence to programs within the shift work structure. As such, additional research is required to investigate the specific mechanisms responsible for cardio-metabolic disorders among shift workers. Further, assessment of exercise as an effective intervention method in terms of effect and adherence requires further exploration.

1.6 Thesis Aims

This thesis is comprised of three studies designed to examine the effect of rotational shift work in conjunction with acute and training exercise interventions on the cardio-metabolic function of apparently healthy volunteers. Specifically, males (25 – 55y) currently employed in rotational shift work (studies 1-3) and non-shift counterparts (study 1 only) were recruited to:

1. Examine the effect of employment in rotational shift work on markers of cardio-metabolic function, sleep quality, body composition, metabolic efficiency, inflammatory status and autonomic modulation;
2. Examine the adherence to and effect of exercise, including variations in mode, intensity, and chronicity (acute vs training), on markers of cardio-metabolic function among rotational shift workers.

1.7 Study Aims and Hypothesis

The following hypotheses and aims were posited to achieve the overall thesis aims:

Chapter Three: Inflammatory status and cardio-metabolic risk stratification of rotational shift work.

Research Aim:

1. To assess the effect of rotational shift work on markers of cardio-metabolic function including sleep quality, body composition, metabolic efficiency, inflammatory status and autonomic modulation.

Research Hypothesis:

1. Employment in shift work will be identified as an independent risk factor for reduced glucose metabolism, decreased insulin sensitivity, increased pro-inflammatory markers, increased body fat percentage, and reduced objective sleep quality.

Chapter Four: A comparison of acute high- and moderate-intensity exercise on cardio-metabolic function and sleep in shift workers

Research Aims:

1. To assess the effect of an acute exercise bout on markers of cardio-metabolic function and sleep among shift workers;
2. Compare the effect of differing exercise intensities on markers of cardio-metabolic function.

Research Hypothesis:

1. An acute exercise bout will improve insulin sensitivity, induce an anti-inflammatory effect, and improve subsequent sleep among rotational shift workers
2. Matched for total work, high intensity interval training (HIT) will induce superior effects in insulin sensitivity, anti-inflammatory proliferation and sleep quality compared to moderate intensity continuous training (MICT).

Chapter Five: Shift work and exercise adherence: the effect of a 12-week mixed-modality training intervention on cardio-metabolic health.

Research Aims:

1. To assess the effect of a 12-week semi-supervised intervention among rotational shift workers on markers of cardio-metabolic function and sleep quality;
2. Compare the effect of MICT to resistance training (RT) for effect on cardio-metabolic risk factors among shift workers.

Research Hypothesis:

1. 12-week training intervention will improve cardiovascular fitness, body composition, glucose metabolism, insulin sensitivity and inflammatory status among rotational shift workers in comparison to non-exercising control.

2. No significant difference will be observed between the exercise modalities for improving cardio-metabolic risk profile among shift workers.

1.8 Limitations:

Due to inherent study design limitations and assumptions, the following factors were acknowledged:

- Due to recruitment issues, the criteria for participation in Study One as a shift worker was expanded to include any type of rotational shifts including night shift. The criteria may limit the extrapolation of results to specific methods of shift scheduling;
- Study One included no assessment of circadian rhythm (core temperature, cortisol or melatonin), limiting the interpretation of results pertaining to circadian disruption;
- For Study Two, participants were instructed to complete a food diary and replicate the exact consumption between each trial. Despite clear instruction and demonstration, participants were not observed recording or replicating diet so compliance could not be verified by the research team;
- Participant's inflammatory responses may at times reflect stimuli other than the effect of shift work or exercise (i.e., influenza or infection); however, all attempts were made to ensure participants are free from such illnesses during testing and data collection;
- Equipment malfunction of HRV transmitters and actigraphy watches during pre- and post-testing excluded several participants' data sets from being included in Study Three, limiting assessment of the 12-week training effect of exercise;
- The diverse shift schedules of the participants in Study Three prevented the standardisation of training opportunities and resulted in a semi-supervised training structure;
- General limitations regarding the use of commercially available enzyme-linked immunosorbent assays (ELISA) kits to assess inflammatory status for all three research projects resulted in numerous participants being below detectable ranges. Consequently, limiting interpretation of some inflammatory based results.

1.9 Delimitations

- All research projects were designed using established scientific approaches to maximise the validity of research observations. Strategies included both experimental and non-experimental research design, randomising of participant prior to interventions and the use of control groups to limit the effect of variables not being tested within the project;
- Prior to all study trials, participants were instructed to abstain from alcohol and physical activity for 24 h, and avoid caffeine consumption for 12 h prior to arrival to standardise pre-exercise conditions;
- Participants attended a familiarisation session detailing each study's testing procedures, measures, and equipment;
- Session times (between each trial or across multiple studies), were standardised for individual participants to avoid diurnal variances in cardio-metabolic measures;
- All studies in this thesis were conducted under controlled laboratory conditions using identical equipment and assessor for each successive trial and data analyses;
- Study Three was provided for free, in a fully functionally commercial gym to minimise the barriers to exercise adherence.

Chapter Two:

Literature Review

2.1 Overview

The development of new technologies, progressive globalisation, and extensions of fundamental health and emergency services, have placed a greater demand on the workforce within modern society (Costa, 2010). Consequently, shift work, designed to extend total labour opportunity beyond conventional daytime hours, has become an essential labour structure, contributing to an estimated 20 % of the workforce (Alterman, Luckhaupt, Dahlhamer, Ward, & Calvert, 2013; Flahr et al., 2018). The diversification of working times and structures have contributed to a general improvement in the quality of life of the broader society (Costa, 2010), however the implications for employee health and well-being are yet to be fully investigated. The first section of the literature review will therefore explore the specific characteristics of rotational shift work to provide context for the potential implications on employees' health.

While being an effective labour structure, shift work has been associated with adverse health conditions, including an increased incidence of cardio-metabolic disorders (Faraut et al., 2012; Kecklund & Axelsson, 2016; Kervezee et al., 2018). However, the current body of research has not established a direct pathogenic role of shift work (Frost et al., 2009; Puttonen et al., 2010). The inconsistent results have been attributed to methodological problems including; lacking a clear definition of shift work and structural characteristics, individual differences in tolerance to the work structure (Saksvik, Bjorvatn, Hetland, Sandal, & Pallesen, 2011), and the potential role of disease co-factors including obesity and sedentary behaviour (Kervezee et al., 2018). Consequently, the identification of specific pathogenic mechanisms is yet to be fully explored, however, the role of circadian desynchronisation, maladaptive sleep and systemic immune responses are emerging as viable mechanisms. The cardio-metabolic system is under homeostatic regulation of several integrated biological systems including circadian rhythmicity, sleep and the immune system (Lange et al., 2010; Rajaratnam & Arendt, 2001). Therefore, disturbed homeostatic regulation, including

misaligned biological cues provided by the structural characteristics of shift work, may facilitate poor health conditions (Flahr et al., 2018). Section 2.4 will examine the pathological effect of shift work, expand on methodological issues and investigate potential mechanisms for shift worker pathogenesis.

The reliance of modern society on shift work, coupled with the hypothesised adverse health effects, highlight the need for intervention strategies to improve employee health. Such interventions may aim to provide primary and/or secondary prevention of the development and progression of cardio-metabolic disorders. Primary prevention may be achieved via limiting the biologically disruptive effect of shift work, including attempting to minimise circadian displacement and/or poor sleep to prevent pathogenic development (Neil-Sztramko et al., 2014). Alternatively, interventions may aim to augment cardio-metabolic function to minimise the potential progression of adverse health conditions (Saner, Bishop, & Bartlett, 2018; Warburton et al., 2006). Section 2.6 will provide theoretical justification for the use of exercise-based interventions to both offset the disruptive effect of shift work and augment the biological pathways implicated in shift work related disorders. Further, section 2.6 will critically evaluate the current exercise interventions conducted among rotational shift workers to provide future directions for research.

2.2 Shift work as a labour structure

Shift work refers to broad spectrum of labour organisation completed both during and outside of the 'normal' structured daytime hours. Typically, labour periods are divided into consecutive shifts, aiming to maximise total work opportunity over a 24 h period (Flahr et al., 2018). Divisions may include: morning, day, evening, night, rotating, on-call, split or overtime with total duration typically 8 to 12 h but ranging up to 24 h per shift. Additional variations in shift structure characteristics include the direction of transition (shifts may follow a clockwise progression from morning, afternoon to night or

counter-clockwise), number of consecutive shifts, speed of rotation as well as the length and distribution of recovery periods (Sallinen & Kecklund, 2010). The specific shift characteristics selected are designed to balance maximising performance during the shift and facilitating recovery for the subsequent work period, which may vary between industries.

The practice of shift work has long been part of the normal work duties in health and emergency services, however the expansion of globalised economies and reliance on 24 h services have resulted in an increased utilisation of shift work across a broader range of industries. Within Australia, industries including hospitality, maintenance, information technology, mining and manufacturing also incorporate shift work (ABS, 2020; Deloitte Access Economics, 2019; Vincent, Kinchin, Ferguson, & Jay, 2018). Consequently, shift work is considered a key labour structure, utilised in approximately 16 % of the Australian workforce, equating to 1.5 million employees (ABS, 2020; Deloitte Access Economics, 2019). However, these statistics, which are based on survey information that identified a further 34% of employees usually work additional hours or overtime, may be a conservative estimate, with global data indicating 20 – 30 % of the workforce are involved in shift work (Alterman et al., 2013; Kecklund & Axelsson, 2016; Schilperoort et al., 2020).

2.3 Does shift work negatively affect cardio-metabolic function?

The potential effect of shift work on health parameters has been a topical research area for close to half a century. Initial research refuted an increased risk of mortality (Taylor & Pocock, 1972), instead identifying reduced sleep (Kogi, 1982), mental fatigue (Colquhoun, 1970) and increased risk of injury as the primary detrimental effects. While the exact economic burden of shift work on health is difficult to determine, the combination of inadequate sleep and increased risk of injury alone has an estimated economic cost of \$66 billion annually (Deloitte Access Economics, 2019). Potentially a conservative

estimate with shift work more recently linked to alterations in metabolic, endocrine and immune responses, collectively increasing the risk of developing cardio-metabolic disorders and health burden (Faraut et al., 2012; Kecklund & Axelsson, 2016; Kervezee et al., 2018). Cardio-metabolic disorders refer to a group of highly preventable and interrelated set of conditions including cardiovascular disease (CVD) and the metabolic syndrome (MetS) (Grandner, 2014). CVD refers to a collection of disorders affecting the heart and blood vessels, with shift work specifically associated with ischemic stroke, myocardial infarction and CVD mortality (Schilperoort et al., 2020). The disorders account for the highest rate of mortality in the developed world (World Health Organization, 2014), and appear to manifest more commonly among shift workers, with a 1.4 and 1.5 fold increase in the relative risk for CVD and MetS, respectively (Anothaisintawee, Reutrakul, Van Cauter, & Thakkinstian, 2016; Bøggild & Knutsson, 1999; Leproult, Holmbäck, & Van Cauter, 2014). However, the pathological association has been criticised as inconsistent, and the aetiological role of shift work in pathogenesis is disputed (Kecklund & Axelsson, 2016; Puttonen et al., 2010). For example, the relative risk of CVD can range from no association (Frost et al., 2009; Hublin et al., 2010; McNamee et al., 1996; Steenland & Fine, 1996; Taylor & Pocock, 1972), slight association with (Wang, Ruan, Chen, Peng, & Li, 2018), and without a dose response (Yadegarfar & McNamee, 2008), to a risk ratio of 1.4 compared to non-shift counterparts (Bøggild & Knutsson, 1999).

Multiple factors may contribute to the inconsistent pathogenic observations among shift workers including methodological issues within research projects. As mentioned, shift work lacks a definitive structural system, with variations in rostering tailored to meet the individual needs of specific industries. Variations in shift characteristics, including shift length, speed and direction of transition, may affect cardio-metabolic function independently (Bøggild & Jeppesen, 2001; Orth-Gomer, 1983; Viitasalo, Kuosma, Laitinen, & Härmä, 2008) and should be reported during research projects. Viitasalo et al., (2008) compared different shift structures for effect on sleep and cardiovascular function under controlled conditions (Viitasalo et al., 2008). A counter-clockwise shift schedule of three evening (E),

three morning (M) and three-night (N) shifts, all separated by two days off, was compared to a fast-forward rotation (one M, E, then N followed by two days off) and a 'flexible' arrangement allowing employees a degree of freedom regarding rotations despite the same total hours worked. The changes in shift rostering resulted in improved subjective sleep assessment for the rapid rotation group and significant decreases in blood pressure (BP) for the 'flexible' group compared to baseline (Viitasalo et al., 2008). Further, comparing direction of shift rotations among 100 nurses demonstrated independent effects of shift work characteristics on sleep quality (Shiffer et al., 2018). Assessed via sleep questionnaires and diary entries, clockwise (M, E, N) nurses reported significantly longer total sleep time (TST) and significantly less frequent awakenings than their counter-clockwise (E, M, N) colleagues (Shiffer et al., 2018). Despite not directly assessing cardio-metabolic function, the highlighted research projects demonstrate that varying structures of shift work may independently affect cardio-metabolic regulators including sleep and autonomic balance.

Therefore, research projects investigating the health effects of shift work must account for labour characteristics and failing to do so may impact the validity of results. Several fundamental research projects that denied an adverse health effect of shift work failed to report or consider the specific characteristics of employment. For example, a population based 22-year follow study concluded that the risk ratio (RR) of shift work and mortality due to coronary heart disease (CHD) were non-significant (RR for males 1.09 and females 1.22) (Hublin et al., 2010). However, the research was based upon questionnaires assessing shift work participation and did not delineate between the characteristics of the shift structures or specify the presence of night shift work. Further, a nested case-control study that was implemented to investigate the relationship between shift work and death from ischaemic heart disease (IHD) concluded that shift work did not increase the risk of mortality (McNamee et al., 1996). However, several methodological issues were present during data collection and subsequent analysis. Shift work was defined as anyone who had worked in shifts, on site, for more than a month.

Resultantly, participants used to assess the potential chronic health effects of shift work may not have been employed in the structure long enough to exhibit the hypothesised chronic health effects. Occupation was additionally based on questionnaires, and in the event no information was recorded, pay slips or an estimation of employment type from a company employee were used to determine shift workers status, and no information was reported regarding the structural characteristics. Finally, the occupational status of participants were only known during the time of employment (average of 10 years), which did not account for people transitioning into or out of shift work (McNamee et al., 1996). Collectively, fundamental research refuting the long-term health risks of shift work have failed to account for key methodological factors regarding employment and shift structuring. The effect of which may impact the ability to refute an adverse effect of shift work on health and support the need for supplementary research projects.

An additional consideration in investigating the pathogenic effect of shift work is the individual variability of tolerance to the labour structure and the potential ‘healthy workers bias’s this may create. Shift worker tolerance was originally defined as the ability to adapt to shift work, free from the commonly associated adverse health consequences such as fatigue (Kecklund & Axelsson, 2016; Saksvik et al., 2011). The variables that may alter an individual’s tolerance centre on genetic adaptations associated with homeostasis and circadian rhythm (Saksvik et al., 2011). Expanded upon in section 2.5.1, the circadian rhythm refers to a temporal organisation of key biological functions including sleep, hormone levels and other bodily functions within a 24 h cycle (Ohdo, Koyanagi, & Matsunaga, 2010). While this multifunctional temporal regulator is ubiquitous in all living systems (Ohdo et al., 2010), individual variability does exist. Chronotypes describe behavioural manifestation of circadian rhythms, with an individual’s propensity to sleep at a particular time is the easiest pattern to identify. Evening and morning types represent the two extremes for preferred sleep periods (Roenneberg, Wirz-Justice, & Mellow, 2003) and individuals identified as ‘evening types’ (assessed via questionnaires) appear to be better equipped to manage shift work. Observational research projects

have previously concluded that evening types report both better perception of adaptation and tolerance to shift work, better flexibility of sleeping habits, and better work performance (Saksvik et al., 2011).

Further, assessment of circadian rhythm among night shift employees support the hypothesis that variation in circadian phases influence the effect of shift work on physiology (Boudreau, Dumont, & Boivin, 2013). Police officers, categorised as ‘adapted’ or ‘non-adapted’ to shift work based on peak salivary melatonin levels, underwent a sleep assessment following seven consecutive night shifts (Boudreau et al., 2013). The ‘adapted’ participants, identified by peak salivary melatonin occurring during the allotted daytime sleep period, demonstrated longer total time and lower sympathetic dominance during daytime sleep compared to their non-adapted counterparts (Boudreau et al., 2013). Results which indicate an influence of individual chronotype on physiological responses to shift work. Conclusively, individuals may both start, and remain in shift work long term because of a higher tolerance for the work structure. As such, a valid assessment of the pathological effect of shift work may be difficult, as those with the highest exposure (longer employment in shift work) may be considered a bias or selected group with an affinity for the work structure (McNamee et al., 1996).

Co-factors for non-communicable disease states associated with shift work may also explain the observed increase in pathogenic conditions (Kervezee et al., 2018). Shift workers commonly report decreased leisure time physical activity (PA) (Atkinson et al., 2008; Peplowska et al., 2014) in comparison to non-shift colleagues. Physical activity facilitates a variety of positive cardiovascular and metabolic health effects including augmenting glucose metabolism, autonomic regulation and inflammatory status in a dose-response manner (Lavie, Ozemek, Carbone, Katzmarzyk, & Blair, 2019; Warburton et al., 2006). Conversely, sedentary behaviour and reduced PA is associated with several negative health consequences, including the development of cardio-metabolic disorders (Lavie et al.,

2019). The labour structure of shift work, including extended and rotating labour periods exacerbate barriers to exercise adherence. Limited break time between consecutive shifts, feelings of residual fatigue associated with limited or out of biological rhythm sleep, and exercise opportunities not aligned with conventional sporting or exercise facilities, combine to limit exercise adherence (Hargens, Kaleth, Edwards, & Butner, 2013; Nea et al., 2017). Further, shift workers are more likely to engage in negative lifestyle behaviours, including consuming levels of alcohol considered to be risky for health (Dorrian et al., 2017; Dorrian & Skinner, 2012) and increased tobacco smoking compared to non-shift counterparts (Bøggild & Knutsson, 1999; García-Díaz et al., 2015). Collectively, the pathological association of shift work may be attributed to lifestyle factors, rather than specific characteristics of the work structure.

Assessment of the general health status among shift workers reveal an additional co-factor for pathogenesis. Shift workers commonly report higher rates of obesity (Liu et al., 2018; O'Brien et al., 2020), an established risk factor for the development of cardio-metabolic disorders (Poirier et al., 2006). Adipocytes are considered active endocrine organs, capable of mediating aspects of metabolic function through production and secretion of inflammatory markers and metabolic hormones including leptin and adiponectin (Greenberg & Obin, 2006). Hypertrophic adipocytes undergo molecular and cellular alterations (Golbidi et al., 2012), facilitating systemic inflammation and mediate pathogenic metabolic functions (Berg & Scherer, 2005) including decreased insulin and leptin sensitivity (Greenberg & Obin, 2006). Hypertrophic adipocytes also reduce adiponectin secretion levels, hypothesised to facilitate atherosclerotic development (Golbidi et al., 2012). Collectively, given the potential pathogenic effect of adverse lifestyle behaviours and independent effect of obesity, investigating the pathological effect of shift work as a labour structure may prove difficult.

In summary, the association of shift work and adverse health conditions is an area of ongoing research with conflicting observations regarding cardio-metabolic disorders (Kecklund & Axelsson, 2016; Puttonen et al., 2010). Methodological issues including not accounting for the independent effect of shift characteristics, the inter-variability of participant adaptability and several pathogenic co-factors have impacted the assessment of a potential adverse health association. Additional exploration is therefore required to assess the direct role of the shift work as a labour structure in the development and progression of cardio-metabolic disorders.

2.4 Shift work and cardio-metabolic pathogenesis

As previously mentioned, cardio-metabolic disorders refer to a group of interrelated disease states including CVD and MetS (Grandner, 2014). An aetiological link to shift work is currently disputed (Frost et al., 2009; Puttonen et al., 2010) with one meta-analysis reporting RR ranging from 0.6- 1.4 for shift work and CVD mortality compared to non-shift employees (Frost et al., 2009). Frost et al., (2009) concluded that most research showed either a weak, or no association between shift work and fatal events (Frost et al., 2009). However, it is plausible that shift work disrupts immune, autonomic and cardiovascular function, facilitating increased CVD and MetS risk, but employees leave the work structure before it manifests as a fatal event. Shift workers have been associated with independent risk factors for CVD including increased body mass index (BMI) (Buchvold, Pallesen, Waage, & Bjorvatn, 2018), impaired glucose metabolism (Suwazono et al., 2009), and increased levels of c-reactive protein (CRP) (Puttonen, Viitasalo, & Härmä, 2011), triglycerides and both total and low-density lipoprotein cholesterol levels (Asare-Anane, Abdul-Latif, Ofori, Abdul-Rahman, & Amanquah, 2015). Further, a meta-analysis including 34 studies with a pooled population of 2,011,935 participants concluded that despite no association identified between shift work and mortality, the work structure is associated with vascular events, specifically ischemic stroke and myocardial infarction (Vyas et al., 2012).

Regarding metabolic function, MetS is the clustering of interrelated metabolic disturbances including obesity, insulin resistance, hypertension and dyslipidaemia (Tasali & Ip, 2008). Shift workers again appear to be at an increased risk, with longitudinal and cross-sectional research associating shift work with both the development of MetS and the separate metabolic abnormalities (De Bacquer et al., 2009; Sookoian et al., 2007). An examination of an international biobank to assess the effect of past and current night shift work on risk of developing type II diabetes mellitus (T2DM) demonstrated the proposed pathogenic effect (Vetter et al., 2018). Conducted in the United Kingdom with over 272000 current and 70000 former night shift workers, multivariable-adjusted analysis concluded that night and rotating shifts are associated with increased risk T2DM risk, which increases with additional shifts per month (Vetter et al., 2018). Assessment of over 7000 male day and night shift workers over a 14 year period concluded that alternating shift patterns is an independent risk factor for both body mass gain (Suwazono et al., 2008) and impaired glucose metabolism (Suwazono et al., 2009). Additionally, when 738 nurses, free from any component of MetS at baseline were annually evaluated over a four year period, the development of MetS was observed to be significantly higher among rotational shift workers in comparison to non-shift colleagues (Pietrojusti et al., 2010). Further, a population-based prospective study of 1529 employees concluded that shift work was associated with an increased RR of 1.77 for developing MetS. Additionally, not only was the risk only marginally effected by multivariate adjustments for lifestyle or work-related cofounders, but a potential dose-response relationship was identified with MetS gradually increasing with accumulated years of shift work (De Bacquer et al., 2009). Cumulatively, shift workers appear to be at increased risk in terms of developing both the independent components and clinical diagnosis, of MetS (Sookoian et al., 2007).

2.5 Disturbed homeostatic regulation, shift work and the link with cardio-metabolic conditions

Collectively, despite several methodological issues and co-risk factors for disease, shift work is associated with cardio-metabolic disorders. However, a problem in establishing a definitive link between shift work and pathogenesis is the incomplete understanding of causal mechanisms (Kecklund & Axelsson, 2016). Multifactorial pathways and behaviours including sleep deprivation, altered food consumption and decreased PA patterns have been proposed as mechanistic causes (Schilperoort et al., 2020). Section 2.5 of the literature review proposes that these mechanisms may be collectively attributed to homeostatic desynchronisation, with circadian misalignment, disturbed sleep and systemic immune responses proposed as the primary mechanisms (Flahr et al., 2018). Investigating the specific mechanisms will provide a better understanding of the pathogenic association and aid in developing intervention strategies to improve employee health.

2.5.1 Circadian Rhythm

To understand the mechanisms responsible for the adverse effect of shift work on cardio-metabolic health, an exploration of how the labour structure impacts physiological function and circadian regulation is required. The circadian system is a rhythmic internal cycling of biological events aimed at optimising human physiology (Antunes, Levandovski, Dantas, Caumo, & Hidalgo, 2010). The primary physiological role of the circadian system is to synchronise and coordinate organ systems, particularly in response to a dynamic environment (Logan & Sarkar, 2012; Morris et al., 2012). Like any timing system, the circadian clock is made up of three operational components; a central oscillator to generate the circadian signal, an input pathway to adjust the timing mechanism, and an output pathway to entrain the peripheral system (Ohdo et al., 2010).

The central oscillator, often referred to as the master clock, is identified as the suprachiasmatic nucleus (SCN) of the anterior hypothalamus. The SCN provides a hierarchical structure of organisation, and synchronises numerous clocks of peripheral cell types throughout the body to provide temporal regulation of physiological function (Levi & Schibler, 2007; Sack et al., 2007). The molecular clockwork within the SCN comprises a transcriptional/translational feedback loop, whereby genes and their protein products interact and feedback to inhibit their own transcription and generate an approximately 24 h cycle (Foster, 2020). The canonical clock genes period (*Per*) and cryptochrome (*Cry*) make up the self-sustaining circadian oscillator, switched on by the clock proteins (CLOCK) and brain and muscle Arnt-like protein-1 (BMAL1), and periodically switched off by a complex of their own encoded proteins; PER and CRY (Najjar & Zeitzer, 2017).

The input pathways, highlighted in *Figure 2.1* are collectively referred to as zeitgebers or ‘timekeepers’, and include exo- and endogenous signals that entrain the SCN. The light-dark cycle is the primary zeitgeber, providing direct adjustments via neural projections (Atkinson, Edwards, Reilly, & Waterhouse, 2007). The retinohypothalamic tract (RHT) projects from a distinct subset of retinal ganglion cells in the retina to the SCN, where its terminals contain glutamate, chemically coded to signal ‘light’, as well as pituitary adenylate cyclase-activating polypeptide, chemically coded to signal ‘darkness’ (Najjar & Zeitzer, 2017). While considered relatively minor compared to the light-dark cycle, the SCN and subsequent circadian rhythms are additionally entrained by non-photoc cues including time of feeding, PA and sleep (Hower, Harper, & Buford, 2018; Yamanaka et al., 2006).

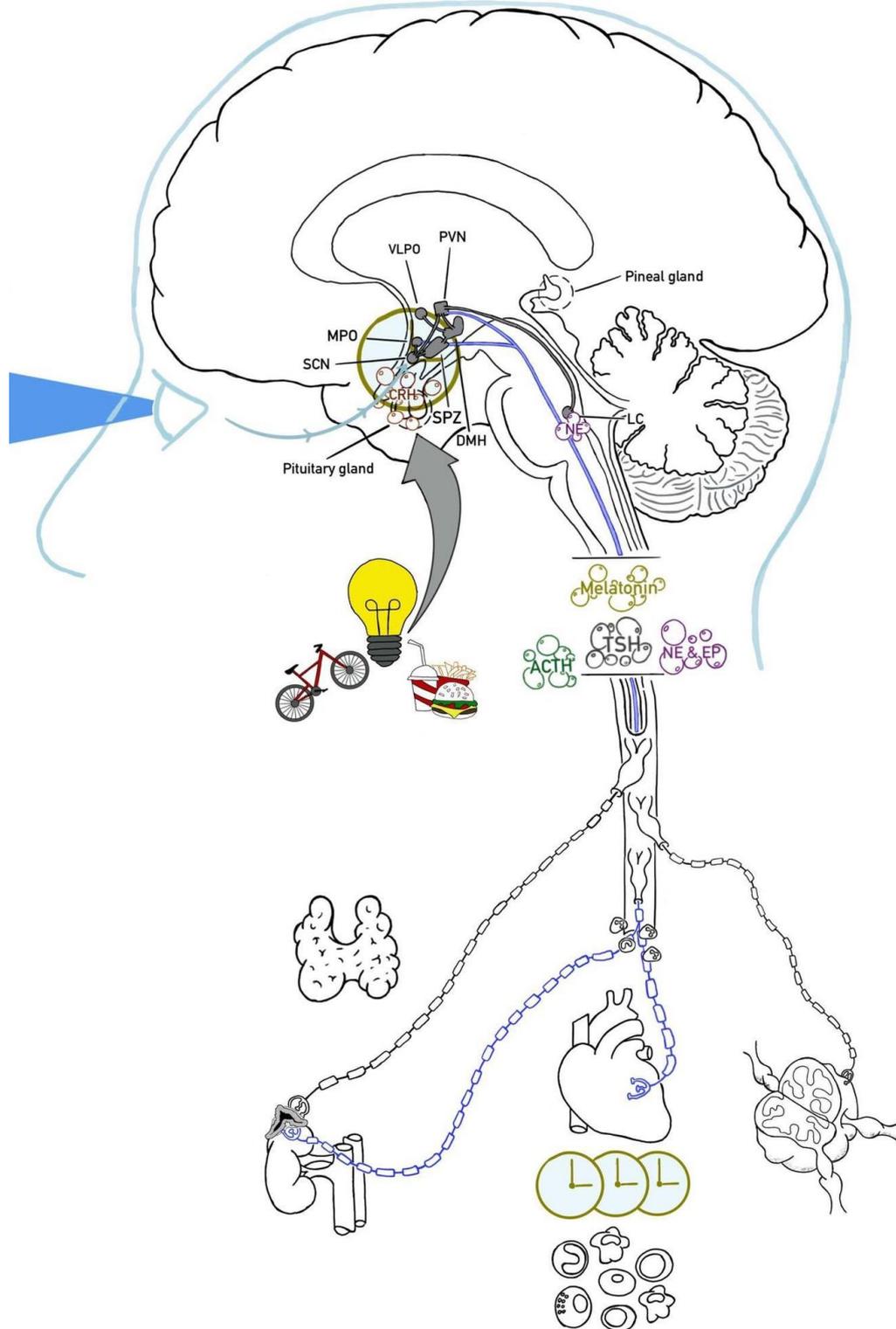
A criticism of PA as a zeitgeber is the difficulty in controlling for light exposure due to exercise’s impact on pupil size and being commonly conducted in well-lit areas (Mistlberger & Skene, 2005). However, acute bouts of PA have previously been demonstrated to produce phase-delays in melatonin (Yamanaka et al., 2006) and the overall shape of exercise-induced phase-response curves (PRC) differ

from photic PRC's (Mistlberger & Skene, 2005). Further, Barger et al., (2004) demonstrated the independent effect of exercise on circadian phase shifts in a 15-day randomized clinical trial within dim light (<5 lux) settings. Eighteen young, fit males had their circadian phase measured via intravenous plasma melatonin levels following a self-selected sleep-wake schedule, and after completing a randomly assigned seven day 9 h delay in sleep-wake schedule protocol with or without exercise (Barger, Wright Jr, Hughes, & Czeisler, 2004). The exercise intervention (3 x 45 min bouts of MICT) showed a significantly greater shift in melatonin onset and offset compared to the non-exercising controls (Barger et al., 2004). Mealtime (feed-fasting cycle) similarly provides entraining signals to circadian rhythmicity. When nocturnal rodents are fed exclusively during the day for a week or longer, circadian phase gene expression of peripheral organs inverts (Levi & Schibler, 2007). A process that uncouples the peripheral rhythms from SCN function and reverts to normal (nocturnal in this case) upon cessation of inverted feeding (Levi & Schibler, 2007). An effect, while not as drastic, that is observed among human participants under laboratory conditions. Wehrens et al., (2017) compared the effect of fixed meal times to delayed feeding opportunity (5.5 hours late) on circadian rhythms (Wehrens et al., 2017). The results indicated a delay in meal time significantly affected plasma glucose rhythms and average glucose concentration (Wehrens et al., 2017). Finally, sleep (expanded upon in section 2.5.3) provides circadian entrainment via shared anatomical structures as well as manipulating the light-dark cycle (closed eyes), physical rest and fasting behaviour (Lange et al., 2010).

The output pathways that entrain the peripheral systems include secretion of hormones as well as neural projections of the parasympathetic (PNS) and sympathetic nervous system (SNS) (Buijs et al., 2006). An overview is provided in *Figure 2.1*, and includes direct and polysynaptic projections from the SCN to numerous brain regions including the paraventricular nucleus (PVN), medial preoptic area (MPO), sub-paraventricular zone (SPZ), dorsomedial nucleus of the hypothalamus (DMH), as well as key sleep mediating areas the ventrolateral preoptic nucleus (VLPO) and locus coeruleus (LC) (Foster,

2020; Fuller et al., 2006; Laposky, Bass, Kohsaka, & Turek, 2008). Further, the SCN exerts regulatory control over the pituitary gland via the PVN and subsequent hormone secretion including corticotrophin releasing hormone (CRH) and thyrotropin-releasing hormone (TRH), to moderate activity of the adrenal and thyroid glands via adrenocorticotrophic hormone (ACTH) and thyroid stimulating hormone (TSH) respectively (Laposky et al., 2008; Miyake, 2012). The adrenal glands are further innervated by neurons connected to the SCN including preganglionic neurons from the spinal cord via the splanchnic and vagus nerves (Levi & Schibler, 2007; Sack et al., 2007). Finally SCN communicates peripherally via diffusible chemical signalling including norepinephrine (NE) and epinephrine production (Levi & Schibler, 2007; Sack et al., 2007).

Figure 2.1 *Circadian system: anatomical structures and entraining mechanisms*



Key anatomical structures of the circadian system include the suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), sub-paraventricular zone (SPZ), dorsomedial nucleus of the hypothalamus (DMH), ventrolateral preoptic nucleus (VLPO), medial preoptic (MPO) and locus coeruleus (LC). Light, physical activity and feeding behaviour provide temporal regulation to the SCN. The output systems includes corticotrophin releasing hormone (CRH), thyrotropin-releasing hormone (TRH), adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), melatonin, neurotransmitters norepinephrine (NE) and epinephrine (EP), and direct neural projections to entrain the peripheral system. Image created by the author.

2.5.2 Circadian Disruption

Through integration of exo- and endogenous signalling, the circadian rhythm provides predictive metabolic and cardiovascular processes, rather than reactive regulation (Atkinson et al., 2007; Levi & Schibler, 2007; Luyster, Strollo, Zee, & Walsh, 2012). Consequently, a fundamental characteristic of the circadian rhythm is the ability to be entrained by external stimuli (Halberg, 1959; R ger & Scheer, 2009). However the temporal integration is also susceptible to desynchronisation via conflicting cues (Kalsbeek et al., 2006; Sack et al., 2007; Schilperoort et al., 2020), a state associated with increased disease risk (Haus & Smolensky, 2006; Leproult et al., 2014). As such, light exposure, PA and feeding behaviour during night shifts, or darkness, physical inactivity and fasting during daytime recovery sleep, provide conflicting cues, and may facilitate biological desynchronisation and pathogenesis (Haus & Smolensky, 2006; Leproult et al., 2014).

The specific mechanisms linking homeostatic desynchronisation and cardio-metabolic disorders may include autonomic dysfunction. The SCN contains compartmentalisation of autonomic motor neurons, which provide the neuroanatomical basis for selective changes in autonomic balance (Kalsbeek et al., 2006). Consequently, reduced activity, or misalignment of the endogenous biological rhythm with the exogenous environment, may result in an unbalanced output towards sympathetic dominance (Kalsbeek et al., 2006). Unbalanced autonomic nervous system (ANS), specifically increased sympathetic drive, is associated with the genesis of cardio-metabolic disorders including obesity, T2DM and hypertension (Kalsbeek et al., 2006). Morris, et al., (2016), recruited fourteen healthy adults to participate in two separate eight day, cross-over design protocols to assess the effect of circadian inversion on cardiovascular function (Morris, Purvis, Hu, & Scheer, 2016). Following the initial eight-day control protocol to establish baseline function, participants underwent circadian misalignment via inverting behavioural and environmental cycles by 12 h (observed in shift work). The results indicated that circadian misalignment significantly increased sleep time BP and reduced cardiac vagal

modulation, assessed via heart rate variability (HRV) (Morris et al., 2016). Despite the limitations of recruiting a small sample size of healthy participants with little or no shift work experience, the results indicate that circadian misalignment is capable of acutely disrupting autonomic function.

Circadian misalignment is associated with adverse metabolic effects including reduced insulin sensitivity. Firstly, glucose metabolism and whole-body insulin sensitivity is mediated by diurnal rhythms (Stenvers, Scheer, Schrauwen, la Fleur, & Kalsbeek, 2019). The CNS influences insulin sensitivity and glucose homeostasis via hypothalamic brain regions including the SCN, preoptic and lateral hypothalamic areas (Ruud, Steculorum, & Brüning, 2017). Glucose concentrations peak during the day, independent of feeding conditions, while glucose transporters, glucagon receptors and gluconeogenesis demonstrate circadian rhythms (Oosterman, Wopereis, & Kalsbeek, 2020). Shift structures may therefore contribute to metabolic disorders via providing feeding opportunity out of sync with metabolic sensitivity. In support, higher postprandial glucose peaks, accompanied by reduced first-phase insulin response were observed during a simulated night shift compared to simulated day shift (Sharma et al., 2017). Twelve, otherwise healthy volunteers currently employed in rotational shift work underwent isotope-labelled mixed meal test during a simulated day and night shift. The results indicated beta cell function is impaired during night shift, which may be attributed to normal circadian variation (Sharma et al., 2017).

Circadian misalignment is additionally associated with a pro-inflammatory responses demonstrated by Morris et al., (2016) when reporting an increase in interleukin (IL)-6, CRP and tumor necrosis factor (TNF)- α among currently healthy volunteers following a misalignment protocol under laboratory conditions (Morris et al., 2016). The same research group further demonstrated the detrimental effect of circadian misalignment on CRP levels among current shift work employees in a randomised, cross-over design protocol (Morris, Purvis, Mistretta, Hu, & Scheer, 2017). Nine, healthy, non-smoking

participants, currently employed in shift work for ≥ 12 months undertook two 3-day laboratory protocols that simulated shift work via 12 h inversions of behavioural and environmental cycles. The research group concluded that circadian misalignment significantly increased 24 h CRP levels among current shift workers (Morris et al., 2017). Further, Bescos et al., (2018) examined the impact of disturbing circadian rhythmicity on metabolic health by evaluating the effect of four consecutive simulated night shifts on insulin sensitivity. The randomised-controlled trial involved the allocation of 17 participants to normal circadian aligned sleep opportunity or simulated night shift (sleep opportunity 0800 – 1700). Glucose tolerance and insulin sensitivity were assessed via glucose tolerance test, hyperinsulinaemic euglycaemic clamp and muscle biopsies, with results identifying a significant reduction in glucose metabolism and peripheral insulin sensitivity (Bescos et al., 2018). Conclusively, the effect of circadian misalignment, albeit in simulated laboratory conditions, may be propagated across multiple systems, adversely effecting autonomic regulation, inflammatory status, and insulin sensitivity,

A key consideration, expanded upon in section 2.5.3, is the independent role of sleep as both a cyclic behaviour and an entraining influence of circadian rhythmicity capable of modulating cardio-metabolic function (Foster & Wulff, 2005; Lavie, 2001; Medic, Wille, & Hemels, 2017). Bescos and colleagues (2018) demonstrated a significant reduction in insulin sensitivity following four days of circadian misalignment (simulated shift work). However, the authors additionally reported reduced TST among the night shift group (1.6 hours less than the day shift group) (Bescos et al., 2018). Given the established detrimental effect of sleep restriction on insulin sensitivity (Donga et al., 2010), differentiating between the effect of disturbed circadian rhythm and poor sleep is crucial in investigating the mechanistic role of homeostatic desynchronisation on cardio-metabolic function. Leproult, et al., (2014) demonstrated such effects when investigating simulated shift work within laboratory conditions. A parallel group design comprising of two 11-day interventions compared

normal sleep, restricted sleep opportunity and circadian misaligned sleep opportunity. The research group concluded that circadian misalignment significantly decreased insulin sensitivity and increased CRP levels, independent of sleep duration (Leproult et al., 2014). The results indicate an independent role of circadian rhythmicity on metabolic function and highlight the adverse effects of shift work induced disruptions.

Collectively, the integration of exo- and endogenous feedback provides dynamic and predictive homeostatic regulation vital for health (Sack et al., 2007). Alternatively, circadian desynchronisation results in acute adverse effects on autonomic regulation (Morris et al., 2016) and metabolism (Gonissen et al., 2013; Leproult et al., 2014). Results supporting the hypothesis that circadian misalignment may induce pathogenic cardio-metabolic function. Further, as shift work is characterised by systemic inversions of the circadian regulatory system, circadian desynchronisation is an intuitive pathological mechanism and warrants further research.

2.5.3 Sleep and the cardio-metabolic system

Despite taking up approximately a third of our lives, the exact physiological role of sleep, has for some time, remained inconclusive. Extensive research has revealed that sleep is a complex biological behaviour comprised of cyclic and active physiological processes (Carskadon & Dement, 2005; Luyster et al., 2012), which independently regulate hormonal, metabolic and autonomic function (Foster & Wulff, 2005; Medic et al., 2017). Consequently, section 2.5.3 of the literature review will explore the fundamental role sleep plays in partitioning metabolic resources, entraining the circadian rhythm (Lange et al., 2010) and providing a designated time for growth and repair (Carskadon & Dement, 2005). An overview of the sleep-specific changes in physiological function, the anatomical structures

that regulate sleep and the adverse effects of disrupting or manipulating sleep that may contribute to shift work related health disorders will also be provided.

The extensive area of sleep research initially focused on the role of the central nervous system (CNS) by tabulating several sleep-specific electroencephalogram (EEG) waveforms and correlating them with physiological changes (Foster & Wulff, 2005; Uchida et al., 2012). Sleep was resultantly categorised into two distinct phases: rapid eye movement (REM) and non-REM (NREM) sleep. REM sleep occupies 20-25% of TST, and is characterised by low voltage, mixed brain wave frequency with variable autonomic activity reflected in variable HR and BP, irregular respiration and rapid rolling eye movements (Schmidt, 2014). NREM sleep comprises the remaining 75 – 80 % of TST, is a state of vagal dominance and is further subdivided into three stages of varying consciousness and physiology (Schmidt, 2014). Stage 1 is defined as a transitional period from wakefulness to sleep and is characterised by reduced HR and respiration. Stage 2 is characterised by sleep onset and further vagal dominance reducing HR, respiration and body temperature (Carskadon & Dement, 2005; Schmidt, 2014); while Stage 3 is referred to as slow wave sleep due to the definitive EEG waveforms observed during this stage, and is characterised by vagal dominance, with HR, respiration and temperature reaching their lowest point. Stage 3 is further characterised by a suppression of stress hormones and peak expression of anabolic hormones including prolactin and growth hormone (GH) (Carskadon & Dement, 2005; Schmidt, 2014). Collectively, REM sleep is associated with an active mind hypothesised to play a role in memory consolidation, while NREM facilitates physical growth and repair (Carskadon & Dement, 2005).

Several anatomical structures have been identified to influence sleep, with the interaction described as mutually antagonistic as inhibitory elements interact in a self-mediating loop (Saper, Scammell, & Lu, 2005). Collectively, referred to as the flip-flop switch (Saper et al., 2005) (*Figure 2.2B*), the wake-

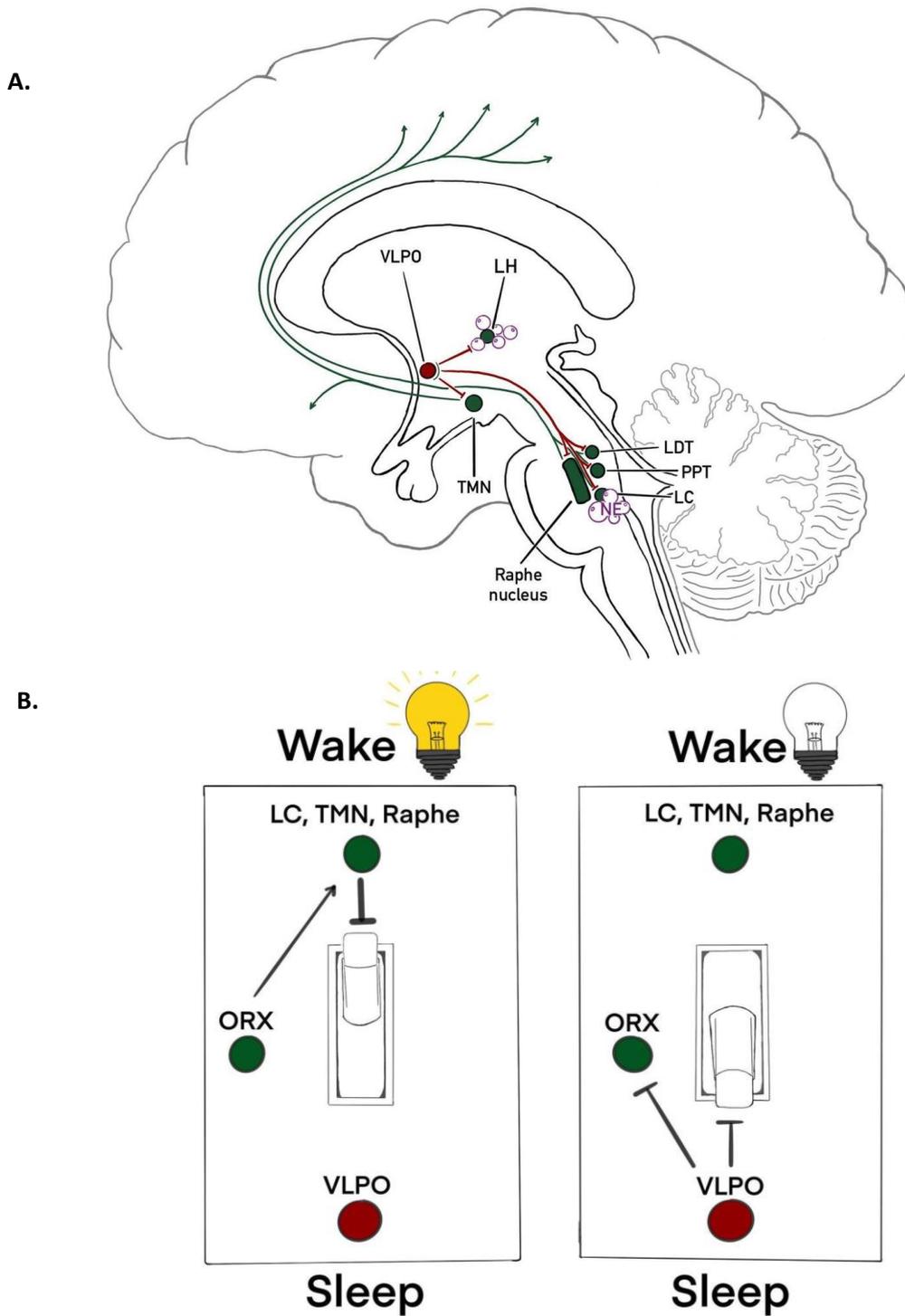
promoting brain structures include cholinergic neurons in the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT), noradrenergic LC, serotonergic dorsal raphe nuclei and histaminergic neurons in the tuberomammillary nucleus (TMN). Axons from these cell groups predominately target the lateral hypothalamus (LH), basal forebrain and cerebral cortex. The LH itself, independently mediates arousal, producing orexin and projects axons to both the TMN and LC (Saper, Chou, & Scammell, 2001; Saper, Fuller, Pedersen, Lu, & Scammell, 2010). Orexin (ORX), a peptide neurotransmitter secreted by neurons in the LH innervate the components of the ascending arousal system and may help maintain wakefulness by increasing activity. The LH additionally has projections to the sleep mediating brain structures and is therefore theorised to stabilize the transitions from wakefulness to sleep (Saper et al., 2001).

The sleep promoting centres are less well defined, with neurons in the preoptic area and basal forebrain contain sleep-active neurons, however the ability of these cells to induce sleep, as opposed to just being active during sleep is debated (Saper et al., 2001; Saper et al., 2010). Conversely, neurons in the VLPO contain inhibitory neurotransmitters gamma-aminobutyric acid (GABA) and galanin, with projections to components of the ascending arousal system including the LC, raphe, LH and TMN. Further, VLPO lesions in both animal studies and pathogenic human conditions including *encephalitis lethargica* result in substantial sleep loss (Saper et al., 2010). Collectively, while it is likely other sleep-promoting neurons participate, VLPO neurons play an important part in the induction and maintenance of sleep (Saper et al., 2001; Saper et al., 2010).

Regulation of sleep therefore involves the integration of multiple neuroendocrine regulatory systems to balance sleep propensity and arousal (Saper et al., 2005). The interaction is effectively conceptualised as a 2 process-oppositional model where by the regulation of sleep depends on propensity and circadian mediated arousal states (Borbély, Daan, Wirz-Justice, & Deboer, 2016). Sleep

propensity increasing during prolonged wakefulness as sleep debt accumulates, alternatively sufficient sleep decreases propensity and leaves the system more susceptible to the influence of arousal centres (Borbély et al., 2016). As outlined in the previous section, the SCN mediate arousal centres in the brain via polysynaptic pathways, predominately through the SPZ and DMH. Conveying circadian information through this polysynaptic network confers less direct control, however allows for both amplification of signalling and a degree of flexibility for altering the timing of sleep and wakefulness (Saper et al., 2010). An overview of the anatomical structures involved in the regulation of sleep and interaction of sleep transition is provided in *Figure 2.2A*.

Figure 2.2 Anatomical structures and interactions governing sleep



A. Key anatomical structures associated with sleep. Wake promoting structures, shown in green, include the pedunculo pontine (PPT) and laterodorsal tegmental nuclei (LDT), locus coeruleus (LC), dorsal raphe nuclei, tuberomammillary nucleus (TMN) and lateral hypothalamus (LH). The main sleep promoting structure is the ventrolateral preoptic nucleus (VLPO) (maroon). **B.** The flip-flop switch demonstrating the interaction between wakefulness and sleep centres in the brain including the role of orexin (ORX) hypothesised to influence both sides of the switch and potentially mediate a smooth transition between the two states.

2.5.4 Poor sleep and health effects

Much of what we know regarding the importance of sleep for health and well-being has been deduced from the pathological effects of poor sleep. Linked to the role in homeostatic modulation (Medic et al., 2017), poor sleep facilitates cardio-metabolic dysfunction (Kecklund & Axelsson, 2016) and increases the relative risk of mortality (Cappuccio, D'Elia, Strazzullo, & Miller, 2010). However, sleep quality can be characterised in terms of duration, continuity, timing and regularity (Medic et al., 2017) with differing effects on health and well-being. As such the literature review will focus on two broad manifestations of poor sleep in the context of shift work, including 1) failure to obtain the necessary amount of sleep (deprivation), and 2) the inability to maintain sleep (fragmentation) including events or misaligned sleep opportunity that disrupt continuity (Medic et al., 2017).

Sleep deprivation is the inability to obtain sufficient sleep and may include either a prolonged time without sleep, or a reduction in sleep opportunity (Knutson, Spiegel, Penev, & Van Cauter, 2007). Fundamental research exploring the detrimental effect of disturbed sleep initially focused on prolonged bouts of sleep deprivation. Frey et al., (2007) demonstrated that 40 h of continual wakefulness significantly increased pro-inflammatory markers including E-selectin, intracellular adhesion molecule-1 (ICAM-1) and interleukin (IL)-1 β (Frey, Fleshner, & Wright Jr, 2007). While studies like Frey et al., (2007), demonstrate the significant effect of sleep deprivation, these prolonged wakefulness protocols may not be reflective of realistic sleep conditions, and potential overstate the pathological effect. More recently, Tobaldini et al., (2013) examined the effect of acute sleep deprivation within more realistic situations by assessing autonomic function and inflammatory profile before and after one night shift (Tobaldini et al., 2013). In total participants remained awake for 26 h (previous day + night shift) which resulted in parasympathetic withdrawal and immune system modulation, increasing interferon (IFN)- γ levels (Tobaldini et al., 2013). The protocol replicated the

sleep deprivation experienced among shift workers and demonstrated the acute adverse effect of such conditions on markers of cardio-metabolic health.

Sleep deprivation is further reported to be comparable to physical inactivity in terms of the relative contribution to developing cardio-metabolic conditions including T2DM (Saner et al., 2018). Partial sleep deprivation, assessed in a randomly assigned, cross-over design study with nine healthy subjects, resulted in reduced insulin sensitivity (Donga et al., 2010). Specifically, one night of 4 h total sleep opportunity (0100 – 0500 h) resulted in both hepatic and peripheral insulin resistance compared to a 'normal' sleep opportunity (2300 – 0730) (Donga et al., 2010). Results which were replicated with consecutive nights of restricted sleep within laboratory conditions. Following 2 nights of baseline sleep (10 h of sleep opportunity) 14 healthy male volunteers had total sleep restricted to 4 h for 5 consecutive nights. The sleep restriction impaired glucose metabolism and increased both cortisol and leptin, consistent with the development of insulin resistance (Reynolds et al., 2012). The potential mechanisms that may contribute to reduced insulin sensitivity following sleep deprivation include modification of regulatory hormone secretion, misalignment of the biological clock and disruptions to cellular signalling and subsequently reduced mitochondrial respiration (Saner et al., 2018). Collectively, laboratory interventions have demonstrated the potential effect of both prolonged wakefulness and restricted sleep opportunity on metabolic pathways among apparently healthy subjects, conditions shift workers are systematically exposed to.

Fragmentation of sleep (disturbed sleep continuity) has also demonstrated an adverse effect on cardio-metabolic function (Stamatakis & Punjabi, 2010) and may independently impact shift workers' cardio-metabolic profile. Stamatakis & Punjabi (2010), assessed the effect of sleep fragmentation on glucose metabolism among 11 healthy volunteers. Following a night of uninterrupted sleep (acted as a baseline), two consecutive nights of fragmented sleep, achieved via noise and mechanical stimuli

across all sleep stages, significantly decreased insulin sensitivity and increased SNS modulation (Stamatakis & Punjabi, 2010). The results are hypothesised to be associated with increased arousal and adrenocortical activity (Stamatakis & Punjabi, 2010). The arousal from sleep elevates cortisol, which even within the normal physiological range, can decrease insulin sensitivity (Stamatakis & Punjabi, 2010). Further, disturbing the stages of sleep associated with parasympathetic dominance (NREM), would alter autonomic modulation and result in increased sympathetic drive (Kalsbeek et al., 2006). Finally, discussed in section 2.5.2, circadian disruption has an independent influence on autonomic output (Kalsbeek et al., 2006), resultantly disturbed sleep may impact circadian modulation of the ANS, facilitating the genesis of cardio-metabolic disorders.

The final consideration for reduced sleep quality regarding shift workers, is the integration of sleep and circadian rhythms effecting sleep continuity. Expanded upon in section 2.5.10, sleep and circadian rhythms are interconnected homeostatic regulators, consequently, irregular, reduced or biological misaligned sleep may impact cardio-metabolic function. The main role of the circadian rhythm is to entrain biological responses, and a regular bedtime schedule can strengthen such entrainment (Kang & Chen, 2009). Alternatively, irregular sleep schedules have been hypothesised to impact sleep quality. Kang and Chen, (2009) demonstrated such effect when assessing the impact of bed-time regularity on subjectively measured sleep quality. The results indicated that irregular bedtime schedule is associated with decreased average sleep time per day (Kang & Chen, 2009). A criticism of the project is the exclusive use of subjective sleep time and quality assessment; however, the results do indicate a potential detrimental effect of irregular or rotating sleep opportunities. A hypothesis supported by Moller-Levet et al., (2013) when exploring the effect of restricted sleep (one week of six h sleep opportunity) on blood transcriptome. The authors concluded that restricted total sleep disrupted transcription of circadian regulatory genes (including PER, CRY and CLOCK molecules), disrupting circadian regulation and impacting molecular metabolism and immune response (Möller-

Levet et al., 2013). Importantly, chronic sleep restriction changed the circadian control of immune function and the inflammatory response to stress (Möller-Levet et al., 2013). Collectively, poor sleep (quality and quantity) not only independently effects cardio-metabolic function but impacts circadian rhythmicity, leading to additionally pathogenic effects.

2.5.5 Shift work specific sleep complaints

Shift workers are exposed to two labour specific characteristics that negatively impact sleep quality (Shantha, 2013). Firstly, rotational shift work provides out-of-sync sleep opportunity, resulting in a transient inability to sustain sufficient sleep continuity and regularity (Niu et al., 2011; Shaker, Samir, Zyada, El-Sharkawy, & Ekladios, 2018). Secondly, the time allocated between successive shifts impact the available time to achieve sufficient sleep (Heath, Dorrian, & Coates, 2019; Korsiak, Tranmer, Leung, Borghese, & Aronson, 2018).

Shift work disrupts and in some cases inverts the regulatory sleep-wake cycle, for example providing daytime sleep opportunity during the peak phase of circadian arousal/alertness (Niu et al., 2011). Out-of-sync sleep opportunity impacts sleep in two primary ways; maladaptive sleep architecture and increased sleep fragmentation. Within laboratory condition, 13 participants underwent polysomnography (PSG) assessment of sleep architecture in time blinded and differing light-entrained circadian phase conditions (Gonnissen et al., 2013). The results demonstrated that both advance and delay circadian misalignment, significantly changed sleep architecture including reduced REM and stage 3 NREM sleep (Gonnissen et al., 2013). Increased awakenings (sleep fragmentation) is additionally hypothesised to be associated with daytime sleep due to increased arousal and daytime environmental factors including noise and temperature (Niu et al., 2011). Sleep fragmentation

(discussed in section 2.5.4) impacts cardio-metabolic function (Stamatakis & Punjabi, 2010) and under chronic conditions may also facilitate pathogenesis.

Laboratory-based interventions have explored the effect of shift work on sleep quality, resulting in significant sleep restriction (Bescos et al., 2018; Lamond et al., 2003). Fifteen healthy subjects were assessed for subjective and objective sleep quality during one week of simulated night shift (Lamond et al., 2003). The results indicated that despite no significant difference in day-to-day TST compared to baseline, participants accumulated 3.5 h sleep debt over the week (Lamond et al., 2003). Interestingly, the study also reported a decrease in sleep latency and wake time after sleep onset, concluding that, in opposition to their initial hypothesis, sleep quality may be improved among rotational shift workers (Lamond et al., 2003). However, decreased sleep latency may be a by-product of the accumulated sleep debt, and as TST is associated with relative risk of mortality, the conclusion of improved sleep quality among shift workers based on sleep latency may be erroneous. Further, four days of simulated shift work adversely effected the subjective sleep duration of 17 healthy adults (Bescos et al., 2018). The night shift group additionally obtained 1.6 h less TST than the day shift group over the four days (Bescos et al., 2018). Sleep quality was objectively assessed by actigraphy, with no significant differences reported. However due to technical difficulties during collection, only seven participants (four completing night shift) had actigraphy recorded. Therefore some doubt is assumed in the power of the observation and questions the validity of the observed non-significant effect (Bescos et al., 2018).

Reduced TST is likewise a direct consequence of shift structuring, with approximately 60 % of shift workers complaining about insufficient sleep (Niu et al., 2011). A key determinant in the reported reduced TST is the total break time afforded between consecutive shifts for rest and recovery (Roach, Reid, & Dawson, 2003; Vedaas et al., 2016). Break time is effected by factors including commute time

to and from work, domestic chores, social obligations and time for self-care, cumulatively reducing sleep opportunity (Vedaa et al., 2016). Assessment of break time and sleep duration among Australian locomotive engineers demonstrated varying break times are associated with significant changes in TST. Break durations of 12, 16 or 24 h resulted in significantly different TST, with mean TST of 5.2, 6.5 and 8.9 h respectively (Roach et al., 2003). Resultantly, it appears a minimum of 16 h is required between consecutive shifts to achieve the recommended sleep duration of seven or more hours (Kurumatani et al., 1994; Roach et al., 2003); however, excluding days off, 12 h remains the conventional break between consecutive shifts (Paech, Jay, Lamond, Roach, & Ferguson, 2010; Roach et al., 2003).

In summary, sleep regulates cardio-metabolic function and sleep disturbances are associated with cardio-metabolic pathogenesis (Covassin & Singh, 2016; Knutson, 2010; Möller-Levet et al., 2013). The structure of shift work exposes employees to specific factors for maladaptive sleep, including inverting the sleep-wake cycle and restricting sleep opportunity. Further, simulated shift work has demonstrated the adverse effect of shift structuring on TST and quality. Cumulatively, shift workers commonly report subjective and objectively measured sleep complaints. *Table 2.1* provides an overview of shift workers sleep assessment, with shorter sleep duration (Ferguson, Shoff, Shreffler, McGowan, & Huecker, 2019; Heath et al., 2019; Korsiak, Tranmer, Leung, et al., 2018; Kwak et al., 2017) and reduced subjective (Burke et al., 2007; Lim, Hoe, Darus, & Bhoo-Pathy, 2018; Yong, Li, & Calvert, 2017) and objective (Kwak et al., 2017) sleep quality commonly reported. Of consideration, variations in shift structure characteristics may independently effect sleep quality, and subjective assessment of sleep may reflect perceived quality rather than TST. As such, objective assessments of sleep quality may be advised in any future research project that aims to investigate the cardio-metabolic health of shift workers.

Table 2.1 Subjective and objective shift work sleep assessments

Subjective Assessment			
Study	Participants and shift structure	Measure	Key Findings
Akerstedt, Ingre, Broman, & Kecklund, 2008	(<i>n</i> =3400) shift and day workers	Karolinska Sleep Questionnaire	Large scale sleep questionnaire showed very few differences in regarding sleep parameters between shift and non-shift workers.
Burke et al., 2007	(<i>n</i> =111) (D, A, N)	PSQI Sleep Apnea Screen	Night shift work is significantly and independently associated with decreased sleep duration (PSQI).
Garde, Hansen, & Hansen, 2009	(<i>n</i> =166) Nurses (D, E, N shifts)	Karolinska Sleep Questionnaire	Nurses on nightshift reported poorer sleep quality than nurses or any other shift.
Holzinger, Mayer & Klosch, 2021	(<i>n</i> =185) mixed cohort (D + N shifts) and (M, A, N)	PSQI ESS	Shift workers who rated their shift schedules as irregular reported significantly lower sleep quality.
Lecca et al., 2021	(<i>n</i> =145) (M, E, N shifts)	PSQI ESS	Poor sleep quality was reported more frequently among night-shift workers
Lim et al., 2018	Night work defined as minimum 8 hr during night-time in either fixed or rotating (3 or 2 shift rotations)	PSQI	Night shift workers reported significantly poorer sleep quality, longer sleep latency, shorter sleep duration, sleep disturbances and daytime dysfunction (PSQI).

Study	Participants and shift structure	Measure	Key Findings
Ohayon, Smolensky, & Roth, 2010	(<i>n</i> =3345) represent population of the state of New York aged ≥ 18 were interviewed via telephone	Stanford Sleep Epidemiology Research Centre Sleep-EVAL	Short-sleep duration (<6 h) was strongly associated with fixed night and rotating morning-afternoon-night shift work.
Shaker et al., 2018	(<i>n</i> =66) shift workers (M, A, N)	Karolinska Sleep Questionnaire	Subjective sleep problems (answering ‘yes’ to sleep problems and report having ‘disturbed sleep’) were significantly higher among night shift workers.
Van de Ven, Hulsegge, Zoomer, de Korte, Burdof & Hengel, 2021	(<i>n</i> =223) mixed cohort (M, D, E, N)	Sleep Questionnaire	Shift workers reported higher levels of fatigue and poorer sleep quality. Increased fatigue was also associated with <16 hours between shifts and backward (anti-clockwise) rotating shifts.
Yong et al., 2017	(<i>n</i> =6338) American workers aged ≥ 18	Independently developed sleep questionnaire	The prevalence of short sleep duration and poorer sleep quality were highest among night shift workers.
Zhang, Sun, Li, & Tao, 2016	(<i>n</i> =513) Rotational shift work nurses	PSQI	After adjusting for sleep co-factors including age and children, multivariate logistic analysis identified participants currently or formerly (last 6 months) involved in shift work had significantly poorer sleep quality (PSQI).

Study	Participants and shift structure	Measure	Key Findings
Zhou, Zhang, Sang, Shen & Lian, 2020)	(<i>n</i> =2453) mixed cohort of non-shift, 2-shift (D + N), 3-shift (M, A, N) and 4-shift (M, early A, late A, N)	PSQI	Regardless of structure, shift workers experienced a higher incidence of sleep disorders than non-shift workers.
Objective Assessment			
Ferguson et al., 2019	(<i>n</i> =27) emergency physicians	Actigraphy	Working the night shift results in significantly less sleep among emergency physicians (significantly less TST).
Feng, Booth, Baldwin-Rodriguez, Osorno & Narayanan, 2021	(<i>n</i> =113) Nurses (D + N shift)	Fitbit PSQI	Shift workers experience significantly reduced total hours of sleep on workdays and report poorer sleep quality.
Flaa, et al., 2021	(<i>n</i> =70) Emergency service helicopter pilots with varying shifts (depending on callouts)	Actigraphy	Workers experienced significantly long wake after sleep onset and reduced sleep efficiency when on call compared to non-work periods (averaged over 7-days).
Heath et al., 2019	(<i>N</i> =52) Shift working nurses 3 shift structure (M, A, N)	Sleep Logbook Actigraphy	TST significantly reduced for morning shifts compared to afternoon and night. Night shift sleep was rated the best. No differences in actigraphy rated efficiency.

Study	Participants and shift structure	Measure	Conclusion
Korsiak, Tranmer Leung, et al., 2018	(<i>n</i> =294) Female Hospital Workers Alternating day/night vs fixed day	Munich Chronotype Questionnaire Actigraphy	Alternating day-night shift work schedule impacts sleep negatively among female hospital workers. Significantly different sleep duration (less than day work) but significantly more nap time resulting in comparable 24 hr total sleep between rosters.
Korsiak, Tranmer, Day, & Aronson, 2018	(<i>n</i> =294) Female hospital employees	Actigraphy	Shift workers reported significantly reduced total sleep duration (332 ± 46 vs 403 ± 40)
Kwak et al., 2017	(<i>n</i> =48) shift vs fixed day nurses	PSQI Actigraphy	No significant differences in PSQI. Additionally, shift workers showed significantly shorter total sleep time and lower sleep efficiency compared to daytime nurses.
Marqueze, Ulhoa, & Moreno, 2014	(<i>n</i> =44) Irregular shifts (night work with flexible finish) and day shift	Actigraphy	No significant differences were observed between shift structures for sleep duration, latency or efficiency.
Onninen, Pylkkonen, Tolvanen & Sallinen, 2021	(<i>n</i> =47) irregular shifts among truck drivers	Actigraphy Karolinska Sleep Questionnaire	Long-haul truck drivers are exposed to severe levels of accumulated sleep loss while working irregular shifts.

Study	Participants and shift structure	Measure	Conclusion
Tremaine et al., 2013	(<i>n</i> =17) nursing midwives (8-10.5 h shifts) Morning (0600-0900 h) Afternoon (1100-1700 h) Night (2000-2200 h)	General health and Sleep Questionnaire Sleep Logbook Actigraphy	Sleep before afternoon shifts was subjectively measured the best quality. Mornings shifts were associated with the lowest sleep durations, lowest subjective sleep quality and highest post-sleep fatigue ratings

n; sample population, A; afternoon shift, D; day shift, E; evening shift, ESS; Epworth Sleep Scale, M; refers to morning shift, N; night shift, PSQI; Pittsburgh Sleep Quality Index.

2.5.6 The Immune System

The immune system plays a vital role in homeostatic regulation, critical for maintaining health and well-being (Del Giudice & Gangestad, 2018; Slavich & Irwin, 2014). Often considered the body's defence system due to the coordinated innate and adaptive inflammatory responses to a challenge (Moser & Leo, 2010), the immune system's fundamental role may instead be considered to maintain normal tissue function (Libby, 2007; Swirski & Nahrendorf, 2018). To achieve this, the immune system integrates several systems and behaviours, including sleep, circadian rhythms and the neuroendocrine system, to partition metabolic resources and provide both somatic maintenance and defence (Lange et al., 2010; Medzhitov, 2008). Under normal conditions, the inflammatory response is tightly regulated (Irwin & Cole, 2011; Pickup, 2004), however, stressful stimuli may result in a chronic elevation of inflammatory markers and acute phase proteins (Motivala, 2011), a state associated with impaired cardio-metabolic function and indicative of future disorders (Dandona, Aljada, & Bandyopadhyay, 2004; Frey et al., 2007; Kaspis & Thompson, 2005; Mathur & Pedersen, 2008; Medzhitov, 2008; Motivala, 2011). This section of the literature review will provide an overview of the immune system and regulatory mechanisms to justify the potential role of shift work in systemic inflammatory responses and cardio-metabolic pathogenesis.

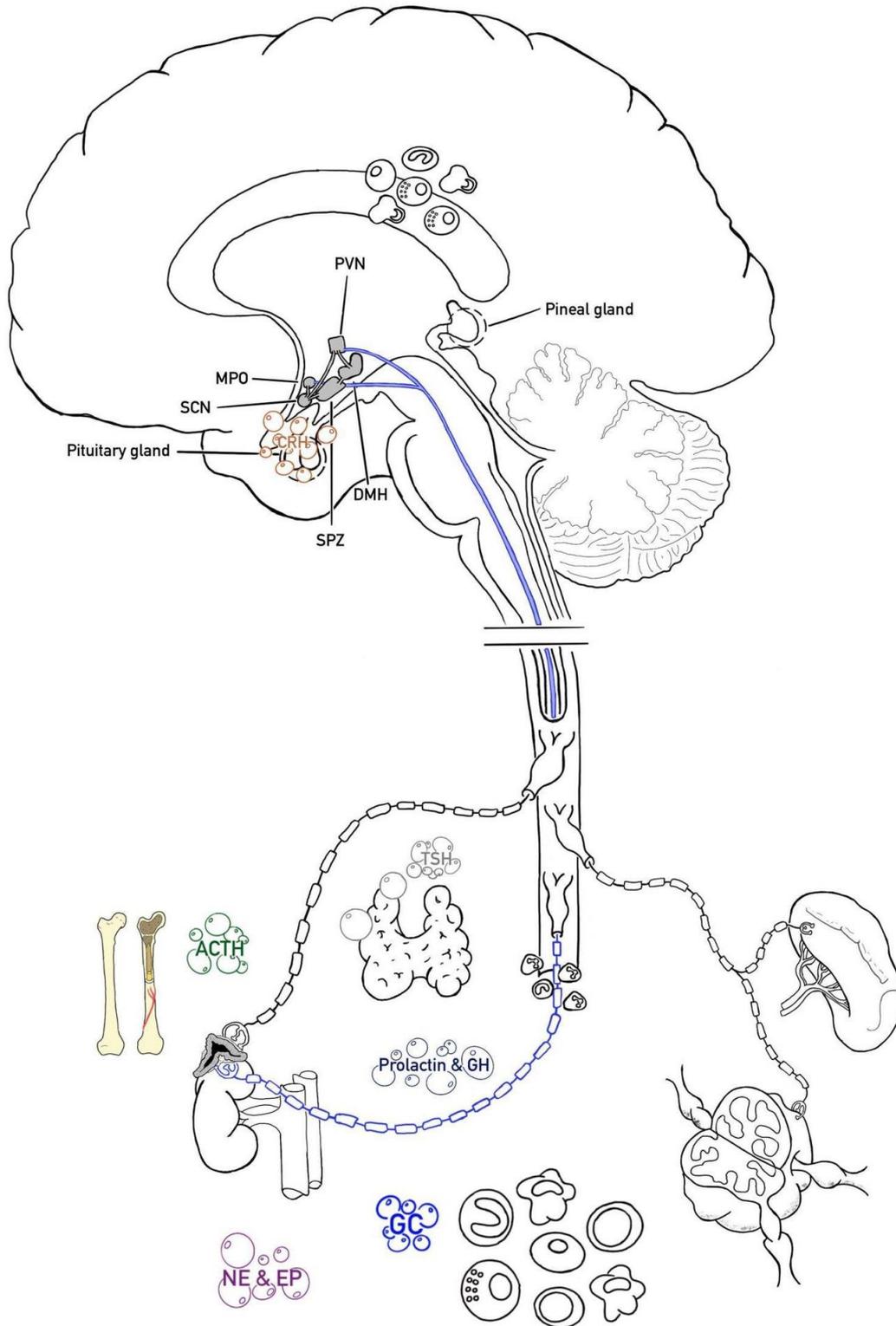
2.5.7 Immune System: anatomical structures and regulatory mechanisms

To provide context for the potential role of shift work in pathogenic inflammatory states, an overview of the immune system, functioning and maladaptive immune responses will be provided. The organs of the immune system, depicted in *Figure 2.3*, are divided into primary lymphoid organs including bone marrow and the thymus, secondary lymphoid organs such as the lymph nodes and spleen, and lymphatic vessels that connect the system (Moser & Leo, 2010). Functionally, the primary lymphoid organs facilitate the proliferation and maturation of lymphocytes, secondary lymphoid organs are

responsible for the initiation of adaptive immune responses and lymphatic vessels provide integration of the immune structures with the cardiovascular system (Moser & Leo, 2010). The effector molecules are differentiated hematopoietic stem cells, and may be viewed as the signalling proteins of the immune system, acting as autocrine, paracrine or endocrine messengers to coordinate inflammatory responses (Del Giudice & Gangestad, 2018). Hematopoietic stem cells mature into lymphoid and myeloid cells, which further differentiate into T and B-lymphocytes, neutrophils, monocytes, macrophages, cytokines and acute phase proteins (Chaplin, 2010; Parkin & Cohen, 2001).

The defensive purpose of the immune system is to detect and remove the source of disturbance, while ultimately, restoring functionality and homeostasis (Medzhitov, 2008). In a functional acute inflammatory reaction, microbial invasion, tissue damage or exposure to foreign particles are detected by molecular patterns and trigger an inflammatory response (Del Giudice & Gangestad, 2018). The inflammatory response involves selective and sequential migration of blood cells into tissue, followed by local activation and interaction with resident tissue cells (Libby, 2007). Anatomical structure that coordinate the immune response are shown in *Figure 2.3*, and include a number of immune signalling proteins, such as the cytokines IL-6 and TNF- α (Del Giudice & Gangestad, 2018) and the neuroendocrine system (Kopp & Medzhitov, 2009), particularly the hypothalamic-pituitary-adrenal (HPA) axis and the LC-norepinephrine (LC-NE) system (Pickup, 2004). Cytokines released by macrophages at the site of inflammation act on the brain through cytokine-induced stimulation of the vagus nerve, circumventricular organs and active transport of cytokines from the periphery into the CNS (Imeri & Opp, 2009). CRH is produced by the hypothalamus, stimulating ACTH from the pituitary gland and resulting in cortisol secretion from the adrenal cortex. The summation is an anti-inflammatory negative feedback loop, suppressing cytokine release and the inflammatory response (Pickup, 2004).

Figure 2.3 Anatomical structures and interactions governing the immune system



Key regulatory structures include the hypothalamus, primary and secondary lymph tissue such as bone marrow, thymus, lymph nodes and the adrenal cortex and humoral modulators including corticotrophin releasing hormone (CRH), thyrotropin-releasing hormone (TRH), adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), glucocorticoids (GC), norepinephrine (NE) and epinephrine (EP). The SCN and sleep likewise exert control over the inflammatory system via shared anatomical structures and hormones including prolactin and growth hormone (GH). Image created by the author.

Therefore, the immune system aims to identify disturbances, initiate and coordinate a self-regulated (negative feedback loop) response, to restore functionality (Medzhitov, 2008; Pickup, 2004). However, if the inflammatory response fails to eliminate the pathogen or restore functionality, the inflammatory process persists and acquires new characteristics (Medzhitov, 2008), which, if sustained, have adverse health outcomes due to the high metabolic requirements and non-specific nature of innate immunity (Libby, 2007). Circadian disruption and sleep disturbances are both capable of disrupting and potentially prolonging the inflammatory response (Castanon-Cervantes et al., 2010). Further, once chronically activated, the inflammatory mediators themselves are capable of facilitating cellular damage and a pro-inflammatory cascade (Libby, 2007). For example, once present and active in tissue including the arterial wall, cells of the innate immune system facilitate the development of reactive oxygen species (ROS), cytokines and pro-coagulants that amplify and sustain the inflammatory response while potentially damaging cellular structures (Libby, 2007).

As the immune system and inflammatory response is predominantly regulated through cell-to-cell communication by localised soluble signalling proteins including cytokines and chemokines (Stenzen & Poschenrieder, 2015; Wang, Wadhwa, Culhane, & Nelson, 2005), measurements of these signalling proteins provides surrogate indicators of inflammatory status and predictive clinical information regarding pathogenesis (Zhou, Fragala, McElhaney, & Kuchel, 2010). Key inflammatory biomarkers including CRP, TNF- α and the interleukin cell group, specifically IL-6, will become a focus of future sections and research projects, (Elmarakby, Abdelsayed, Liu, & Mozaffari, 2010; Petersen & Pedersen, 2006). CRP is a hepatocyte derived, acute phase protein which plays a key role in triggering the innate immunity pathway (Sproston & Ashworth, 2018). CRP upregulates the expression of adhesion molecules (ICAM-1 and vascular cell adhesion molecule (VCAM)-1), mediates chemoattractant protein induction and activates complement cells (Blake & Ridker, 2002). TNF- α is a pleiotropic cytokine produced during acute inflammation and stimulates the production of acute-phase proteins including

CRP (Laveti et al., 2013). Regarding cardio-metabolic function, TNF- α independently effects glucose and insulin sensitivity, and is hypothesised to induce insulin resistance under chronic conditions (Ruan & Lodish, 2003). The interleukins describe a group of cytokines, derived from leukocytes, with complex immunomodulatory functions including cell proliferation, maturation, migration and adhesion (Febbraio, 2014). IL-6, has a dual inflammatory role, in the presence of TNF- α , IL-6 can initiate the pro-inflammatory cascade and CRP production (Laveti et al., 2013), alternatively, when derived from muscle contraction, such as exercise, IL-6 mediates anti-inflammatory pathways and suppresses TNF- α (Petersen & Pedersen, 2006). Resultantly, systemic upregulation of pro-inflammatory TNF- α , CRP and IL-6 are associated with an increased risk of CVD (Elmarakby et al., 2010), diabetes and MetS (Elmarakby et al., 2010; Petersen & Pedersen, 2006) among the general population and shift workers (Khosro, Alireza, Omid, & Forough, 2011; Puttonen et al., 2011).

A currently expanding area of research is the direct aetiological role inflammatory markers play in the development and progression of cardio-metabolic conditions (Dandona et al., 2004). Due to the high metabolic cost of the immune response, immune cells can initiate a decline in skeletal insulin sensitivity (Medzhitov, 2008). A process that facilitates the redistribution of glucose from one of the system's major consumers (skeletal muscle) to immune cells with an increased energy demand during infection and tissue repair (Medzhitov, 2008; Schenk, Saberi, & Olefsky, 2008). The metabolic influence of immune cells was initially hypothesised by Hotamisligil et al., (1993) when demonstrating that Zucker rat adipocytes constitutively express the pro-inflammatory cytokine TNF- α and that neutralisation of TNF- α by soluble receptors resulted in decreased insulin resistance (Hotamisligil, Shargill, & Spiegelman, 1993). Feingold et al., (1989) and Grunfeld et al., (1991) additionally observed that administration of TNF- α lead to an increased serum glucose concentration, prompting suggestions that hyperglycaemia may be exacerbated by cytokine overproduction (Feingold et al., 1989; Grunfeld & Feingold, 1991). Further, Rotter et al., (2003) demonstrated the inflammatory effect

on insulin sensitivity among human participants when examining abdominal subcutaneous adipose tissue biopsies from 10 obese and 10 non-obese volunteers. The results indicated that IL-6, like TNF- α , can induce insulin resistance through multiple phosphorylation pathways (Rotter, Nagaev, & Smith, 2003). While requiring further elucidation, insulin signalling can be negatively regulated (decreased sensitivity) via phosphorylation of serine residues impeding the insulin receptors and downstream propagation of insulin signalling. Several of the serine kinases involved in this process are integral components of pro-inflammatory pathways and suggest a dual role of inflammation (Schenk et al., 2008). The immune system is therefore capable of redistributing metabolic process to achieve the somatic role, however a chronic shift in insulin sensitivity may lead to metabolic disorder including T2DM (Medzhitov, 2008).

Further supporting the direct pathogenic role of inflammatory markers is the recognition of atherosclerosis being more than a lipid storage disease, with ongoing inflammatory response mediating all stages of disease from initiation and progression to thrombotic complications (Libby, Ridker, & Maseri, 2002). Under normal conditions the endothelium cells resist prolonged contact with leukocytes, however on exposure to an activating stimulus (including pro-inflammatory cytokines), the endothelium express VCAM-1 as well as P and E-selectin. The combined effect is binding and attraction of monocytes, T lymphocytes and neutrophils, which after adhesion to the endothelial surface migrate into the artery wall (arterial intima). Monocytes are recruited to the area, differentiate into macrophages, and begin to proliferate. Over-expression of scavenger receptors engulf modified lipoprotein particles, resulting in accumulation of cholesteryl ester within the macrophages and create foam cells (Libby, 2007). Cumulatively, the immune system has a vital role in maintaining normal tissue function, however the non-specificity and metabolic cost of the inflammatory response may facilitate adverse health effects under chronic conditions.

2.5.8 Sleep and Immune System

Identifying the neuro-immunological basis of integrated immune responses have highlighted the potential role of sleep in mediating immune function. Sleep and the immune system share anatomical structures (Besedovsky, Lange, & Born, 2012) and intercellular signals including hormones, neurotransmitters and cytokines (Besedovsky et al., 2012). Additionally, the brain regions that are implicated in the regulation of sleep-wake behaviour, notably the hypothalamus, the hippocampus and the brainstem have neurons that are immune-creative and have signalling receptors for IL-1 and TNF- α (Imeri & Opp, 2009). Further, nocturnal sleep is hypothesised to mediate cell growth, differentiation and restoration of immune cells (Besedovsky et al., 2012; Bollinger, Bollinger, Oster, & Solbach, 2010). A process achieved via down-regulation of the HPA axis and SNS, reducing humoral levels of cortisol, while upregulating pro-inflammatory signals including pituitary GH, prolactin and the pineal hormone melatonin (Besedovsky et al., 2012; Bollinger et al., 2010). An effect best demonstrated by the reduction in immune cell activity and number following partial sleep deprivation (Irwin et al., 1996). Forty-six healthy male volunteers underwent baseline laboratory testing to evaluate immune variables before and after a night of sleep deprivation and subsequent recovery sleep. Sleep deprivation (average 198 ± 17 min) resulted in significant reduction in natural killer and lymphokine-activated killer cells, an effect that was not observed following a night of recovery sleep (437 ± 62 min) (Irwin et al., 1996). While the research project did not assess cortisol, GH, prolactin, or melatonin to provide potential mechanistic context for reduced immune cell activity, the project does demonstrate the role of 'normal' sleep in immune responses.

The neuro-immunological relationship appears to be bi-directional, with the immune system providing regulatory feedback and mediation of sleep. As mentioned, sleep regulatory regions in the brain have signalling receptors for IL-1 and TNF- α (Imeri & Opp, 2009), while LPS (pathogen-associated molecular pattern lipopolysaccharide) administration studies in humans have linked changes in NREM sleep to

elevated levels of circulating type 1 IFNs and pro-inflammatory cytokines (Irwin & Cole, 2011). Moreover, pharmacological administration of IL-6 and IFN- α have resulted in decreases in NREM slow-wave sleep and complimentary increases in REM sleep (Irwin & Cole, 2011). Thirty-one patients without pre-existing sleep disorders underwent night-time PSG, daytime multiple sleep latency testing and serial blood sampling at baseline and following 12 weeks of IFN- α administration in a randomised and controlled trial (Raison et al., 2010). Administered subcutaneously once a week, the IFN- α group reported significantly increased wake after sleep onset (WASO), significantly decreased slow wave sleep and efficiency, conclusively reducing sleep continuity and depth (Raison et al., 2010). Finally, elevated daytime levels of TNF- α have also been linked with sleepiness, fatigue and altered sleep architecture, while pharmacological antagonism of TNF can reduce these effects (Irwin & Cole, 2011).

Reduced sleep quality impacts immune function, initially suppressing inflammatory cytokines before adrenergic signalling increases inflammatory gene expression and increases daytime levels of innate immunity and inflammation (Irwin, 2015). Upregulation of systemic immune propagation facilitates further pro-inflammatory cascades including a de-sensitisation of anti-inflammatory mediators such as cortisol as a consequence of persistent or repeated activation of the HPA axis (Irwin & Opp, 2017). While the exact mechanisms require further investigation, numerous research projects have demonstrated the inflammatory effect of sleep disturbances. Irwin, et al., (2006), compared the baseline inflammatory profile of 30 healthy adults to the inflammatory profile following a night of partial sleep loss. The protocol involved a four-day protocol with three days of baseline data with sleep opportunity between 11 pm and 7 am with routine blood sampling, followed by one night of partial sleep deprivation with sleep opportunity limited to 3 am to 7 am. In the morning after partial sleep deprivation, monocyte production of IL-6 and TNF- α was significantly greater in comparison to the morning levels following uninterrupted sleep (Irwin, Wang, Campomayor, Collado-Hidalgo, & Cole, 2006). In support, Meier-Ewert et al., (2004) assessed both the impact of total sleep deprivation and

sleep restriction on CRP levels among 10 health adults. In the first experiment, blood samples were taken every 90 minutes for 88 continuous hours of wakefulness. The second experiment involved the random assignment of normal (8.2 h) or restricted (4.2 h) sleep opportunity. The research project concluded that both acute total and short-term partial sleep deprivation result in elevated levels of CRP concentration (Meier-Ewert et al., 2004). Critically, while 88 continuous hours of wakefulness is extreme, shift workers are exposed to both acute total sleep deprivation and short-term sleep restriction making the results of the research project particularly pertinent. Expanding on the previous section regarding sleep, the results support the hypothesis that shift work, maladaptive sleep and immune responses may facilitate cardio-metabolic disorders.

2.5.9 Circadian Rhythmicity of the Immune System

Circadian rhythm likewise mediates the immune system through shared anatomical structures and signalling pathways. Peripheral immune cells contain molecular clock components which mimic the SCN transcription-translation feedback loops to modulate cellular processes (Morris et al., 2012). Further, the SCN conveys circadian information to the immune system by mediating autonomic and endocrine activity (Logan & Sarkar, 2012). Specifically, neurons of the PVN govern autonomic input to peripheral inflammatory tissue (via dorsal nucleus of the vagus nerve), including the thymus and spleen, mediating activity of natural killer cells, macrophages and other lymphocytes (Logan & Sarkar, 2012). Endocrine mediation is primarily achieved via melatonin, a key chronobiotic hormone regulating circadian rhythms, which additionally facilitates increased proliferation of immune cells including T lymphocytes (Rogers, Szuba, Staab, Evans, & Dinges, 2001). Reciprocally, immunological signals are perceived by the pineal gland and provide regulatory feedback to mediate immune responses (Calvo, Gonzalez-Yanes, & Maldonado, 2013).

Consequently, the circadian system plays a predominant role in regulating the distribution of immune cells with a discernible regulatory peak and nadir of cytokines such as IL-1, IL-6 and TNF- α (Besedovsky et al., 2012; Labrecque & Cermakian, 2015). Circadian desynchronisation, as experienced by shift workers, may therefore impact immune regulation and lead to maladaptive and pathogenic immune responses, including upregulating inflammatory gene expression and systemically increasing the immune response (Cuesta, Boudreau, Dubeau-Laramée, Cermakian, & Boivin, 2016; Lange et al., 2010; Potter et al., 2016). For example, melatonin facilitates proliferation of immune responses, conversely cortisol has an established immunosuppressive effect both *in vitro* and *in vivo* (Rogers et al., 2001). However, the combined effect of the two hormones (present during circadian disruption) resulted in a greater reduction in proliferative response than cortisol alone, initially suppressing the immune response (Rogers et al., 2001). An acute effect that transitions into an upregulated immune response under chronic conditions. As outlined in section 2.5.2, Morris et al., (2016) compared two eight-day laboratory protocols investigating the effect of inverted behavioural and environmental cues including sleep opportunity, on markers of cardiovascular function. The protocol resulted in increased 24 h levels of serum IL-6, CRP and TNF- α (Morris et al., 2016). Circadian disruption increasing markers of systemic inflammation was further demonstrated within a randomised cross-over study (Morris et al., 2017). Volunteers undertook two three-day protocols separated by a minimum of three weeks comparing simulated day shift (circadian aligned) and simulated night shift (12 h inverted environmental cycle) for effect on inflammatory status. The results demonstrated a significant effect for circadian desynchronisation on 24 h CRP levels (Morris et al., 2017). Given the integrated nature of circadian rhythms, sleep and immune function, it is again difficult to identify the independent influence of circadian rhythmicity. However, Leproult et al., (2014) reported similar findings regarding the independent nature of circadian-immune regulation. A parallel-group design involving 26 health adults compared the impact of sleep restriction (five h sleep opportunity) to circadian misaligned sleep restriction (Leproult et al., 2014). No difference was observed in daily TST; however, a significant difference was observed in CRP levels following circadian misaligned sleep (Leproult et al., 2014).

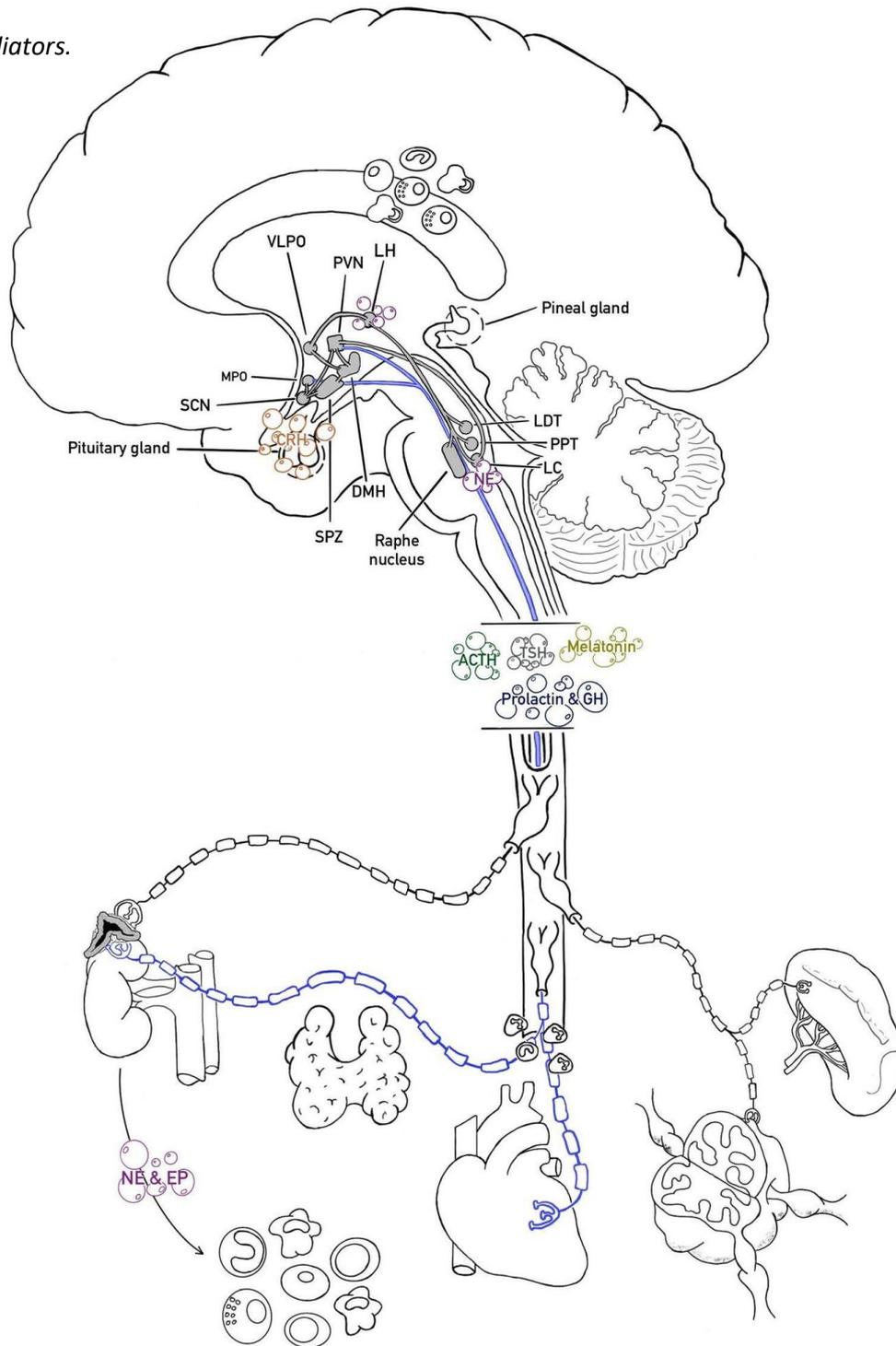
Circadian desynchronisation may not produce comparable inflammatory responses to those that occur in acute infections, but have been linked to chronic states of low grade activation of the same biochemical pathways (Del Giudice & Gangestad, 2018). In support, 244 male employees of a manufacturing company, aged 20-39 years old, were recruited to assess the effect of rotational shift work on health and inflammatory status. Sub-categorised as daytime, former shift and current shift workers, chi-square, and analysis of variance (ANOVA) identified a significant difference in both CRP and leukocyte count among current shift workers. The authors concluded a significant association exists between shift work and inflammatory markers associated with increased CVD (Kim et al., 2016). Additionally, former shift workers (previously worked shift work including nights but currently work fixed days) had significantly higher leukocyte counts in comparison to daytime workers (Kim et al., 2016). Finally, Puttonen et al. (2011), performed a cross-sectional analyses of 1877 airline company employees who utilised varying rotational and fixed shifts (Puttonen et al., 2011) with non-shift workers used as a control for comparison to former shift workers, two-shift workers (morning and evening) and three-shift workers (morning, evening, night). Results indicated that rotating morning, afternoon and night shifts were associated with significantly higher levels of CRP in comparison to other work schemes (Puttonen et al., 2011). Collectively, the acute effects of circadian disruption on inflammatory status observed within laboratory conditions are similarly present within observational research conducted among permanent shift workers.

2.5.10 Shift work, mixed biological signals and adverse health outcomes

Section 2.5 of the literature review sought to explore the hypothesis that while circadian rhythms, sleep and the immune are often discussed separately, the systems have shared anatomical structures and humoral mediators. The cumulative effect is an integrated homeostatic regulation of cardio-metabolic function to meet the demands of a dynamic biological system. The shared systems are

likewise vulnerable to desynchronization, which under chronic conditions, is capable of facilitating cardio-metabolic disorders (Flahr et al., 2018). Shift work is associated with increased incidence of cardio-metabolic disorders, and it may be the structural labour characteristics that facilitate the pathological relationship (Flahr et al., 2018). *Figure 2.4* depicts the integrated nature of the cardio-metabolic system including shared anatomical structures and humoral modulators to further support this summation.

Figure 2.4 Homeostatic regulation of the cardio-metabolic system; shared anatomical structures and humoral mediators.



Shared anatomical structures of circadian rhythm, sleep regulation and the immune system including structures within the hypothalamus and brain stem, direct neural innervations to peripheral organs, and shared humoral factors. Suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), sub-paraventricular zone (SPZ), dorsomedial nucleus of the hypothalamus (DMH), medial preoptic (MPO), ventrolateral preoptic nucleus (VLPO) locus coeruleus (LC), corticotrophin releasing hormone (CRH), thyrotropin-releasing hormone (TRH), adrenocorticotrophic hormone (ACTH), thyroid stimulating hormone (TSH), melatonin, norepinephrine (NE), epinephrine (EP), pedunculopontine (PPT), laterodorsal tegmental nuclei (LDT), dorsal raphe nuclei, tuberomammillary nucleus (TMN), lateral hypothalamus (LH), glucocorticoids (GC), growth hormone (GH). Image created by the author.

2.6 Exercise as a health intervention

Given the longevity and necessity of shift work in various industries and professions, the development of intervention strategies is required to improve shift workers' health and sustain employment. In theory, interventions should aim to improve circadian adaptation, sleep quality or the functioning of cardio-metabolic system (Neil-Sztramko et al., 2014), with previous strategies including controlled light and dark exposure, manipulating shift scheduling and pharmacological aids to promote sleep (Neil-Sztramko et al., 2014). This section of the literature review will explore the concept of exercise as a valid intervention strategy capable of both offsetting the biological risk factors of shift work (circadian disruption and poor sleep) and augmenting cardio-metabolic function (Warburton et al., 2006). Due to the limited body of research exploring exercise interventions among shift workers, the literature review will provide theoretical support for exercise, and where possible, analyse relevant shift work research.

Exercise is a structured component of physical activity (PA) that elicits physiological adaptation, augments biological function (Myers et al., 2004) and is inversely related to all-cause mortality in a dose-response manner (Myers et al., 2004). Specific to shift work, exercise augments the biological pathways implicated in cardio-metabolic disorders including autonomic balance, inflammatory status and metabolic efficiency (Saner et al., 2018; Warburton et al., 2006). Furthermore, prominent sleep theories indicate that exercise may have potent sleep-promoting and circadian entraining effects (Driver & Taylor, 2000), improving sleep architecture, and reducing the risk of developing disease states (Chennaoui, Arnal, Sauvet, & Léger, 2015). Despite its advantages, cross-sectional research indicates that shift workers currently engage in lower levels of leisure time (Peplonska, Bukowska, & Sobala, 2014; Peplowska et al., 2014) and occupational PA (Hulsege et al., 2017), highlighting the potential novelty of exercise interventions. However, several considerations must be explored

including the effect of variations in exercise mode, timing, intensity, and duration as well as the labour specific characteristics of shift work that may impact exercise adherence.

2.6.1 Exercise and sleep quality

The structure of shift work exerts specific limitations on optimising sleep duration and quality (*Section 2.5.5, & Table 2.1*), with low PA levels identified as potential co-factor for increased incidence of sleep complaints (Gerber, Lindwall, Börjesson, Hadzibajramovic, & Jonsdottir, 2017). Alternatively, exercise hours per week are inversely related to the severity of sleep disorders (Peppard & Young, 2004) and numerous exercise intervention studies have improved subjective and objective sleep quality (Chennaoui et al., 2015; Reid et al., 2010; Yang, Ho, Chen, & Chien, 2012). However, there are several considerations for both the general exercise-induced improvements in sleep, and advocating exercise as a sleep remedy for shift workers. The empirical evidence supporting exercise interventions are impacted by several factors including methodology issues and a lack of definition in regards to what constitutes sleep improvement (Uchida et al., 2012). Further, a lack of research currently exists specifically investigating shift work interventions, and a heavy reliance is placed on subjective assessments. Finally, shift workers face unique logistical issues regarding exercise interventions including impeding on an already tight break time schedule, and the potential impact of exercise intensity and timing on sleep opportunity (Flahr et al., 2018). This section will therefore explore exercise as a sleep intervention, detail key considerations among shift workers, and critically evaluate the current literature.

Cross-sectional epidemiological research has associated total exercise time with the severity of sleep-disordered breathing, with an inverse relationship noted (Peppard & Young, 2004). Peppard, (2004), performed PSG assessments of sleep quality as well as assessment of exercise participation

(questionnaire) among 1104 men and women. The results indicated that the likelihood of a moderate or worse sleep-disorder breathing (apnoea) significantly decreased as the level of weekly exercise increased (Peppard & Young, 2004). The results appear to support exercise as preventative measure for the presence and severity of sleep disorders. However, cross-sectional observational research supporting the association between self-reported exercise and sleep precludes an understanding of the temporal nature of the relationship. Individuals may experience better sleep because they are more physically active, alternatively individuals may engage in more PA because of greater restorative sleep (Buman & King, 2010). Of additional consideration is the observation that decreased levels of PA tend to cluster with other health behaviours and conditions associated with poor sleep for example smoking and being overweight (Buman & King, 2010). The effect of which may make distinguishing the independent effect of exercise or lack thereof on quality of sleep difficult.

Sleep is hypothesised to serve as a time of reduced metabolic requirements and peripheral repair (Driver & Taylor, 2000). Consequently, as exercise acutely increases energy expenditure and chronically raises basal metabolic rate (Miles, 2007), a proportional increase in TST and/or in NREM sleep, would be required to fulfil such a role (Driver & Taylor, 2000). Exercise interventions have been demonstrated to improve objective measures of TST and sleep architecture. Chronic exercise training (six months three days/week) improved sleep quality among middle-aged sedentary participants with chronic primary insomnia (Passos et al., 2011), with the exercise intervention significantly decreasing sleep onset latency and increasing sleep efficiency measured via PSG (Passos et al., 2011). Furthermore, King, et al (2008) demonstrated a moderate-intensity exercise intervention improved objective sleep quality, increasing stage two sleep and reducing awakenings during the first third of the sleep period (King et al., 2008). Participants also reported feeling more rested in the morning, greater improvements in sleep disturbance subscale scores, and sleep diary-based sleep latency in comparison to the control group (King et al., 2008). Wong et al., (2013) supported the positive effect of exercise on sleep while simultaneously demonstrating the independent effect of exercise intensity

among sedentary, but healthy adults. Exercise at 45, 55, 65 or 75% of maximum oxygen consumption (VO_{2max}) was compared to a non-exercising control on PSG assessed sleep quality. The results indicated an increased time spent in NREM sleep (stage 1 and 2) (Wong, Halaki, & Chow, 2013), the sleep phase associated with vagal dominance and reduced metabolic requirements, following exercise at 65 and 75% of VO_{2max} (Carskadon & Dement, 2005; Schmidt, 2014). However, no other changes were observed in additional sleep variables associated with objective sleep quality including TST, latency or efficiency. Interestingly, the significant results (increase in NREM stage sleep) associated with increased intensity potentially indicates a role of intensity-based adaptation that may influence future exercise prescription for shift workers.

Regarding the role of sleep in growth and repair, exercise would be expected to acutely affect sleep architecture, anabolic hormonal balance and cellular function (Driver & Taylor, 2000). Previous research has supported this hypothesis, including a balanced crossover study designed to assess the impact of long-duration exercise (LDE) at different intensities on nocturnal anabolic profiles (Kern, Perras, Wodick, Fehm, & Born, 1995). Conducted among 10 trained healthy men, low- and moderate-intensity LDE was compared to a non-exercising control to assess the effect of daytime exercise on temporal patterns of hormone release during subsequent sleep. Moderate-intensity exercise decreased the amount of REM sleep, and varied the temporal levels of testosterone, cortisol and GH throughout the night in comparison to baseline. Collectively, while not significantly effecting the total secretion of anabolic hormones, exercise did effect the temporal regulation of participant anabolic profile (occurring later during the sleep period) (Kern et al., 1995).

Many of the mechanisms linking poor sleep and cardio-metabolic pathogenesis (particularly insulin signalling and mitochondrial dysfunction) share biochemical signalling pathways with exercise, further supporting exercise as an intervention (Saner et al., 2018). In addition to offsetting the long-term

detrimental effects of sleep loss and shift work, singular bouts of exercise may be utilised to offset acute disruption in function. Twenty-four healthy, young men matched for age, BMI, sleep duration and aerobic fitness were randomly assigned a normal (eight h sleep opportunity), restricted (four h sleep opportunity) or restricted with exercise (four h sleep opportunity with three sessions of HIT) (Saner et al., 2020). Muscle biopsies were obtained pre- and post-intervention to assess myofibrillar protein synthesis and molecular markers of protein synthesis and degradation. Myofibrillar protein synthesis was observed to be reduced in the restriction group but not among the normal or exercise group (Saner et al., 2020). Results which suggest sleep restriction impacts cellular function, and that acute bouts of exercise may be utilised to offset such adverse effects.

Predominant sleep theories additionally consider exercise as an exogenous zeitgeber, believed to entrain the biological circadian rhythm (Driver & Taylor, 2000). The theorised chronobiological mechanisms include exercise-induced elevations in core temperature as well as phase shifts in melatonin (Atkinson et al., 2007), cumulatively regulating the circadian system with potential sleep benefits (Driver & Taylor, 2000). Criticism of this theory cite the potential difficulty of isolating a circadian effect of exercise, given exercise commonly takes place outside or in well-lit areas (Atkinson et al., 2007; Buman & King, 2010). However, as discussed in section 2.5.1, acute bouts of exercise are capable of producing phase-delays in melatonin (Yamanaka et al., 2006), that differ from those induced by photic stimuli (Mistlberger & Skene, 2005). In support, 18 young and fit males completed a 15-day randomised clinical trial to assess the effect of nightly exercise bouts on circadian phase within dim light laboratory conditions (Barger et al., 2004). Plasma samples of melatonin were used to assess the effect of exercise (3 x 45-min bouts of cycling per night for seven days) or control condition on circadian phase. The exercise group reported a significantly greater shift in dim light melatonin onset, offset and midpoint of melatonin profile (Barger et al., 2004). The results indicate that when standardised for total wakefulness and light exposure, an independent effect of PA on

circadian regulation is observed. Of interest, but currently unexplored, is the distinction between the effect of planned and incidental PA on circadian regulation. Labour intensive industries utilising night shifts may facilitate circadian regulation similar to that induced by planned and structured PA, however this observation requires further research.

A key consideration regarding shift workers is if exercise is capable of mediating circadian phase shifts, poorly timed exercise interventions may exacerbate circadian desynchronization. An observation supported by Thomas, et al., (2020) when investigating the effect of morning or evening exercise on circadian regulation (Thomas et al., 2020). Conducted within dim light laboratory conditions, 52 young, sedentary adults had circadian phase assessed via salivary melatonin sampling pre- and post-five days of morning or evening exercise. The results indicated that morning exercise induced significantly greater phase advance shifts than evening exercise, however, individual chronotypes were identified as an additional factor. Specifically, early chronotypes, assessed via the Morningness-Eveningness Questionnaire, reported increased circadian misalignment following evening exercise compared to later chronotypes (Thomas et al., 2020). One criticism of the project however is the lack of objective sleep measures to assess the potential relationship between circadian rhythm and sleep regulation. Consequently, questions may be asked whether the reported observations are impacted by the proximity to sleep opportunity. For example, is it the 'evening' exercise that exacerbates circadian misalignment in early chronotypes or the proximity of exercise to nocturnal sleep opportunity? This question is particularly pertinent to shift workers that have the biological 'evening' regarding circadian rhythm and an 'evening' period regarding the time before sleep.

The relationship between sleep and circadian regulation may make distinguishing the individual effects difficult, however some research has been conducted regarding exercise timing and sleep. The timing of exercise was initially hypothesised to impact sleep architecture via physiological arousal, and

if performed too close to sleep opportunity may negatively impact sleep quality (Irish, Kline, Gunn, Buysse, & Hall, 2015). A hypothesis yet to be cogently substantiated, with evening (Larsen et al., 2019a) and late-night (Myllymäki et al., 2011) exercise among middle-aged and fit young participants, respectively, having little to no adverse effects on sleep quality. Further, Larsen et al., (2019) reported significant increases in NREM sleep, measured via PSG following morning and evening exercise. Eleven inactive men undertook sleep monitoring to determine baseline sleep stages before completing HIT in the morning, afternoon, and early evening (Larsen et al., 2019a). No difference was observed for total sleep; however, morning exercise significantly increases stage 3 sleep compared to baseline and evening exercise reduced REM sleep and increased NREM compared to baseline. Furthermore, exercise-induced improvements in thermoregulation and acute elevations in core body temperature may also improve sleep latency (Irish et al., 2015). Supported by the observation that acute aerobic exercise, performed within three h of sleep opportunity, decreased objectively measured sleep onset latency among middle-aged (44 ± 8 years) individuals with difficulty initiating sleep (Passos, Poyares, Santana, Tufik, & Tú, 2010).

2.6.2 Shift work, exercise, and sleep quality

Shift workers face unique challenges for both obtaining sufficient sleep and the application of exercise as an intervention. Highlighted in section 2.5.5, shift work labour characteristics specifically limit time available to sleep and facilitate misaligned sleep opportunity due to some rotational structures. The structural limitations effecting sleep duration (break time afforded for sleep) are fixed, as such advocating exercise during periods of restricted sleep opportunity may exacerbate the adverse health effects (Atkinson et al., 2008). However, sleep quality refers to a multi-facet assessment including regularity, continuity and factors such as the time take to fall asleep (Medic et al., 2017). Consequently, while perhaps further limiting total sleep opportunity, exercise may improve sleep quality, via reduced fragmentation, improved regularity or decreased time taken to fall asleep (King

et al., 2008; Passos et al., 2011). Given the lack of time to attain sufficient sleep and inability to extend sleep opportunity due to shift work schedules, improvements in sleep quality may be of greater importance and a more realistic outcome for shift workers. Section 2.6.1 additionally provided support for exercise as an intervention to improve circadian rhythm and sleep architecture, further reducing the health effects associated with homeostatic desynchronising.

2.6.3 Shift work exercise intervention and sleep outcomes

Regarding shift work and exercise-based intervention for sleep, a scarce body of research currently exists and of that research, shift work is poorly defined, not specifically targeted or subjectively measured (*Table 2.2*). Section 2.6.3 will critically review the predominant research currently undertaken among rotational shift workers regarding the overall effect of exercise, exercise modality and validity of observation.

Of the limited research, several studies have demonstrated significant improvements in sleep following exercise-based interventions. Fang and Li (2015) examined the effect of yoga among 120 nurses currently employed in rotational shift work. The yoga group performed 50-60 min of yoga, twice a week for six months and demonstrated a significant improvement in subjectively measured sleep quality (Fang & Li, 2015). Similarly, Harma et al., (1988) demonstrated that four months of two – six aerobic exercise sessions per week had a positive effect on questionnaire-assessed sleep-wakefulness (Härmä, Ilmarinen, Knauth, Rutenfranz, & Hänninen, 1988). Finally, Atlantis et al, (2006) concluded that six months of moderate-to-vigorous exercise improved subjective sleep assessment among both shift and non-shift participants (Atlantis, Chow, Kirby, & Fiatarone Singh, 2006). Results that lend support to the potential effect of targeted exercise interventions to improve the sleep quality of shift workers. However, large epidemiological surveys indicate that the therapeutic and sleep promoting benefits of regular PA are accepted by the public regardless of empirical evidence (Driver

& Taylor, 2000; O'connor & Youngstedt, 1995; Urponen, Vuori, Hasan, & Partinen, 1988). Therefore, studies that demonstrate subjective improvements in sleep in the absence of objectively measured sleep improvements may have the validity of the results questioned. Consequently, the limited research assessing the effect of exercise and sleep quality among shift workers, combined with a reliance on subjective sleep assessment has not fully explored exercise as an intervention.

Collectively, predominant sleep theories, cross-sectional and intervention-based research support exercise as a valid intervention strategy to improve the sleep quality of rotational shift workers with potential augmentation of cardio-metabolic health. However, limited research currently exists substantiating such observations among shift workers, who are exposed to specific barriers to both exercise adherence and structural limitations on sleep. Consequently, future research should aim to assess the adherence to shift worker interventions and the objectively assesses the effect of exercise on sleep quality.

2.6.4 Exercise and Inflammation

Inflammation is recognised to play an etiological role in the development of adverse cardio-metabolic conditions (Libby, 2007; Medzhitov, 2008). Conversely, exercise is hypothesised to facilitate acute and chronic anti-inflammatory responses capable of offsetting the pathogenic role of systemic inflammation (Petersen & Pedersen, 2005). However, the interaction between exercise and inflammation is complex and in need of further research before being advocated as an intervention for shift workers. As previously mentioned, (Section 2.4), exercise is a broad term describing structured PA, but can vary in mode, intensity, duration, and regularity. Variations that independently affect the immune system and inflammatory responses (Wisløff, Ellingsen, & Kemi, 2009; Yao & Basner, 2019) in need of consideration.

Acutely, exercise is associated with increased oxidative metabolism and stress, potentially leading to oxidation of cell components and inducing a pro-inflammatory cascade (Kasapis & Thompson, 2005). Additionally, acute muscle and connective tissue damage caused by unaccustomed or prolonged exercise may induce inflammation (Woods et al., 2012). Consequently, exercise at high intensities or over an extended period (relative to participant conditioning) may induce a pro-inflammatory response, exacerbating the risk of adverse health conditions among shift workers. Conversely, the acute and chronic anti-inflammatory effect of exercise (Brown et al., 2015) may reduce prevalent disease states through inhibiting pro-inflammatory cascades and subsequent biological effects (Lavie, Church, Milani, & Earnest, 2011; Mathur & Pedersen, 2008; Petersen & Pedersen, 2005; Starkie, Ostrowski, Jauffred, Febbraio, & Pedersen, 2003). Starkie et al. (2003), demonstrated that moderate-intensity continuous training (MICT) could blunt the pro-inflammatory cascade, specifically inhibiting the proliferation of TNF- α (Starkie et al., 2003). Comparing the immune response to an injected endotoxin capable of inducing low-grade inflammation (administration among control group significantly increased TNF- α), both MICT and recombinant human IL-6 attenuated any endotoxin-induced TNF- α response (Starkie et al., 2003). Of additional interest regarding cardio-metabolic function is the observed relationship between IL-6 and glucose metabolism. Carey, et al., (2005) demonstrated that infusion of recombinant human IL-6 increased the phosphorylation of adenosine monophosphate-activated protein kinase (AMPK) in healthy humans and stimulated glucose disposal via increased glucose transport (GLUT)-4 translocation (Carey et al., 2006). Contracting skeletal muscle induced IL-6 has additionally been hypothesised to contribute to mediating hepatic glucose output necessary to maintain blood glucose homeostasis during the increased muscular demands of glucose during prolonged exercise bouts (Febbraio & Pedersen, 2002). Exercise induced changes in inflammatory balance may therefore have wider repercussions for cardio-metabolic function, including increased glucose metabolism.

As stated, acute bouts of exercise have resulted in the propagation of anti-inflammatory mediators including IL-6, IL-1Ra and IL-10 with subsequent inhibition of pro-inflammatory mediators (Brown et al., 2015; Starkie et al., 2003). The exact mechanisms responsible for the acute anti-inflammatory effect of exercise are yet to be fully elucidated. Proposed mechanisms highlight muscle-synthesised anti-inflammatory mediators, termed myokines, and downstream inhibitory effects on pro-inflammatory cytokines (Gleeson et al., 2011; Mathur & Pedersen, 2008; Petersen & Pedersen, 2005). Exercise-induced reduction in the expression of Toll-like receptors on monocytes and macrophages further inhibits downstream pro-inflammatory propagation and may contribute to the observed anti-inflammatory effect (Gleeson et al., 2011). Finally, impulses from the motor centres in the brain as well as afferent impulses from active muscle elicit intensity-dependent increases in sympathoadrenal activity (Gleeson et al., 2011). Neural signals which facilitate the secretion of pituitary hormones including ACTH, which combined with the increased release of catecholamines, proceed a rise in plasma cortisol, with established anti-inflammatory effects (Gleeson et al., 2011). Thus, hormones, myokines and neurotransmitters may all contribute to the anti-inflammatory effect of exercise in an intensity-dependant manner.

As a result, exercise stimulus is cited as a key element in anti-inflammatory synthesis and secretion by skeletal muscle (Brown et al., 2015). Secretion of myokines, particularly IL-6, have been described as being sensitive to intensity, with exercise duration, total mass recruited and type of contraction (eccentric verses concentric) also identified as factors in myokine synthesis (Pedersen & Febbraio, 2008). MICT has previously induced a significant anti-inflammatory response, with protocols ranging from 45 min to 3 h (Kaspar et al., 2016; Scott et al., 2011; Starkie et al., 2003). Starkie et al., (2003) reported a blunted pro-inflammatory cascade following a 3 h cycle intervention at 75% VO_{2max} , while Scott et al., (2011) significantly increased IL-6 and IL-1Ra following 60 mins treadmill running at 75%

VO_{2max} and Kaspar et al., (2016) significantly decreased monocyte chemoattractant protein (MCP)-1 two days after a 45 min bout of cycling at 62.5% of HR_{max} . These results suggest that a minimum of 45 min is required to elicit a significant anti-inflammatory effect when exercise is performed at moderate intensities.

However, shift workers commonly identify a lack of time as the key barrier to exercise adherence (Nea et al., 2017), potentially limiting the effectiveness of MICT interventions. Alternatively, high-intensity interval training (HIT) is hypothesised to induce a superior anti-inflammatory response compared to MICT via increased muscle contraction and force (Brown et al., 2015). Kaspar et al., (2016) additionally reported significant increase in IL-6 30 min after HIT, a result not replicated in the MICT session. Scott et al., (2011), likewise reported a difference in intensity-based inflammatory responses with the 60 min of treadmill running at 75% VO_{2max} inducing significantly higher anti-inflammatory response compared to 55% and 65% VO_{2max} bouts. Directly comparing MICT and different bouts of HIT further indicate the independent role of exercise intensity and duration on inflammatory responses. Cullen et al., (2016) compared 35 min of MICT at 50% VO_{2max} , to two different HIT protocols utilising bouts of high-intensity (80% VO_{2max}) exercise interspersed with varying lengths of recovery intervals (performed at 50% VO_{2max}). The results indicated that the HIGH HIT protocol (5 x 4 min intervals of 80 % interspersed with 3 min intervals of 50 %) elicited a significantly higher IL-6 response than the MICT bout (Cullen, Thomas, Webb, & Hughes, 2016). Based on the research cited above, 35 min at 50% VO_{2max} may have not been enough stimulus to elicit an inflammatory response, however the results still indicate superior responses associated with high intensity bouts of acute exercise. Cumulatively, individual bouts of exercise may provide an anti-inflammatory effect, acutely mediating the pro-inflammatory state associated with shift work. However, variables of acute exercise, particularly the effect of duration and intensity require further research.

Regular exercise likewise exhibits an anti-inflammatory effect, with higher physical fitness levels associated with reduced basal and post-exercise pro-inflammatory markers (Kleiven et al., 2018). This chronic effect of exercise may be attributed to several direct and indirect factors, including modulation of the ANS, improved body composition and endothelial function (Wallberg-Henriksson & Zierath, 2015; Warburton et al., 2006). Exercise augments parasympathetic tone and vagus nerve secretion of acetylcholine, theorised to suppress pro-inflammatory cytokines (Papathanassoglou, Miltiadous, & Karanikola, 2015). Furthermore, fat cells are recognised as active endocrine organs that produce adipokines capable of initiating the pro-inflammatory cascade (Lavie et al., 2011). Regular exercise alters body composition through reducing fat cell tissue, suppressing adipokine activity and subsequent inflammation (Woods, Vieira, & Keylock, 2009). In addition, myokine production in response to exercise directly remodels adipose tissue, improving metabolism and decreasing pro-inflammatory markers (Schnyder & Handschin, 2015; Wallberg-Henriksson & Zierath, 2015). Exercise may also mitigate inflammation via ameliorated endothelial function (Kasapis & Thompson, 2005), with reports that regular exercise enhances antioxidant capacity of cells, upregulates antioxidant enzymes and increases the bioavailability of nitric oxide (NO) (Beavers, Brinkley, & Nicklas, 2010; Kasapis & Thompson, 2005). The combination of which decreases the susceptibility of low-density lipoprotein (LDL) oxidation, preventing endothelial injury and associated inflammation response (Kasapis & Thompson, 2005). Further, chronic exercise interventions such as the aforementioned studies would involve participants being no more than 48 h post-acute exercise bouts. Consequently the beneficial adaptations derived from PA may, in part, be attributed to chronic short-term or acute responses to exercise (Brown et al., 2015). Theoretical and experimental findings support exercise as a plausible intervention method to downregulate systemic inflammation and offset pathological conditions, however, this hypothesis needs empirical research among the shift work population.

2.6.5 Exercise and cardio-metabolic conditions

The previous sections have outlined, critiqued and validated the potential mechanisms propagating poor health among shift workers, while advocating exercise as an intervention to improve cardio-metabolic function. Section 2.6.5 will now explore the exercise interventions specifically targeting cardio-metabolic conditions, where possible among shift workers or similar population groups.

The positive physiological effect of exercise on cardiovascular outcomes has previously been demonstrated among permanent night shift workers (Lim, Min, Kwon, Park, & Park, 2015). Lim et al., (2015) sought to evaluate the effect of a 10-week exercise intervention among 30 males currently involved in rotational shift work on cathepsins and arteriosclerosis adhesion molecules including MCP-1 and E-selectin. Dysregulated cathepsins, a group of lysosomal proteases (Yadati, Houben, Bitorina, & Shiri-Sverdlov, 2020), are associated with a diverse range of pathogenic conditions including MetS and CVD (Lim et al., 2015; Yadati et al., 2020), while arteriosclerosis adhesion molecules serve as independent biomarkers of future CV risk (Lim et al., 2015). Results demonstrated significant pre-to-post decreases in cathepsins, MCP-1 and E-selectin, decreasing the relative risk of future cardio-metabolic disorders (Lim et al., 2015). Faria and Faria (1991), reported a 30 min combination of aerobic activity and circuit training three days/week for 32 weeks improved coronary heart disease risk among firefighters working in rotational shifts (Faria & Faria, 1991). The intervention increased aerobic capacity, including both anaerobic threshold and VO_{2max} , while improving cholesterol profile through increased high-density lipoproteins (HDL) (Faria & Faria, 1991). Despite inconsistent exercise modes, the results support aerobic activity as a valid intervention, improving key physiological mechanisms associated with cardio-metabolic pathology and reducing the risk of future adverse cardiovascular events (Lim et al., 2015). However, the project did not specifically outline the shift characteristics, while firefighting is associated with rotational shift it is also plausible that the participants may not have been solely employed in rotation shifts.

As with CVD and systemic inflammation, a plethora of observational and intervention-based research support exercise as an intervention to mediate the development of MetS, independent of occupation (Church, 2011). Exercise has an established inverse relationship with the development of MetS, physical inactivity is identified as an independent risk factor for pathogenesis (Golbidi et al., 2012), and exercise augments shared biological pathways with established pathophysiological mechanisms. One example is the relationship between exercise, excessive adipose tissue and MetS. As previously outlined (section 2.3), adipose tissue, initially viewed as benign tissue functioning as storage of excess energy, is now recognised as an active endocrine organ capable of producing acute phase proteins and propagating low-grade systemic inflammation (Lavie et al., 2011). Obese hypertrophic adipocytes and stromal cells within adipose tissue directly augment systemic inflammation, mediating multiple pathogenic mechanisms associated with MetS (Berg & Scherer, 2005). Exercise has been shown to remodel subcutaneous fat tissue (Wallberg-Henriksson & Zierath, 2015), augments the oxidative capacity of skeletal muscles, improves lipid metabolism (Golbidi et al., 2012) and supplements weight loss while maintaining lean body mass, essential for fat metabolism and glucose transport (Golbidi et al., 2012).

Of the limited exercise-based interventions specifically targeting shift workers, several projects have supported the salutary effect of exercise on body composition. Kim et al., (2015) examined the effect of an exercise intervention or exercise and abdominal ultrasound on body composition. The protocols involved four weeks of either exercise five days a week, or exercise twice a week with three days of deep ultrasound therapy (Kim, Lee, Lee, & Lee, 2015). The results indicated that both protocols significantly improved body composition via reduced body fat mass. However, the intervention lacked a control group to establish a non-intervention effect on body composition, and the protocol (a total session time of 60 min, conducted five days a week) may not be a realistic protocol for all shift work

industries. Lim et al., (2015) demonstrated the effectiveness of a 10 week training intervention for improving body composition and biomarkers of cardiovascular risk in night shift workers (Lim et al., 2015). Conducted over 10 weeks, participants were prescribed 30 minutes of brisk walking (60-79 % maximal HR) which could be accumulated in three 10 min bouts, on three days a week. The intervention resulted in improved body composition via significant reductions in fat mass compared to baseline (Lim et al., 2015). Additionally, by allowing the accrual of total exercise minutes throughout the day, the intervention may have been perceived as more flexible for a rotational work structure rather than attempting to fit extended exercise bouts into limited rest opportunity. Finally, a workplace-based weight loss program for overweight male shift workers resulted in significant pre-to-post weight loss (Morgan et al., 2011). Regarding assessing the effectiveness of a workplace intervention on weight loss the research project validates the potential effect of exercise. However, the program incorporated information seminars, website tutorials, dietary feedback, and financial incentives to ensure the best possible outcomes. A situation that, again, may not be an applicable long-term intervention among rotational shift workers.

Substantial evidence also validates exercise as an effective method of enhancing insulin sensitivity and upregulating glucose transport (de Souza, Dáttilo, De Mello, Tufik, & Antunes, 2017; Golbidi et al., 2012; Miles, 2007), which is particularly pertinent to shift workers given the increased risk of MetS throughout the progression of their employment. Specifically, de Souza et al., (2017) demonstrated that two weeks of HIT, increased insulin sensitivity among healthy males despite 24 h of total sleep deprivation (de Souza et al., 2017). The intervention consisted of repeated bouts of sleep deprivation with one laboratory session of 24 h continual wakefulness before and after two weeks of HIT (total of six training sessions). Pre-intervention, sleep curtailment induced insulin resistance, however HIT minimised this deleterious effect (de Souza et al., 2017). While not conducted among shift work, it is

demonstrated that exercise can be an effective intervention among voluntary sleep curtailment, like that observed among shift workers.

While the mechanisms associating exercise with increased insulin sensitivity require further investigation, it is likely to be mediated by both acute and chronic adaptations. Acute exercise facilitates muscle contraction, activating glucose transport independent of insulin-stimulated pathways and resulting in increased rates of glucose uptake and utilisation (Henriksen, 2002). At a molecular level, AMPK is considered an important sensor of cellular energy balance, which when activated, is capable of stimulating adenosine triphosphate (ATP) generating processes and increasing glucose uptake (Kjøbsted et al., 2017). Acute muscular contraction and hypoxia associated with acute bouts of exercise are capable of facilitating such a response (Kjøbsted et al., 2017) and offer one potential mechanisms for exercise to mediate metabolic function. The chronic effect of exercise increases insulin sensitivity through enhanced glycogen synthesis, increase mitochondrial biogenesis, enhanced β -oxidation and increased expression and translocation of the muscle GLUT-4 to cell surface (Henriksen, 2002). A process that is partly mediated by the transcriptional co-activator peroxisome proliferator-activated receptor γ (PGC-1 α), which is considered the primary regulator of mitochondrial biogenesis due to its role in the transcriptional co-activation of several transcription factors (Saner et al., 2018). PGC-1 α is likewise up regulated in response to exercise and facilitates improved metabolic function.

Exercise may also be beneficial in reducing the risk of MetS through favourable changes in plasma lipids and lipoproteins profiles (Golbidi et al., 2012). Chronic exercise augments oxidative capacity of skeletal muscle (including increased PGC-1 α and mitochondrial biogenesis), improving fat oxidation capacity and removal of plasma free fatty acids (Golbidi et al., 2012). Furthermore, HDL-cholesterol (HDL-C), associated with decreased risk of CVD (Boden, 2000) is generally responsive to aerobic

exercise and increases in a dose-dependent manner with increased energy expenditure (Miles, 2007). In support, Kim et al. (2015) reported physical intervention of exercise or combined exercise and deep abdominal ultrasound therapy was sufficient stimulus to improve body composition and augment blood lipid profile among shift workers (Kim et al., 2015). Consequently, exercise ameliorates lipid metabolism (Kim et al., 2015), positively affects body composition (Lim et al., 2015), increases insulin sensitivity and glucose transport, as well as exerting an anti-inflammatory effect. Critical observations in support of exercise as a strategy to address the individual characteristics of MetS and improve the health of rotational shift workers.

2.7 Exercise barriers and considerations for shift workers

The thesis has provided cogent support of exercise as a health intervention, however the implementation and maintenance of an active lifestyle among shift workers must address several key factors. Firstly, shift workers exercise participation is limited by the structural characteristics of their employment. In addition to the previously discussed considerations regarding exercise during limited sleep opportunity (Atkinson et al., 2008), and the potential effect of temporal proximity to sleep (Irish et al., 2015), is the independent effect of break time afforded between successive shifts. Limited total break time, effected by factors including industry standards, commute time and social obligations, often results in the prioritisation of sleep and a lack of time for exercise participation (Atkinson et al., 2008; Blake et al., 2017). Surveys of 361 nursing and medicine students on placement explored the perceived benefits and barriers to exercise over 12 months, with the most notable barrier to exercise identified as a lack of time (Blake et al., 2017). Conversely, the current PA guidelines advocate the accumulation of at least 150 minutes of moderate activity exercise to achieve significant health benefits (Gebel et al., 2015). Advancement in research regarding high-intensity interval training (HIT) and superior adaptations associated with increased intensity (Gibala, Little, MacDonald, & Hawley, 2012) may provide time-efficient alternatives; however, limited research exists among shift workers

(Flahr et al., 2018). Additionally, the structural characteristics of shift work may interfere with developing a consistent exercise routine due to the erratic work schedules (Nea et al., 2017). Focus groups totalling 109 participants were interviewed to gain an understanding of the issues facing shift workers in relation to their lifestyle practices. The results indicated that not only did shift work provide exercise opportunities out-of-sync with gym or team sport participation but the rotating structure of shift work was perceived as a barrier to developing a consistent exercise routine (Nea et al., 2017). Collectively, shift workers are exposed to structural labour characteristics that appear to negatively influence exercise adherence and may impact the potential validity of exercise as an intervention.

Of additional concern are recent investigations into shift work, leisure-time and occupational PA levels. Hulsegge et al., (2012) assessed the leisure-time and occupational PA levels of 812 employees aged 18-65 via actigraphy accelerometers over four successive days (Hulsegge et al., 2017). After categorising activity levels into sedentary, light and moderate-to-vigorous, the results indicated that shift and non-shift workers had similar objectively measured leisure-time PA patterns (Hulsegge et al., 2017). Loef et al., (2017) further reported shift workers had increased PA levels during work compared to non-shift colleagues, however the project did not assess any physiological measures to investigate relative health associated with PA (Loef et al., 2017). Finally, a cross-sectional study of fixed and irregular shift workers concluded that while subjective leisure-time PA was generally low in both groups, the irregular shift workers were more physically active than day workers despite a significantly higher BMI (Marqueze et al., 2014). While acknowledging that PA levels may differentiate between occupation (Prince, Elliott, Scott, Visintini, & Reed, 2019), the aforementioned research outcomes suggest that shift workers may be engaging in comparable levels of PA, yet remain more prone to cardio-metabolic disease states. Therefore, the current PA guidelines may be a conservative prescription for shift workers and not enough to elicit meaningful health changes.

Finally, the effect of excessive daytime sleepiness, fatigue, and the development of health disorders related to shift work may also impact the effectiveness and appropriateness of an exercise intervention (Hargens et al., 2013). While a shift worker may have the opportunity to participate (days off or extended break periods), increased fatigue and plausible negative effects or experience of exercising at a time out of sync with circadian rhythmicity may deter participation (Hargens et al., 2013). If exercise exacerbates or improves feelings of fatigue for an individual, that experience will affect future participation (Atkinson et al., 2008). Therefore, exercise may mitigate the risk factors associated with shift work; however, the effectiveness of exercise intervention with consideration of the above factors requires further investigation.

2.8 Review of exercise-specific interventions

Despite the strong theoretical and researched-based evidence, many shift workers fail to achieve the recommended PA guidelines (Flahr et al., 2018) and few exercise-based interventions have been implemented among shift work population groups (summarised in *Table 2.2*). The one systematic review of shift work and exercise-based interventions identified seven studies between 1988 and 2017 (Flahr et al., 2018). The inclusion criteria were comprised of the use of randomised control trials or protocols, shift workers as the target population and PA listed as the primary intervention component (Flahr et al., 2018). Of these interventions, standardised exercise modality and workloads, as in the case of both Kim et al., (2015) and Lim et al., (2015), have resulted in improved body composition (Kim et al., 2015; Lim et al., 2015), blood lipid profiles (Kim et al., 2015) and decreased biomarkers of CVD (Lim et al., 2015). Additionally, improvements in CV fitness, BP and subjective measures of sleep quality and fatigue among male (Atlantis, Chow, Kirby, & Fiatarone Singh, 2006), female (Härmä et al., 1988), healthy (Atlantis, Chow, Kirby, & Singh, 2004) and overweight (Morgan et al., 2011) shift workers have supported exercise as an intervention. However, the inconsistent use of interventions including mixed exercise modalities, different workloads, incorporation of a variety of behavioural

interventions including health tutorials and seminars used concurrently with exercise, and a high dependence on subjective sleep measures limit interpretations. As such, while exercise may be effective to mitigate the intermediate risk factors associated with shift work, limited interventions studies, comprised of inconsistent measures and small participant groups prevent the findings informing exercise intervention guidelines (Flahr et al., 2018). Further research is required to investigate the success and potential mechanism responsible for exercise as an intervention among shift workers.

Table 2.2 Workplace intervention strategies among shift workers

Study	<i>n</i>	Participants Characteristics	Shift System	Intervention	Measures	Findings and Conclusions
Atlantis, Chow, Kirby, & Singh, 2006	73	Male and female, healthy but sedentary casino employees (73% SW)	<i>Not reported</i>	6 mo 20 min aerobic moderate-to-high intensity (50-75% HRmax) 3 d/wk + 30 mins resistance 3 d/wk	VO _{2max} Anthropometry	Significantly improved waist circumference and aerobic fitness amongst healthy but sedentary SW
Atlantis et al., 2004	73	Male and female, healthy but sedentary casino employees (73% SW)	<i>Not reported</i>	6 mo 20 min aerobic moderate-to-high intensity (50-75% HRmax) 3 d/wk + 30 mins resistance 3 d/wk	DASS SF-36 Health Status Survey	24-week intervention significantly improved QOL, depression and stress outcomes
Atlantis, Chow, Kirby, & Singh, 2006	73	Male and female, healthy but sedentary casino employees (73% SW)	<i>Not reported</i>	6 mo 20 min aerobic moderate-to-high intensity (50-75% HRmax) 3 d/wk + 30 mins resistance 3 d/wk	PSQI	Both SW and non-SW subgroups displayed greater post-test PSQI improvements following exercise intervention.
Christensen et al., 2011	98	Females, overweight health care workers	<i>Not reported</i>	3 mo of combined dietary plan, strengthening exercises and leisure time aerobic fitness	Anthropometry VO _{2max} BP	Significant reduction in body weight, body fat %, waist circumference and BP. <i>Not all SW population</i>

Study	<i>n</i>	Participants Characteristics	Shift System	Intervention	Measures	Findings and Conclusions
Fang & Li, 2015	120	Female nurses 25-51 years	Morning, evening, and night (8 h)	Minimum of 2 yoga sessions per wk (50-60 min) after work hours	PSQI QMWS	Regular yoga can improve subjective sleep quality and reduce work stress
Faria & Faria, 1991	38	Male firefighters 42.7 ± 6.4 years	<i>Not reported</i>	Aerobic activity including running, rowing, cycling + circuit weight training for 30 min, 3 d/wk, 32 wk	Cholesterol VO _{2max} AT Body Composition	Moderate exercise intensity increased AT, VO _{2max} , and HDL-C. <i>Does not specifically target SW.</i>
French et al., 2010	696	Bus drivers recruited from Metropolitan Transit Council in Minneapolis	<i>Not reported</i>	Worksite intervention: access to fitness facilities, educational programs regarding diet and exercise + access to healthy food options	Anthropometry Behavioural Q	Changes in BMI, eating behaviours and PA did not reach statistical significance. <i>Does not specifically target SW.</i>
Gamble, Boreham, & Stevens, 1993	14	Male ambulance employees	<i>Not reported</i>	Physical training 2 d/wk for 10 wk. Including indoor soccer and circuit training	Anthropometry VO _{2max} AT LS	Exercise intervention programme effectively improved physical fitness levels. <i>Does not specifically target SW.</i>

Study	<i>n</i>	Participants Characteristics	Shift System	Intervention	Measures	Findings and Conclusions
Green & Crouse, 1991	24	Male firefighters 30.1 ± 7.7 years	<i>Not reported</i>	Warm up, 20 min running/walking + body weight circuit every 3 rd day for 5 y	Cholesterol VO _{2max} HR Anthropometry	Total improvements regarding fitness and body composition over 5 y period were minimal. <i>Does not specifically target SW.</i>
Harma et al., 1988	75	Female nurse/nursing aids, ≥ 1.5 y experience in SW, 20-49 years	Morning, evening (8 h) or night (10 h)	4 mo, 2-6 sessions per wk, jogging, swimming, skiing, walking or gymnastics @ 60-70% HRmax.	Q	Moderate physical activity had a positive effect on sleep-wakefulness and symptoms of SW
Kim et al., 2015	30	SW employed at an undisclosed company with a BMI >25	<i>Not reported</i>	4 wk, 5 d/wk either 5 exercise session including 30 min aerobic + 20 min resistance or 2 days exercise + 3 days of 30 min deep ultrasound therapy	Anthropometry Body Composition Blood lipid profile	Exercise effectively improved body composition while the use of additional ultrasound therapy improved blood lipid profile through increases of HDL-C.
Lim et al., 2015	30	Healthy male night SW	<i>Not reported</i>	10 wk, 3 d/wk of 3 x 10 min walking @ 60-79% HRmax.	Anthropometry Venous blood analyses	Exercise intervention improved body composition while decreasing levels of cardiovascular risk biomarkers in night SW

Study	<i>n</i>	Participants Characteristics	Shift System	Intervention	Measures	Findings and Conclusions
Matsugaki et al., 2017	30	Female, healthy full-time nurses aged 20-40	<i>Not reported</i>	12 wk, 2 d/wk combining aerobic (20 min) and resistance (4 exercises 10 reps/3 sets) supervised vs unsupervised	Anthropometry Blood lipid Blood Glucose	Supervised training is better in terms of adherence however the interventions failed to significantly improve health measures.
Morgan et al., 2011	110	Overweight/obese males aged 18-65	<i>Not reported</i>	14 wk, information seminars, tutorials and handbooks regarding exercise and healthy diet	Anthropometry BP HR Health Qs	Program resulted in significant weight loss and improved health-related outcomes and behaviours amongst overweight male SW

AT; anaerobic threshold, BP; blood pressure, BMI; body mass index, CV; Cardiovascular, d; day, DASS; Depression anxiety and stress scales, h; hour, HDL-C; high density lipoprotein cholesterol, HR; heart rate, HRmax; heart rate maximum, LS; leg strength, min; minute, mo; month, PA; Physical Activity, PSQI; Pittsburgh sleep quality index, Q; questionnaire, QMWS; Questionnaire on medical worker's stress, QOL; Quality of life, SW; Shift work, SF; short form, VO_{2max}; maximum oxygen consumption, wk; week, @; at.

2.9 Summary

In summary, shift work is a key labour structure that dysregulates homeostatic function and increases the prevalence of cardio-metabolic disorders. The specific mechanisms responsible for the increased disease risk among shift workers are yet to be fully elucidated. However, the integrated relationship of circadian rhythms, sleep and systemic inflammation with cardio-metabolic function is an area of emerging interests that warrants further investigation. Conversely, exercise is viable intervention method with a myriad of physiological health adaptations. However, several factors may affect the feasibility of an exercise intervention among shift workers, including limited time and potentially adverse effects on already dysregulated homeostatic function. A key variable in the inability to evaluate exercise as an intervention is the limited body of research currently conducted among shift workers. Therefore, further research is required to investigate the feasibility of exercise as an intervention method to improve shift worker health.

Chapter Three:

Methodology

3.1 Overview

Shift work encompasses a range of labour structures, disturbs multiple interrelated regulatory systems, and is associated with pathogenic lifestyle behaviours (Kervezee et al., 2018; Sallinen & Kecklund, 2010). Consequently, research projects looking to explore independent pathogenic mechanisms must display a clear rationale for research methodology. This chapter will provide the rationale for research methods including measures of cardio-metabolic function, recruitment strategies and intervention designs employed within the research projects.

3.2 Methods and Measures:

As outlined, moderating the cardio-metabolic system involves integration of several systems, humoral mediators, and oscillatory interactions. Consequently, a variety of collections techniques may be utilised to assess the function of the cardio-metabolic system. Section 3.2 will investigate the key sampling methods to assess cardio-metabolic function, including critical analysis of accuracy, cost and pragmatic use of common measures.

3.2.1 Inflammation markers of disease progression

Sample Collection

Quantifiable levels of circulating cytokines can be impacted by a variety of factors prior, during and post sample collection. The sample matrix used in biological and clinical studies is a key consideration as plasma and serum are both derived from whole blood that undergoes different biochemical processing. Serum preparation involves coagulation (approximately 20-30 min) of the whole sample before centrifuging to separate the serum from cellular elements including fibrinogen, platelets, and other circulating proteins. During this process, platelets release proteins including pro-inflammatory cytokines into the serum which may affect cytokine levels and should be considered (Yu et al., 2011).

Plasma is obtained using an anticoagulant such as ethylenediaminetetraacetic acid (EDTA) or heparin which is added before the separation of blood cells via centrifugation. Comparison of serum and plasma measures have indicated that while absolute reproducibility value is significantly higher for plasma, both matrices present high reproducibility; 0.83 (plasma) and 0.80 (serum) respectively (Yu et al., 2011). Higher metabolite concentration is found in serum samples, making the serum analysis more sensitive regarding biomarker detection. However, as long as standardised blood preparation procedures are used, serum and plasma have high correlations (mean $r = 0.81 \pm 0.1$) in clinical and biological studies (Yu et al., 2011).

Enzyme-linked immunosorbent assay

Enzyme-linked immunosorbent assay (ELISA) is a widely used diagnostic and analytical tool for the detection and quantification of specific antigens or antibodies in a given sample (Gan & Patel, 2013). ELISA utilise the basic immunology concept of an enzyme-labelled antigen binding to its specific antibody, which allows detection of very small quantities within a fluid sample. Fundamentally, the antigen in fluid phase is immobilised and allowed to bind to a specific antibody, which itself is subsequently detected by a secondary, enzyme-coupled antibody. A chromogenic substrate for the enzyme yields a colour change indicating the presence of the antigen. Quantitative and qualitative measures can be assessed based on colorimetric reading (Gan & Patel, 2013).

Multiple based immunoassay

Multiplex bead array assay has been developed from traditional ELISAs with the purpose of measuring multiple cytokines in the sample at the same time. They differ methodologically depending on the utilization of flow cytometry, chemiluminescence or electrochemiluminescence technology. Flow cytometric multiplex arrays, also known as bead-based multiplex assay are the most common used

format. This format utilised bead sets which are coated with a specific capture antibody, and fluoresce or streptavidin-labelled detection antibodies bind to the specific cytokine-capture antibody complex on the bead set, which are distinguishable under flow cytometry. Multiple cytokines in a biological liquid sample can thus be recognised and measured by the differences in both bead sets, with chromogenic or fluorogenic emissions detected using flow cytometric analysis (Leng et al., 2008). Compared with ELISA, multiplex arrays have several advantages including higher throughput multiplex analysis, less sample volume needed and time/cost efficiencies. Additionally the ability to evaluate the levels of one given inflammatory molecule in the context of others, ability to perform repeated measures of the same cytokine panels for the same participants under the same experimental assay condition and the ability to reliably detect different proteins across a broad dynamic range of concentrations are considered advantageous (Leng et al., 2008). The major consideration of multiplex assays, by their nature, is the potential effect of interactions between multiple different antibodies and cytokines (antigens) in the assay solution. One cannot assume that a reliable uniplex assay can just simply be added to a functioning multiplex assay (Leng et al., 2008).

3.2.2 Glucose metabolism and insulin sensitivity

Glucose Metabolism

The oral glucose tolerance test (OGTT) is considered the gold standard surrogate marker of glucose metabolism and intolerance for research purposes (Singh & Saxena, 2010). Glucose test assesses the efficiency of glucose utilisation, indicated by how rapidly glucose is cleared from the blood stream (Borai, Livingstone, & Ferns, 2007; Singh & Saxena, 2010). The distinct advantage of the OGTT is the imitations of the normal glucose and insulin flux (Singh & Saxena, 2010). Additionally the OGTT method is considered a minimal risk procedure that is applicable for large scale screening in research protocols (Chen, Aguirre, & Hannon, 2018). Criticism of the OGTT includes the involvement of an

overnight fast for participants, time consuming, labour intensive and low reproducibility resulting in the requirement for multiple tests before diagnosis (Bennett, Guo, & Dharmage, 2007).

Indices of Insulin Sensitivity

While dynamic techniques can provide valuable information on insulin sensitivity, the invasive, technically demanding, and expensive procedures are not well suited for large scale investigations. Therefore, interest has developed in markers that correlate with insulin sensitivity and may provide effective alternatives. Quantitative insulin sensitivity check index (QUICKI) is an index of insulin sensitivity calculated from a single fasted glucose and insulin measurement. $QUICKI = 1 / [\log \text{insulin } (\mu\text{U}\cdot\text{mL}^{-1}) + \log \text{glucose } (\text{mg}\cdot\text{dL}^{-1})]$. QUICKI model expresses the intercepts of the data by taking both the logarithms and the reciprocal of fasting glucose and insulin product. Consequently the QUICKI method is considered more accurate than the Homeostasis Model Assessment (HOMA) over a broad range of insulin sensitivities (Borai et al., 2007). Fasting glucose may also be expressed as a ratio of insulin concentration to provide a screening test for insulin resistance, referred to as fasting glucose/insulin ratio (FGIR) (Borai et al., 2007).

HOMA is the result of a computed model in which fasting glucose and insulin values are plotted to allow assessment of the expected β -cell response and insulin function. The product of fasting glucose and fasting insulin is divided by a constant: $\text{fasting plasma glucose } (\text{mmol}\cdot\text{L}^{-1}) \times \text{fasting serum insulin } (\mu\text{U}/\text{mL}^{-1}) / 22.5$. The denomination reflect normalisation, based on normal fasting values as the result of the product of $5 \mu\text{U}\cdot\text{mL}^{-1}$ insulin levels and $4.5 \text{mmol}\cdot\text{L}^{-1}$ glucose levels (Antuna-Puente et al., 2011). HOMA strongly correlates with the euglycemic clamp ($r = 0.91$) (Gungor, Saad, Janosky, & Arslanian, 2004), and has been found to be more reliable and appropriate for large scale studies than the QUICKI method (Keskin, Kurtoglu, Kendirci, Atabek, & Yazici, 2005). In addition to the validated analysis of

insulin resistance, HOMA is predictive of adverse cardiovascular health. The finding from a meta-analysis suggest that the relative risk of CVD was higher with adverse HOMA concentration (increase of one standard deviation) compared to traditional deviation in fasting glucose or insulin concentration (Gast, Tjeerdema, Stijnen, Smit, & Dekkers, 2012). However, the key criticism of these simple indices based on fasted samples is the inability to distinguish between certain physiological circumstances. For example, it is possible for subjects to be significantly insulin resistant without having fasting hyper-insulinaemia. Furthermore, some individuals may be euglycaemic when fasted by hyperglycaemic following a 75 g oral glucose load. Finally, fasting blood glucose and insulin measures generally assess hepatic insulin resistance more than peripheral insulin sensitivity and assume the two measures are equal which is not always the case (Borai et al., 2007).

3.2.3 Body Composition

With the shift of all-cause mortality and morbidity to obesity related disorders and non-communicable diseases, the measurement of body composition has become an important component of health assessment (von Hurst et al., 2016). Simple measures of body mass or calculations of BMI are unsuitable to assess health status as researchers investigate and intervene in metabolic related disorders. As the risk of mortality has been shown to increase in relation to adiposity, the measurement of body fat percentage and distribution has become a key criteria for assessing risk and measuring the success of interventions aimed at risk reduction (von Hurst et al., 2016). Anthropometry is commonly used method to assess body composition that continues to play an important role in clinical practice and an essential field method in large population based studies (Ball, Altena, & Swan, 2004).

Dual energy x-ray absorptiometry (DEXA) passes filtered x ray beams at two different photon energies through the participant that are attenuated differentially by the material in their path. With the participants laying on the scanning table, the process maps the mass and composition of each pixel in terms of bone mineral, fat (FM) and fat-free soft tissue. FM is determined by the ratio of soft-tissue attenuation at the two energies and in vivo elemental composition supports the underlying physical concept of this being accurate. Multi-component models as a reference method to validate DEXA are extensive with standard error estimates (SEE) for predicting fat falling between 2 and 3 % representing a major advance in laboratory and clinical practice for estimating body composition. DEXA has been criticised for assuming consistent segment tissue composition, despite regional variation in water and lipid content of skin, adipose, muscle and bone tissue being identified (Ackland et al., 2012). Despite low radiation dose, caution is expressed regarding the cumulative radiation dose of multiple scans within a given time frame. Finally the calculation algorithms used to calculate composition may differ between manufacturers and are often not published (Ackland et al., 2012).

3.2.4 Heart rate variability

Heart rate variability (HRV) is a non-invasive cardio graphic maker measuring beat-to-beat fluctuations of cardiac contraction. Mediated by the ANS, HRV is reflective of neurocardiac function with healthy biological systems exhibiting temporal variation and responsiveness to stimuli (Shaffer & Ginsberg, 2017). Conversely, abnormalities of the ANS have been demonstrated in pathological conditions such as diabetes, coronary heart disease and ventricular arrhythmias (Sztajzel, 2004). Pathological cardiovascular function is associated with compensatory neural, and hemodynamic mechanisms to offset loss of function (Mendes-Ribeiro et al., 2009). The compensatory adjustments include increasing the activation of the SNS in an attempt to maintain cardiac function, however prolonged SNS activation is associated with increased risk of mortality (Mendes-Ribeiro et al., 2009; Shaffer &

Ginsberg, 2017). Conversely, vagal-mediated HRV is associated with an adaptive and self-regulatory ANS (Shaffer & Ginsberg, 2017).

The analysis of HRV can be separated into two broad approaches. The first of which involves the use of global descriptive statistics to characterise the distribution of intervals between successive heart contractions. This approach considers the entire population of contractions or the differences between adjacent contractions, as isolated or independent data samples and includes the use of range and standard deviation. The second approach involves extracting specific frequency components of variance, which describes the average power of the signal at a given frequency. This is achieved by modelling periodic patterns that relate to functional processes or physiological mechanisms and focus on the serial linkages among the entire data set (Berntson et al., 1997).

Time Domain

The first and computationally simplest HRV method assesses the variation in HR through statistical or geometric approach. First, the HR at a point in time, or the intervals between successive normal complexes are determined in continuous electrocardiographic (ECG) record. Each QRS (depicts ventricular depolarisation) complex is detected, and normal-to-normal (NN) intervals, difference between successive QRS complex's resulting from sinus node depolarisation is determined. The statistical method then uses the NN interval to determine simple descriptive time domain variables including the mean NN interval, mean HR and the range for a given time interval. More detailed information is provided by the statistical analysis of a continuous sequence of NN intervals for the time period of interest of which two subdivisions of statistical time domain methods exist. The first method is the standard deviation (SD) (the square root of variance) of the NN interval (SDNN), while

the second method involves measures derived from the differences between NN intervals; square root of the mean squared differences of successive NN intervals (RMSSD) (Task Force, 1996).

Frequency Domain

While time domain methods are computationally simple, spectral analysis provides insight into the modulatory effects of autonomic neural influences and mechanisms on the sinoatrial node (Acharya, Joseph, Kannathal, Lim, & Suri, 2006). Power spectral density (PSD) analysis describes the periodic oscillations of the HR signal decomposed at different frequencies and amplitudes providing information on the amount of variance in the hearts sinus rhythm (Sztajzel, 2004). The spectral power for a given frequency can then be quantified by determining the area under the curve within a specific frequency range. The two most common spectral analysis approaches are fast Fourier transform (FFT) analysis and autoregressive (AR) modelling. FFT is based upon the assumption that a time series is composed of only deterministic components (Billman, 2011). Conversely, AR view the data as being composed of both deterministic and random components. For shorter term recordings (2-5 minutes) there are three main peaks: very low frequency (VLF) 0.003-0.04Hz, low frequency (LF) 0.04-0.15Hz and high frequency (HF) 0.15-0.4Hz components. However the distribution of the power and the central frequency of LF and HF is not fixed and may vary in relation to changes in autonomic regulation of HR (Task Force, 1996). For example, HF analysis is shifted to a higher frequency range (or spectral band) of 0.24-1.04 Hz when measuring HRV during exercise (Billman, 2011).

The physiological explanation attributed to the VLF component of spectral analysis is not well defined and the existence of a specific physiological process attributable to this frequency may be questionable (Task Force, 1996). The VLF band has been interpreted as indicative of thermoregulation, kidney function (Reyes del Paso, Langewitz, Mulder, van Roon, & Duschek, 2013) and hormonal influence over HRV (Zhong, Jan, Ju, & Chon, 2006). However, it is commonly accepted that a major

constituent of VLF is the non-harmonic component, affected by algorithms of baseline or trend removal. Therefore VLF assessment during short term recordings is an unsupported measure and should be avoided when interpreting PSD (Task Force, 1996).

LF component of spectral analysis can be interpreted as a marker of cardiac sympathetic outflow (Malliani, Pagani, Lombardi, & Cerutti, 1991), partially explained by the metabolic properties of the sympathetic neurotransmitter, norepinephrine. Distinct to the rapid catabolism of acetylcholine, norepinephrine is metabolised relatively slowly and would therefore theoretically operate at lower frequencies (Pumprla, Howorka, Groves, Chester, & Nolan, 2002). However, β -adrenergic blockade results in reduced LF power and some conditions associated with sympathetic over excitation, report drastically diminished LF power (Sztajzel, 2004). Collectively, the LF component of HRV represents both sympathetic and parasympathetic regulation. Unlike the above frequency components there is broad evidence supporting the vagal influence on HF spectral analysis (Sztajzel, 2004; Task Force, 1996; Zhong et al., 2006). Parasympathetic nerve fibres regulate cardiac function on a beat-to-beat basis via releasing the acetylcholine from postganglionic terminals on the sinoatrial node. The sinoatrial node is rich in acetylcholinesterase, thus allowing a rapid breakdown of acetylcholine (Pumprla et al., 2002), therefore possessing a very short latency period and occurring at a high frequency (Task Force, 1996). An overview of spectral domain measure is provided in *Table 3.1*.

Table 3.1 Summary of Heart Rate Variability (HRV) collection methods commonly utilised during research

Measure	Description	Indicates
Time Domain		
SDNN (ms)	Standard Deviation (SD) of all the NN intervals	Square root of variance reflects the cyclic components responsible for overall variability
SDANN (ms)	SD of the average NN intervals calculated over short time periods (usually 5 mins) for the entire recording period (usually 24 h)	Measure long-term fluctuation and is less subject to editing error than SDNN
RMSSD (ms)	The square root of the mean squared differences between adjacent NN intervals	Average change in interval length between beats reflects short term variability
SDNN index (ms)	Mean of the SD of the NN intervals calculated over short periods of time (usually 5 mins) for the entire recording period (usually 24 h)	Reflects the average of changes in NN intervals over recording period
NN50	The number of pairs of adjacent NN intervals that differ by more than 50 ms	In the presence of normal sinus rhythm and atrioventricular-nodal function, these measures indicate ventilator driven PNS modulation in the short term
pNN50 (%)	NN50 divided by the total number of NN intervals x 100	
Frequency Domain		
Very Low Frequency (ms ²)	Power in the very low frequency (≤ 0.04 Hz)	Not clearly defined, may include artefact/noise
Low Frequency (ms ²)	Power in the low frequency (0.04-0.15Hz)	Both SNS and PNS influence
High Frequency (ms ²)	Power in the high frequency (0.15-0.4Hz)	Respiratory sinus rhythm and PNS
LF/HF	Ratio of LF (ms ²)/HF (ms ²)	ANS balance
ms: millisecond, h: hour, N-N: consecutive intervals of 'R' spike in an electrocardiogram defined as normal (N) via sinoatrial node origin %: percentage. Adapted from (Billman, 2011; Task Force, 1996)		

3.2.5 Sleep Assessment

Sleep entails multiple levels of behavioural, physiological, and neural activity characterised and assessed by multiple measures. These include duration, quality, brain activity patterns or sleep architecture and the phase or circadian aspects (Sadeh, 2011). As such, with multiple aspects of sleep in combination with the widely accepted relevance of sleep research and associated health benefits, both subjective and objective measures have been developed to evaluate sleep quality in both research and clinical settings (van de Water, Holmes, & Hurley, 2011).

Objective sleep assessment

Polysomnography (PSG)

As a diagnostic reference tool and objective measures of sleep, polysomnography (PSG) is considered the gold standard and conventional method. Recordings are obtained from at least four channels measuring brain activity (electroencephalogram; EEG), eye movements (electro-oculogram; EOG), muscle activity (electromyogram; EMG), and cardiac activity (electrocardiogram; ECG), breathing efforts and oxygen saturation (oximetry) which are then scored either automatically or manually by appropriately trained technician (Sadeh, 2011). Information including total sleep time, sleep-onset latency, wake after sleep onset, sleep efficiency, sleep fragmentation index, number of awakenings, time in each sleep stage and sleep stage percentages is then obtained and enables clinical research and diagnosis of a variety of sleep disorders. Criticism of PSG highlight impractical use in a clinical environment, labour-intensive to set up and examine and expensive due to its specialised equipment and requiring a certain level of expertise to be assessed correctly. Additionally, Type 1 PSG requires the participant to sleep in unique, unfamiliar environment (laboratory setting) with an assortment of attached electrodes and sensors which could both, hypothetically, effect sleep (Sadeh, 2011; van de Water et al., 2011). While the development of portable home PSG technology (Type 2) allows analysis

from a familiar sleep setting and potentially reduces PSG-related costs (Bruyneel & Ninane, 2014), critique remains that the attachment of electrodes and sensors may affect sleep quality.

Actigraphy

The invasive and expensive nature of PSG recording has led to the development and validation of alternative sleep assessment methods. Actigraphy is a non-invasive and relatively easy data collection method combining recorded body movement and activity via a wristwatch, considered with data from sleep diaries. Activity levels are measured via accelerometers, either analogue system or piezo-electric beam within the device which are translated to digital counts accumulated across pre-designed epoch intervals and stored in the internal memory (Sadeh & Acebo, 2002). Threshold based algorithms (set post hoc) use activity counts calculated from each epoch during the monitoring period, which combined with subjective sleep diaries recording activity, are used to estimate sleep and wakefulness, (Kosmadopoulos, Sargent, Darwent, Zhou, & Roach, 2014). All time is scored as awake unless the sleep diaries indicate the participant was lying down attempting to sleep, this combined with a sufficiently low activity count recorded via the monitor is scored as time asleep (Halson, 2014). The activity-based criteria for sleep are the main distinction between actigraphy and PSG, which relies on stereotypical brain electrical activity to define sleep. Period of wrist inactivity may or may not align with changes in brain wave activity resulting in actigraphy overestimating sleep periods and an inability to diagnose certain sleep complaints including insomnia where wakefulness may not be associated with movement (Marino et al., 2013).

Actigraphy is the most widely used and validated alternative to PSG to objectively record general sleep patterns in the non-laboratory setting (van de Water et al., 2011). PSG-actigraphy correlations between whole night measures of sleep and sleep efficiency are reasonable (≥ 0.80) for normal

individuals and participants suffering from sleep disorders (Kushida et al., 2001; Sadeh & Acebo, 2002). High correlation is also reported and validated among shift workers for all sleep periods, however sleep efficiency correlations (Spearman ranked correlation coefficient) were extremely variable (0.72-0.15). The research concluded that while some caution should be used for specific measure such as sleep efficiency, actigraphy is a valid measure of sleep/wake activity and sleep duration (Reid & Dawson, 1999). Additional evaluation of actigraphy in comparison to PSG including 77 participants of varying health status, sleep complaints and night work concluded that actigraphy is useful and valid means of measuring total sleep time and wakefulness after sleep onset. However some limitations included the ability to identify periods of wakefulness especially in sleep disorders where wakefulness may not be associated with movement (Marino et al., 2013).

Subjective sleep assessment

Although sleep and sleepiness can be measured by objective means such as PSG and actigraphy, these methods are often impractical as clinical screening or research tools. Self-report questionnaires are cost-effective alternatives used to assess subjective sleep quality, subjective effect of interventions and supplement objective data to determine the risk of sleep conditions (O'donnell et al., 2009). Many different instruments have been developed to measure sleep quality, insomnia, and daytime sleepiness, but two of the most widely used are the Pittsburgh Sleep Quality (PSQI) and the Epworth Sleepiness Scale (ESS). The PSQI is a 19-item self-rated questionnaire for evaluating subjective sleep quality over the previous month. The 19 questions are combined into seven clinically derived component scores, each weighted equally from 0-3. The seven component scores are added to obtain a global score ranging from 0-21, with higher scores indicating worse sleep quality. The clinical and psychometric properties of the PSQI have been formally evaluated with results indicating an 89.6% sensitivity and specificity of 86.5% for identifying cases with sleep disorders, using a threshold of five. Validity is further supported by similar differences between groups using PSQI or PSG sleep measures

(Buysse et al., 2008). The ESS consists of eight self-rated items, each scored from 0-3, that measure a subjects habitual 'likelihood of dozing or falling asleep' in common situations of daily living. No specific time frame is specified. The ESS score represents the sum of individual items, and ranges from 0-24. Values >10 are considered to indicate significant sleepiness. The ESS is sensitive to change in clinical status, as evidenced by improvements following treatment of sleep apnea with continuous positive airway pressure. The psychometric analyses of the ESS support its internal consistency and uni-dimensionality, although factor analyses have indicated some variation in the number of identifiable factors (Buysse et al., 2008). An overview of sleep assessment procedures is provided in *Table 3.2*.

Table 3.2 Objective and subjective sleep assessment methods commonly used in sleep-related research

	<i>Advantages</i>	<i>Disadvantages</i>
Polysomnography (PSG)	<ul style="list-style-type: none"> • Provides the most detailed information on sleep architecture and clinical diagnosis • Provides objective assessment of daytime sleepiness • Ambulatory polysomnography may increase the advantages in normative samples 	<ul style="list-style-type: none"> • Expensive and labour-intensive scoring requiring expertise • Usually an intrusive unnatural sleep environment • Less informative for daytime sleep, regular night waking, behavioural insomnia and schedule disorders • Insufficient reliability and validity of some data parameters and safety issues
Actigraphy	<ul style="list-style-type: none"> • Enables cost-effective, non-intrusive 24 h monitoring at home for extended periods • Requires no installation and sleep-wake scoring is relatively easy • Provides good data for extended periods and therefore recommended for follow-ups and assessment of schedule disorders 	<ul style="list-style-type: none"> • Only measures activity • Does not provide data on sleep staging, breathing or specific behaviours • Artefacts related to induced external motion, device removal, and motionless wakefulness are threats to validity
Subjective Reports	<ul style="list-style-type: none"> • Questionnaires and diaries are easy, cost-effective, and can measure a wide range of sleep parameters in various contexts • While the correspondence between subjective and objective measures of quality is modest, subjective report provides important and unique information. 	<ul style="list-style-type: none"> • Information is influenced by response biases, compliance and subject burden • Lack of standardisation and norms stratified by age, gender, ethnicity for comparative purposes.

Adapted from (Sadeh, 2011).

3.3 Recruitment and study protocol

Sex characteristics of shift work

To limit potential co-founding factors and independent influences on markers of cardio-metabolic function female shift workers were excluded from the research projects presented in this Thesis. Shift work employs up to 1.5 million Australians (ABS, 2020) across a range of industries with both female and male employees contributing to the total labor force. However, the menstrual cycle has previously been demonstrated to independently effect the serum concentrations of hs-CRP in both normal-weight and overweight subjects (Blum et al., 2005). Further, Attarchi and colleagues (2013) demonstrated that night and rotational shift work can disrupt normal physiological response of the menstrual cycle. In total, 406 female employees were sub-divided by shift or fixed daywork occupation and compared in terms of the frequency of menstrual disorder and hormonal values. The odds ratio for menstrual disorder among shift workers was 5.54 compared to non-shift workers, despite no significant differences observed in hormonal values (Attarchi, Darkhi, & Kashanian, 2013). Similarly, a longitudinal study assessing the effect of 12 hour rotating shifts on menstrual cycles compared menstrual cycle characteristics of rotating shift workers and fixed daytime office workers (Su, Lu, Kao, & Guo, 2008). The project concluded that the prevalence of cycle irregularities was significantly higher in the shift work group (Su et al., 2008).

Consideration of menstrual phase is therefore highlight as a key ethical and methodological factor, with research projects advised to identify the specific phase and standardize testing times (Portaluppi, Smolensky, & Touitou, 2010). Collectively, the research group acknowledge that female shift workers make up a substantial percentage of shift work demographics and research is needed to explore pathogenic mechanisms and intervention strategies. However, given the difficulty in testing shift workers with rotating availabilities, the disruptive influence of rotating shift work on

menstrual cycles and independent effect of the menstrual cycle on key markers (inflammation and insulin sensitivity), female participants were excluded from the research projects.

Study Protocol – Recruitment

Participants were recruited from the local Bathurst area from multiple industries including but not limited to manufacturing, health, and emergency services. *Section 2.3* of the Literature Review previously outlined the independent effect of shift length, direction of rotation and structure of recovery days on cardio-metabolic risk factors (Viitasalo et al., 2008). Due to required participants following G power analysis (*Study 1 – Chapter 4*) and difficulties with recruitment, the inclusion criteria were expanded to allow males currently employed in rotating shift work regardless of occupation or specific structure. As such, the recruitment of participants from multiple industries with variations (outlined in *Study 1 – Chapter 4*) of shift structure may impact the interpretation of results.

Study Protocol - Intervention

As outlined in *Figure 1.3*, Thesis studies were designed to investigate both potential mechanistic causes of impaired cardio-metabolic function and exercise-based intervention among male shift workers. Consequently (outlined in *Appendix B*) shift workers recruited for *Study 1* were offered the opportunity to participate in the exercise interventions if they met the subsequent inclusion criteria. To limit the potential effect of lifestyle related behaviors, pre-existing medical conditions (diagnosed cardiovascular, metabolic or sleep) and exclude conditions limiting physical capacity (to participate in exercise training) subjective screening took place pre-inclusion. Screening consisted of several questionnaires including the ESSA Adult pre-exercise screening system (APSS) and general health questionnaire. Historically, a medical clearance has been used as the recommended criteria to

commence exercise participation, however this places the responsibility on physicians not often provided with the training or time (in standard consultation) to effectively perform the role. Further, the necessity of medical clearance may create a cost burden and potential barrier to the uptake of exercise (Maiorana et al., 2018). In Australia the Adult Pre-Exercise Screening System (APSS) was developed through a collaborative venture between the peak bodies for exercise science and exercise physiology, sports medicine, and personal training (Norton & Norton, 2011). The general health questionnaire was further used to subjectively identify any diagnosed cardio-metabolic or sleep disorders.

Testing and exercise intervention were conducted on site at Charles Sturt University in the Exercise Physiology Laboratory and Gym facilities. Testing times were standardized between 0600 – 0900 across the three studies. Consistent timing was selected to limit the potential effect of circadian rhythms on key variables (inflammatory markers and insulin sensitivity) with the morning selected as participants had to arrive in a fasted state (~12 hours) to complete the OGTT. Testing was also conducted following a day off or previous day shift to limit the acute effect of circadian disruption or sleep restriction following a night shift (Donga et al., 2010; Reynolds et al., 2012). While previous research has demonstrated one night of recovery sleep may not be sufficient to attenuate effects on inflammation and insulin sensitivity, prolonged disruption is dependent on the restriction or disruption protocol (Faraut et al., 2012). Given participants were recruited from several industries and labour structures, one day of recovery was selected to be both realistic and reflective of 'normal' shift work effect.

Exercise training

Exercise is proposed as viable intervention among shift workers because of the broad health benefits including augmented cardio-metabolic function (Warburton et al., 2006). However, the physiological adaptations and subsequent decreases in pathogenic risk are mediated by stimulus (Baar, 2009). The current exercise guidelines advise adults (aged 18-64 years) to undertake regular PA, with health benefits observed when average weekly volumes of moderate-intensity aerobic activity exceed 150 minutes (Bull et al., 2020). Moderate-intensity describes a range of exercise below the anaerobic threshold, with 60-75% of age-predicted HR maximum previously prescribed among shift workers (Atlantis, Chow, Kirby, & Fiatarone Singh, 2006; Atlantis et al., 2004; Lim et al., 2015). Muscle-strengthening activities at moderate or greater intensity is additionally advocated on 2 or more days per week. An update on previous recommendations is the exercise bouts may be accrued in bouts of any duration with total exercise volume rather than bout length a key factor in health benefits (Bull et al., 2020). The labour structure of shift work appears to impose organisations limitations on exercise participation (Nea et al., 2017). For example, limited break time between consecutive shifts may fail to provide sufficient time to meet the recommended PA guidelines. Further shift rotations may provide exercise opportunities outside gym facility operating hours (Blake et al., 2017; Nea et al., 2017), and prevent a set routine for exercise participation (Blake et al., 2017; Nea et al., 2017). Specific to shift workers, limited exercise research has currently been conducted to further inform exercise guidelines for protocol design (Flahr et al., 2018). Consequently, an exercise intervention was designed to investigate potential differences in exercise-based adaptation (specific to stimulus) while meeting the prescribed exercise guidelines (minimum of 150 minutes). Resultantly, both the effect of acute exercise bouts at different intensities and chronic training adaptation with two conventional training approaches (aerobic and resistance) were designed. Strategies to minimise the perceived barriers to exercise adherence include providing a free, pre-programmed, individualised (regarding resistance and intensity) training intervention to be completed at any time during staffed hours at a local fully function gym.

Chapter Four:

Inflammatory status and cardio-metabolic risk stratification of rotational shift work.

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Title: Inflammatory Status and Cardio-metabolic Risk Stratification of Rotational Shift Work.

Running Title: Cardio-metabolic risk of shift work

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Abstract

Purpose: To investigate the physiological effects of rotational shift work on measures of cardio-metabolic function.

Methods: Sedentary, healthy men ($n = 87$; age 37 ± 9 y; BMI: 30.7 ± 5.1 kg·m²) were recruited and categorised via occupation. SHIFT group: currently employed in rotational shift work defined by 8-12 h morning, afternoon, and night rotations; or NSHIFT: working fixed daytime hours. Testing procedures included baseline objective sleep assessment and laboratory testing, conducted between 0600-0900 hours to assess body composition, cardiorespiratory fitness (VO_{2peak}), inflammatory status (C-reactive protein (CRP), interleukin (IL)-6 and tumor necrosis factor-alpha (TNF- α)), glucose metabolism, heart rate variability (HRV) and self-reported leisure time physical activity (PA).

Results: SHIFT reported significantly less leisure time PA ($p = 0.019$), reduced VO_{2peak} ($p = 0.007$), higher body fat percentage (BF %) ($p = 0.021$), increase response time to oral glucose tolerance test (OGTT) ($p = 0.016$) and higher IL-6 values ($p = 0.008$) compared to NSHIFT. A significant difference was observed in actigraphy measured total sleep time (TST), with SHIFT recording reduced sleep following a night shift ($p = 0.001$). No group difference was observed in HRV or average sleep parameters ($p > 0.05$). Linear regression identified a significant association between occupation and inflammatory status ($p = 0.006$).

Conclusion: Rotational shift work is associated with increased risk factors for cardio-metabolic disorders, despite no differences in sleep quality and quantity. The results suggest rotational shift work has a detrimental effect on the health and well-being of employees; with homeostatic desynchronisation identified as potential pathogenic mechanisms.

Key words: shift work, cardiovascular disease, metabolic syndrome, cardio-metabolic, pathogenesis.

What's important about this paper?

Rotational shift work is associated with an increased incidence of cardio-metabolic conditions; however, several methodological inconsistency and disease co-factors limit specific mechanistic interpretations. The current project demonstrated that when matched for co-factors including behavioural factors, current health status and sleep, employment in rotational shift work significantly increased markers of future cardio-metabolic disease risk including fat mass, inflammatory markers and response time to a glucose challenge. Results which highlight the potential role of homeostatic desynchronisation in disease states and may help shape future interventions aimed at improving the health and well-being of employees.

Introduction

Shift work is a labour structure associated with increased incidence of cardio-metabolic disorders (Sookoian et al., 2007; Torquati, Mielke, Brown, & Kolbe-Alexander, 2018; Wang, Armstrong, Cairns, Key, & Travis, 2011). The cardio-metabolic system is moderated by several biological systems including circadian and sleep-wake cycles, with shared anatomical structures and oscillatory interactions integrating endo and exogenous signals to provide predictive function (Irwin & Cole, 2011; Zimmet et al., 2019). Within this integrated system, conflicting biological cues are capable of disrupting homeostatic regulation (Irwin & Cole, 2011), acutely impacting function and facilitating pathogenesis under chronic conditions (Dandona et al., 2004; Leproult et al., 2014). While the exact mechanisms require further research, the labour characteristics of shift work, specifically rotational shifts and night work, provide conflicting biological cues capable of disrupting homeostatic regulation (Flahr et al., 2018; Kecklund & Axelsson, 2016; Kervezee et al., 2018). Therefore, homeostatic desynchronization has emerged as a viable pathogenic mechanism facilitating the adverse health conditions observed among shift workers.

The acute cardio-metabolic effect of homeostatic desynchronization has previously been explored within laboratory conditions, with simulated shift work decreasing insulin sensitivity (Gonnissen et al., 2013; Leproult et al., 2014) and vagal autonomic modulation (Morris et al., 2016). Acute effects that appear to manifest as chronic health conditions, with observational research identifying impaired glucose metabolism (Suwazono et al., 2009) and increased relative risk of cardiovascular disease (CVD) and metabolic syndrome (MetS) (Sookoian et al., 2007; Torquati et al., 2018; X. Wang et al., 2011). Further, pro-inflammatory cytokines have shown acute (Leproult et al., 2014; Wright Jr et al., 2015) and chronic (Puttonen et al., 2011) responses to shift work. Cytokines coordinate inflammatory responses, and are capable of acute redistribution of resources in response to stressful stimuli (Del Giudice & Gangestad, 2018). Consequently, cytokines have been used to indicate

inflammatory status, are predictive of future disease risk (Lange et al., 2010), and, within chronic systemic conditions, are hypothesised to have an aetiological role in cardio-metabolic pathogenesis (Berg & Scherer, 2005).

However conflicting results have been reported regarding shift work and the long-term health effects. Previous meta-analysis have observed inconclusive or non-significant association for both CVD (Hublin et al., 2010) and MetS (Canuto, Garcez, & Olinto, 2013). Consequently, a direct causal role is disputed, with criticisms including an inconsistent definitions of shift work, methodological problems and misclassification of measures potentially inflating the risk of adverse health effects (Kecklund & Axelsson, 2016). Specifically, variations in shift direction, speed of rotation, length of shifts and number of recovery periods (days off) have independent effects on shift work physiology (Bøggild & Jeppesen, 2001) and must be considered. Further, while robust support exists for circadian misalignment and disturbed sleep facilitating pathogenic immunological and cardio-metabolic states (Kecklund & Axelsson, 2016), confounding factors including obesity and reduced physical activity (PA) levels may have independent cardio-metabolic effects (Torquati et al., 2018).

Therefore, further investigation of shift workers cardio-metabolic health and potential pathogenic mechanisms are required. The study aims to assess the effect of rotational shift work on biological risk factors for the development of cardio-metabolic disorders among currently healthy shift workers compared to non-shift counterparts. It is hypothesised that employment in shift work will be identified as an independent risk factor for reduced sleep time, sleep quality and cardiorespiratory fitness, impaired glucose metabolism and increased inflammatory markers and adiposity.

Methodology

Participants:

The participant inclusion criteria required volunteers to report as currently inactive but healthy men, defined as: non-smoking, no known cardio-metabolic, sleep or mood disorders, however not meeting the recommended 150 minutes (min) of PA per week. Resultantly, following the screening process conducted initially via interview and confirmed during the familiarisation session, 11 of the 98 volunteers were excluded for a total study population (n) = 87 (data in Table 1). Following recruitment, participants were categorised based on occupational status. Shift work participants (SHIFT; $n=44$): currently employed in rotational 8-12 hour (h) shifts across a variety of industries including health, emergency services and manufacturing. The 8 h rosters included clockwise rotations of four days on and four days off, cycling through morning (0600-1400), afternoon (1400-220) and night (220-0600) shifts (n =10). The 12 h shift workers complete two days (0600-1800) and two nights (1800-600), followed by four days off (n =19), and 15 participants worked a combination of 8 h (weekday morning, afternoon and night) and 12 h (weekend day and night) shifts, however all groups had optional overtime. Non-shift work (NSHIFT; $n=43$) were categorised as participants employed in fixed 'normal' daytime hours. All participants gave their written and informed consent before commencing the study which was approved by the Institutional Human Ethics Committee in line with the principles of the Declaration of Helsinki.

Study Overview:

Following pre-screening and recruitment, eligible participants attended a familiarisation session where they completed initial screening (Adult Pre-exercise Screening Tool, Depression Anxiety Stress Scales and a general health and wellbeing questionnaire) anthropometry assessments and a maximal exercise test. A wrist-worn actigraphy device (Actiware 2, Philips Respironics, Andover, MA, USA) and sleep diary were issued to be recorded over a 14-day (d) period, before returning in a fasted state (~12 h) for a laboratory testing session. The session included baseline venous blood sample for assessment

of inflammatory markers, oral glucose tolerance test (OGTT) and dual x-ray absorptiometry (DEXA) scan for body composition.

Familiarisation Session

Following the explanation and demonstration of measures and procedures, participants completed an Adult Pre-exercise Screening Tool (Norton & Norton, 2011), Depression Anxiety Stress Scales (DASS21) (Lovibond & Lovibond, 1996) and a general health and wellbeing questionnaire to determine current health status and medication use. Body mass was recorded wearing minimal clothing, using calibrated electronic scales (A and D HW-PW200, Thebarton, SA, Australia). Height was recorded using stadiometer in an upright position with head in the Frankfort plane. Waist circumference were measured at the mid-point between the costal arch and the iliac crest and hip circumference were measured in line with the greater trochanter (steel tape; EC P3 metric graduation, Sydney, NSW, Australia)(World Health Organization, 2011). The graded maximal exercise test was conducted on a stationary cycle-ergometer (Wattbike Trainer, Smeaton Grange, NSW, Australia), had an initial power output of 50 W, before incremental increase of 25 W per one minute stage until volitional exhaustion. Expired gases and flow volumes were collected during the GXT and analysed by a calibrated metabolic cart (TrueOne 2400, ParvoMedics, Salt Lake City, Utah) to provide peak oxygen uptake (VO_{2peak}).

Sleep Quality Assessment

Actigraphy was recorded in 30 s epochs over a 14-d period and analysed using Actiware v5.70 software (Philips Respironics) in correspondence with participant's sleep diary recorded during the collection period. Variables obtained included bedtime, wake time (identified by participant in sleep diary), time in bed (TIB), total sleep time (TST), sleep latency (period between bedtime and sleep onset), sleep efficiency (percentage of time in bed spent sleeping), total time awake after sleep onset (WASO), and number of awakenings during total sleep period which were averaged across the collection period. If the actigraphy device was removed (assessed via sleep diary and a zero-activity measurement), or

technical difficulties were experienced within an hour of documented bed or rising time, data of the effected time and subsequent sleep period was manually excluded (Ancoli-Israel et al., 2015).

Laboratory testing session

Participants arrived between 0600 and 0900 h in fasted state for standardised testing. Shift workers were tested following either a day off or previous day shift to minimise the potential residual effects from acute sleep deprivation on night shift. Participants underwent an OGTT with blood samples taken at baseline and in 30 min increments for a total for 120 min, before undertaking a DEXA scan and 5 min rested heart rate variability (HRV) analyses.

Measures:

Venous Blood Collection and Analysis

Participants underwent venepuncture procedures with a cannula inserted into a medial antecubital vein. Blood was drawn manually by syringe and ejected into pre-chilled tubes treated with ethylenediaminetetraacetic acid and serine protease inhibitor (Pefabloc SC, Sigma-Aldrich, Sydney, Australia) to prevent cytokine degradation. Drawn blood was also ejected into serum separator tubes and left to clot for ~20 min. The pre-chilled and clotted serum tubes were centrifuged for 10 min at 4° C, before being stored at -80° C until analyses. Analyses was performed with commercially available enzyme-linked immunosorbent assays (ELISA kit). Serum was analysed for C-reactive protein (CRP) (Human CRP ELISA kit/catalogue no. EK-0040; Crux Biolabs, Scoresby, VIC, Australia), TNF- α (Human TNF-alpha ELISA kit/catalogue no. EK-0001; Crux Biolabs, Scoresby, VIC, Australia) and insulin (Human Insulin ELISA kit/catalogue no. ELH-Insulin-1; Raybiotech, Scoresby, VIC, Australia). Plasma was analysed for IL-6 (Human IL-6 ELISA kit/catalogue no. EK-0012; Crux Biolabs, Scoresby, VIC, Australia). The manufacturer's specified assay and analytical methods were used throughout. All samples were completed in duplicate with minimum detectable cytokine levels for each kit: IL-6 (<5 pg/ml), TNF- α (<5 pg.ml⁻¹), CRP (<0.1 ng.ml⁻¹) and Insulin (<4 uIU.ml⁻¹) respectively. Duplicate readings for each

standard, control and sample were averaged and the average zero standard optical density reading was subtracted. A standard curve was created using a 4-parameter logistic curve fit. Concentration values were then determined, using this curve for control and sample average optical densities per the ELISA kit instructions. The Pearson product-moment correlation coefficient (r) was determined by comparing the known standard concentration with the curve fit; r was shown to be greater than 0.98 for all the assays.

Oral Glucose Tolerance test

Testing procedures were designed in accordance with the American Diabetes Association guidelines (American Diabetes Association, 2010). Morning blood glucose levels were obtained via venepuncture following an overnight fast \sim 12 h. Participants consumed a 75 g oral glucose load dissolved in 300 ml carbonated water within 5 min (Carbotest, Thermo Fisher Scientific, Australia). Venous whole blood levels were drawn pre glucose load and incrementally every 30 min for 120 min. Glucose response was measured via Accu-Chek Performa 2 blood glucose monitoring system (Roche Diagnostics, Castle Hill, NSW, Australia).

Heart Rate Variability

Assessment of HRV was conducted and analysed following standardised procedures (Task Force, 1996). Participants were instructed to avoid strenuous exercise for 48 h prior to testing, and stimulants such as caffeine or alcohol 12 h prior to testing. Arriving in a fasted state (\sim 12 h), participants wore a heart rate (HR) chest strap and rested in a semi-recumbent position for 5 min before HR was recorded using Polar Team Sport 2 system (Polar Team 2, Polar Electro Oy, Kempele, USA). R-R interval data were then exported as a text file to be visually analysed for ectopic beats. If abnormal R-R intervals were identified, manual editing or interpolation was performed by replacing abnormal R-R interval with the average of the two adjacent R-R intervals. HRV recordings were excluded from further analysis if they had frequent atrial or ventricular ectopic beats, which was defined as more than 3 per cent of all heartbeats (no participant was excluded). Data were then exported to the HRV analysis

software (Kubious heart rate variability version; Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio, Kuopio, Finland) for analysis of the following HRV parameters (1) time domain: resting heart rate (RHR), R–R intervals, standard deviation of normal-to-normal intervals (SDNN), mean square root differences of the standard deviation (RMSSD), percentage of beats that changed more than 50 ms from the previous beat (pNN50); and (2) frequency domain: low-frequency normalised units (LF nu) and high-frequency normalised units (HF nu).

Dual-energy x-ray Absorptiometry and anthropometric assessment

A supine DEXA scan was undertaken to estimate whole-body composition (GE Lunar Prodigy, GE Healthcare, Madison, WI, USA). Participants were positioned centrally on the bench of the DEXA machine and a whole-body scan was completed with a resolution of 4.5×9.0 mm and scanning speed of $130 \text{ mm}\cdot\text{sec}^{-1}$. The scan was then analysed with customized software (Illuminatus DXA, version 4.2.0, Turnbull, CT, USA) for lean mass (LM), fat mass (FM) and fat free mass (FFM) in absolute (kg) and relative (%) terms

Data Analysis

Data are reported as mean \pm standard deviation (SD) with statistical significance accepted when $p < 0.05$. Analyses were performed using SPSS Statistics v 26.0 (IBM Corp., Armonk, NY, USA). Glucose area under the curve (AUC) was assessed using the absolute sum trapezoidal method (Metcalfe, Babraj, Fawcner, & Volvaard, 2012). Independent samples *t*-tests were completed to determine significant differences in reported exercise participation, BMI, waist-to-hip ratio (WHR), inflammatory status, glucose AUC and both relative and absolute maximal oxygen consumption ($\text{VO}_{2\text{peak}}$). Multiple analyses of variance (MANOVA) models were used to determine significant differences for HRV, glucose metabolism (response to OGTT), body composition and objective (actigraphy) sleep assessment. If a significant difference was observed, tests of between-subject effect were assessed using a Bonferroni adjustment. A standard multiple regression analysis was conducted to assess the interrelationship and independent predictive value of occupation on

markers of cardio-metabolic health, with occupation, body fat %, interleukin 6 and VO_{2peak} used as test variables. Following the initial regression analysis, beta coefficients were inspected for comparison of the contribution for each independent variable.

Results

Descriptive Characteristics

Baseline descriptive characteristics of participants are shown in Table 1. A significant difference between groups was observed for peak relative oxygen consumption (VO_{2peak} ml.kg.min⁻¹); $t(85) = 2.782$, $p = 0.007$, eta squared = 0.08, with lower values reported among SHIFT. Additionally, a significant reduction in reported minutes of PA per week; $t(85) = 2.392$, $p = 0.019$, eta squared = 0.063 was observed among SHIFT. No significant differences were observed for WHR, BMI, absolute VO_{2peak} (L.min⁻¹) or BP ($p > 0.05$).

Objective Sleep Assessment

23 participants were excluded due to technical difficulties experienced during data collection, resulting in a total sample size of SHIFT ($n = 31$) and NSHIFT ($n = 33$). No significant differences were observed between SHIFT and NSHIFT groups for the combined dependant variables of time in bed, total sleep time, latency, efficiency, WASO and number of awakenings (Table 2) $F(6, 57) = 1.103$, $p = 0.372$; Wilks Lambda = 0.89; partial eta squared 0.104. A significant difference was observed within the SHIFT group comparing the same variables for days on night shift vs days off or day shift (Table 2) $F(6, 55) = 2.520$, $p = 0.032$; Wilks Lambda = 0.78; partial eta squared 0.216. Following Bonferroni adjustment alpha level of 0.008, significant difference was observed for TIB ($p = 0.001$) and TST ($p = 0.001$).

Inflammatory Profile

Multiple participants reported metabolite levels below detectable range, therefore total population values (n) are indicated with inflammatory results in Table 3. SHIFT recorded significantly higher concentrations of IL-6 $t(22.49) = 2.936, p = 0.008, \eta^2 = 0.15$; while no significant differences were observed for CRP or TNF- α ($p > 0.05$).

Glucose Metabolism

A significant difference was observed between occupation groups $t(82) = 3.623, p = 0.001, \eta^2 = 0.138$, with SHIFT reporting higher total glucose AUC in response to OGTT (Table 3). Figure 1 shows the blood glucose values in response to the OGTT. A significant difference was observed between SHIFT and NSHIFT employees for the combined dependant variables (time points), $F(5, 78) = 3.007, p = 0.016$; Wilks Lambda = .84; partial $\eta^2 = 0.162$. No difference was observed at pre ($p = 0.966$), however significant differences were observed at T30 ($p = 0.002$), T60 ($p = 0.002$), T90 ($p = 0.008$) and T120 ($p = 0.009$) with SHIFT values higher at each time point. Insulin values among NSHIFT group were below detectable ranges and were therefore removed from analysis.

Body Composition

Table 4 depicts body composition measures in which a significant difference was observed between SHIFT and NSHIFT employees for body fat (kg), lean mass (kg) fat free mass (kg) and body fat (%), $F(4, 76) = 4.893, p = 0.001$; Pillai's Trace = 0.205; partial $\eta^2 = 0.205$. A significant difference was identified between groups for fat mass ($p = 0.028$) and fat mass as a percentage ($p = 0.021$) with SHIFT reporting higher values for both.

Heart Rate Variability

Total sample size for HRV were $n = 31$ for SHIFT and $n = 26$ for NSHIFT (Table 5). No significant differences were observed between shift and non-shift groups for SDNN, RMSSD, pNN50, HF (n.u.) and LF (n.u.), $F(5, 51) = 1.217, p = 0.315$; Wilks Lambda = 0.89; partial $\eta^2 = 0.107$.

Regression Analysis

Regression analysis resulted in a significant value for occupation ($f(3, 43) = 4.999$, $p = 0.005$, $R^2 = 0.259$). A significant interaction was identified between occupation and IL-6 (Beta = 0.384, $t(46) = -2.91$, $p = 0.006$), but no significant interaction was observed between occupation and BF% ($p = 0.933$) or relative VO_{2peak} ($p = 0.096$).

Discussion:

The key finding of the present study is the significant increase in cardio-metabolic risk factors evident among 'healthy' rotational shift workers compared to their non-shift counterparts. Specifically, cardiovascular fitness was significantly lower, while fat mass, inflammatory markers (IL-6) and response time to an OGTT were significantly increased among the SHIFT group. Given the influential effect of the cytokine IL-6 on cardio-metabolic health, regression analysis was used to assess a relationship with occupation, with a significant association observed. Collectively, the present findings support previous laboratory interventions (Gonnissen et al., 2013; Leproult et al., 2014; Morris et al., 2016; Wright Jr et al., 2015) and cross-sectional studies (Sookoian et al., 2007; Torquati et al., 2018; X. Wang et al., 2011), suggesting an increased relative risk of cardio-metabolic disorders among shift workers. Further, our results add to the body of knowledge by identifying specific variables related to inflammatory and metabolic pathways.

In support of previous observations (Liu et al., 2018), the SHIFT group reported increases in both total fat mass and body fat as a percentage (Table 4). Increased adiposity is identified as a risk factor for the development of cardio-metabolic disorders (Poirier et al., 2006), with hypertrophic adipocytes hypothesised to have a direct mechanistic role in pathogenesis (Berg & Scherer, 2005). A number of factors may contribute to the observed increased levels of adiposity, including

behavioural factors such as poor diet and insufficient PA levels (Liu et al., 2018). However, sleep deprivation and circadian desynchronization also impact metabolic regulation (McHill et al., 2014), including altered hunger and satiety hormonal levels, and reduced energy expenditure (Liu et al., 2018). Specifically, simulated night shift work among healthy participants has previously resulted in decreased 24 h levels of satiety hormones, decreased daily energy expenditure and decreased carbohydrate and protein utilisation (McHill et al., 2014). As such, it is possible that the increased fat mass observed among the SHIFT group is a by-product of chronic homeostatic desynchronization facilitated by the labour structure. The current project aimed to limit the potential effect of excess adiposity co-factors including matching participants for reported PA levels (neither group meet the current activity guidelines). However, a significant difference in subjectively reported PA was observed (Table 1) and no data was collected regarding dietary profile, both limiting mechanistic observations.

Homeostatic desynchronization may further facilitate the decreased metabolic efficiency observed among shift workers (Gonissen et al., 2013; Kervezee et al., 2018; Leproult et al., 2014). Inclusion criteria for the current study excluded any participants with a current cardio-metabolic disorder, consequently, neither group met the criteria for impaired glucose tolerance (American Diabetes Association, 2010). However significant group differences were observed among the SHIFT group in both OGTT plasma glucose levels (Figure 1) and AUC (Table 3, Figure 1). The OGTT imitates the normal physiology of the glucose/insulin influx and may be used to evaluate glucose tolerance and future risk of developing type II diabetes mellitus (Singh & Saxena, 2010). Further, the glucose AUC indexes the whole glucose excursion after a glucose challenge, and has been widely used for calculating glycaemic index and sensitivity (Sakaguchi et al., 2016). Consequently, the significant differences observed may indicate a reduced metabolic response and predict future cardio-metabolic health problems.

The present study also identified a significant group difference and relationship (regression analysis) for occupation with increased IL-6 levels. IL-6 is unanimously regarded as an inflammatory biomarker, used to assess the presence and potential severity of low-grade inflammation (Del Giudice & Gangestad, 2018). Further, IL-6 has been implicated in the development of cardio-metabolic disorders including both insulin resistance (Dandona et al., 2004) and the earliest stages of atherosclerosis (Berg & Scherer, 2005). Several factors mediate proliferation of IL-6, however given the integration of the circadian and immune systems, circadian disruption may facilitate an immune response (Lange et al., 2010). Shift work systematically disrupting circadian rhythm may therefore contribute to the observed increase in IL-6 among the SHIFT group (Lange et al., 2010; Puttonen et al., 2011), and contribute to the adverse cardio-metabolic effect of rotational shift work. However, emerging evidence indicates IL-6 may also serve as a biological mediator, facilitating metabolic and somatic maintenance (Del Giudice & Gangestad, 2018). As such increased IL-6 in the absence of acute phase proteins (CRP) and associative pro-inflammatory markers (TNF- α) may indicate a different role of IL-6 in shift work metabolism (Del Giudice & Gangestad, 2018), an observation in need of further exploration.

While endocrine and inflammatory responses provide insight into the health of shift workers, co-factors for non-communicable disease states associated with shift work may also explain the pathogenic association (Kervezee et al., 2018). In agreement with previous literature, SHIFT reported significantly reduced leisure time PA (Peplonska et al., 2014), as well as reduced cardio-respiratory fitness (Table 1). Reduced fitness levels are independently associated with metabolic disturbances (Leite, Monk, Upham, Chacra, & Bergenstal, 2009) and increased body fat percentage (Bradbury, Guo, Cairns, Armstrong, & Key, 2017) among asymptomatic individual, potentially explaining the adverse results observed within the current study. However, reduced PA levels may be mitigated by feelings of fatigue (Hargens et al., 2013) and negative experiences of exercising at a time out-of-sync

with circadian rhythmicity. Consequently, reduced PA levels may be an additional consequence of circadian disruption and maladaptive sleep. In addition, the implicated pathogenic role of reduced PA may provide context for the design of future health interventions. Exercise presents as a plausible intervention strategy to improve shift worker health outcomes, however to date, limited research has been conducted to substantiate that hypothesis (Flahr et al., 2018).

Sleep plays an essential role in regulating cardio-metabolic function, with restricted or fragmented sleep promoting adverse health effects (Kecklund & Axelsson, 2016). Shift work systematically misaligns and, in some cases, inverts the sleep-wake cycle, theorised to disrupt sleep architecture and reduce TST (Lamond et al., 2003). Resultantly, it was hypothesised that the SHIFT group would be associated with reduced sleep quality, however no significant differences were observed (Table 2). Of note, a total of 23 participants had their sleep data excluded due to technical difficulties during data collection, which may impact the specific actigraphy observations. However, the lack of observable differences is supported by previous research (Åkerstedt et al., 2008; Marqueze et al., 2014), and may be explained by the use of additional sleep strategies to supplement sleep loss, for example increased naps or extended sleep on recovery days (Wickwire, Geiger-Brown, Scharf, & Drake, 2017). Further analysis of sleep opportunity following a night shift compared to day shift/day off identified significant difference in TIB and TST among the SHIFT group (Table 2). The results suggest that shift workers may be exposed to acute, significant reductions in sleep quality following night work or shift rotations, however when averaged across a 14 d collection period, the differences become non-significant. Further, despite no significant group differences, TST was less than seven hours which increases the risk for both ischemic stroke and the development of coronary heart disease (Luyster et al., 2012). Therefore, the reported total sleep duration among SHIFT (370 ± 43.8 min) may still play an aetiological role in the observed cardio-metabolic risk factors.

Of additional interest is the observation of a non-significant effect of occupation on supplementary markers of systemic inflammation (CRP and TNF- α) and autonomic function which contradicts the initial hypothesis and previous research (Morris et al., 2016; Puttonen et al., 2011). Simulated shift work has demonstrated a maladaptive effect on autonomic modulation (Morris et al., 2016) and pro-inflammatory markers (Leproult et al., 2014; Wright Jr et al., 2015), however the current project did not replicate those findings. The results may suggest a divergence in acute and chronic cardio-metabolic effects of rotational shift work. Circadian rhythm and sleep interact to provide oscillatory modulation of autonomic and immune function (Lange et al., 2010). Consequently, the acute effect of circadian disruption and sleep deprivation may be transient increases in inflammatory markers (TNF- α and CRP) as well as decreases vagal autonomic modulation (Leproult et al., 2014; Morris et al., 2016; Wright Jr et al., 2015). The chronic effect of repeated acute disruptions may however result in the observed significant differences in cardio-metabolic risk factors (Rüger & Scheer, 2009). Namely, steering the inflammatory profile towards a pro-inflammatory state (Lange et al., 2010), impacting glucose metabolism (Kervezee et al., 2018) and increase feelings of fatigue and reduced PA (Hargens et al., 2013). Participants were tested following a day off or previous day shift to limit the potential acute effects of sleep deprivation following a night shift. A design element aimed to ensure the project reflected the 'average' health of the cohort, however, may have missed the acute cyclic effect of shift work on cardio-metabolic health.

Conclusion

The current research project adds to the expanding body of literature associating shift work with adverse health conditions. Significant differences were observed for key cardio-metabolic health markers including cardiorespiratory fitness, body composition, inflammatory status and TST following a night shift. Additionally, the absence of statistical differences in average group sleep quality, autonomic control and acute inflammatory markers may indicate a difference in the acute

and chronic effect of shift work that requires further investigation. However, the research group do acknowledge the inclusion criteria of currently healthy men employed in a variety of shift structures and industries may impact the application of specific observations.

The necessity of shift work combined with identified labour induced health effects have resulted in the development of interventional programs aimed at improving health among shift workers.

Interventions should aim to reduce circadian disruptions and improve both sleep quality and cardiometabolic function of employees. Current interventions conventionally fall into four categories; controlled light and dark exposure, shift schedule changes, behavioural or lifestyle interventions aimed at improving sleep hygiene and pharmacological aids to promote sleep (Neil-Sztramko et al., 2014). Exercise may also be a valid interventions strategy capable of offsetting the biological risk factors and augmenting cardio-metabolic function, however a limited research currently exists to substantiate such a hypothesis (Flahr et al., 2018).

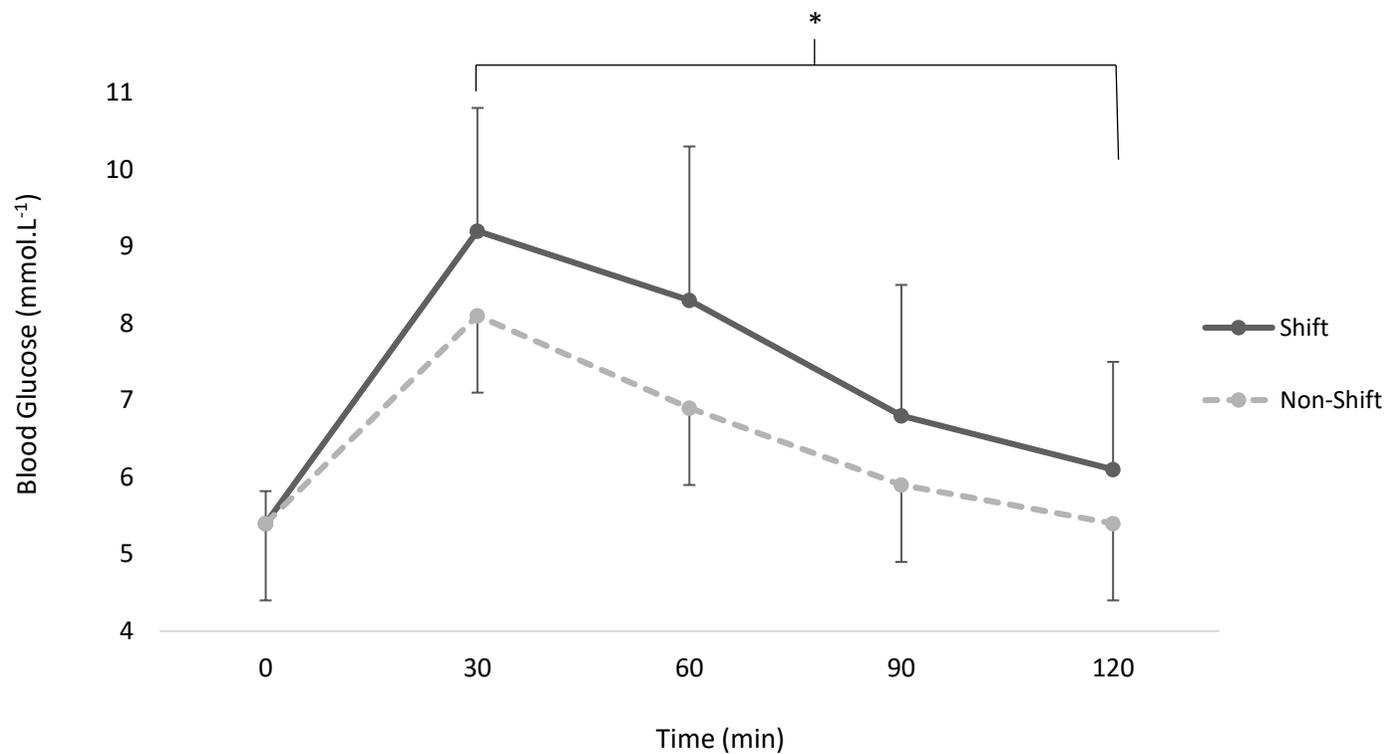


Figure 4.1 Blood glucose response to an oral glucose tolerance test (OGTT). 0; pre intervention, 30; 30 min, 60; 60 min, 90; 90 min 120; 120 min post ingestion. Values are mean \pm SD (error bar). * denotes significant group differences ($p < 0.05$).

Table 4.1 Baseline characteristic comparison of shift and non-shift workers.

Measure	Shift (<i>n</i> = 44)	Non-Shift (<i>n</i> = 43)
Age (y)	37.7 ± 9.5	36.2 ± 8.0
BMI (kg.m ²)	31.5 ± 6.1	29.9 ± 3.7
WHR	0.94 ± 0.09	0.93 ± 0.07
Systolic BP	131 ± 11	130 ± 10
Diastolic BP	87 ± 10	87 ± 9
Reported Ex. (min.week)	66 ± 56 *	94 ± 53
VO _{2peak} (ml.kg.min ⁻¹)	33.21 ± 6.2 *	36.8 ± 5.7
VO _{2peak} (L.min ⁻¹)	3.32 ± 0.7	3.59 ± 0.7

Data presented are mean ± standard deviation (SD) for age expressed in years (y), body mass index (BMI) expressed as kilograms (kg) per metre (m) squared (kg.m²), waist-to-hip ratio (WHR), blood pressure (BP), reported minutes of exercise per week (min.week) and peak measured oxygen uptake (VO_{2peak}) in relative and absolute terms.

* denotes significant difference (p<0.05).

Table 4.2 Sleep quality assessment comparing shift and non-shift workers (top) as well as sleep attained by shift workers while on shift vs days off (bottom)

Measure	Shift (<i>n</i> = 31)	Non-Shift (<i>n</i> = 33)
TIB (min)	442.9 ± 42.3	460.2 ± 37.3
TST (min)	370 ± 43.8	392.7 ± 32.5
Efficiency (%)	84.5 ± 6.8	85.6 ± 4.9
Latency (min)	16.6 ± 11.6	13.7 ± 9.1
WASO (min)	33.8 ± 14.5	36.4 ± 13.2
Awakenings (#)	32.3 ± 11.3	34.4 ± 9.6
	Shift Days on	Shift Days off
TIB (min)	401.2 ± 56.0 *	447.0 ± 46.4
TST (min)	333.23 ± 56.8 *	378.5 ± 49.4
Efficiency (%)	83.3 ± 8.9	84.4 ± 6.2
Latency (min)	16.0 ± 13.2	16.3 ± 13.2
WASO (min)	30.9 ± 14.9	35.1 ± 14.8
Awakenings (#)	29.1 ± 11.5	33.1 ± 11.9

Data are presented as mean ± standard deviation (SD). Min; minutes. TIB; time in bed. TST; total sleep time. WASO; wake after sleep onset. %; percent. #; number.

* denotes significant difference ($p < 0.05$).

Table 4.3 Inflammatory profile and glucose response comparison between shift and non-shift workers.

Measure	Shift	Non-Shift
CRP ng.ml ⁻¹ (<i>n</i> = 40 & 42)	1.98 ± 1.97	2.01 ± 2.6
IL-6 pg.ml ⁻¹ (<i>n</i> = 23 & 25)	255.9 ± 351.0 *	39.8 ± 38.9
TNF-α pg.ml ⁻¹ (<i>n</i> = 19 & 27)	5.19 ± 5.8	4.45 ± 4.2
Glucose AUC mmol.L.min ⁻¹	253.0 ± 146.2 *	143.6 ± 124.3

Data presented are means ± SD. AUC; area under the curve. CRP; C-reactive protein. IL-6; Interleukin-6. (*n*); denotes participants in each group due to non-detectable values. TNF-α; tumour necrosis factor alpha.

* denotes significant difference ($p < 0.05$).

Table 4.4 Dual-energy x-ray absorptiometry (DEXA) body composition comparison between shift and non-shift workers.

Measure	Shift (<i>n</i> = 44)	Non-Shift (<i>n</i> = 43)
Total Weight (kg)	102.1 ± 19.0	97.3 ± 12.8
Lean Mass (kg)	64.8 ± 8.7	64.8 ± 6.8
Fat Mass (kg)	33.9 ± 11.7 *	28.9 ± 7.8
Fat Mass (%)	33.5 ± 6.3 *	30.5 ± 4.9

Data presented are mean ± SD. Kg; mass expressed in kilograms. %; percent

* denotes significant difference ($p < 0.05$)

Table 4.5 Time domain heart rate variability (HRV) comparison between shift and non-shift workers.

Measure	Shift (<i>n</i> = 44)	Non-Shift (<i>n</i> = 43)
SDNN	208.7 ± 158.7	285.5 ± 161.3
RMSSD	308.5 ± 275.5	422.6 ± 290.7
pNN50	36.5 ± 26.9	43.1 ± 25.6
HF (n.u.)	68.1 ± 27.4	76.7 ± 27.5
LF (n.u.)	31.1 ± 27.8	22.4 ± 27.9

Values are means ± SD. pNN50; proportion of N-N interval above 50 ms divided by the total number of NN intervals. RMSSD; root mean square of successive differences between contractions. SDNN; standard deviation of N-N intervals. HF (n.u.); high frequency expressed in normative units. LF (n.u.); low frequency expressed in normative units. No observed group difference ($p > 0.05$).

Chapter Five:

A comparison of acute high- and moderate-intensity exercise on cardio-metabolic function and sleep among shift workers.

The research project presented in *Chapter Four* identified significant differences in markers for future cardio-metabolic risk among male rotational shift workers. Specifically, reported minutes of PA and cardiorespiratory fitness (measured by VO_{2max}) were significantly reduced, while IL-6 levels and response time to a glucose challenge were significantly increased. Given the necessity of shift work and apparent detrimental effects on cardio-metabolic function, Chapter Five will explore the potential effect of acute bouts of exercise on markers indicative of cardio-metabolic function.

Title: A comparison of acute high- and moderate-intensity exercise on cardio-metabolic function and sleep among shift workers.

Running Title: Exercise and shift worker cardio-metabolic health.

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Abstract

We examined the acute effect of moderate and high-intensity exercise on markers of cardio-metabolic function among rotational shift workers. Sedentary healthy men ($n = 26$, age: 38 ± 8 y; BMI: 32.2 ± 6.0 kg·m², VO_{2peak} 32.6 ± 6.7 ml.kg.min⁻¹) employed in rotational shift work were recruited and underwent objectively assessed sleep quality (~7 days actigraphy) prior to reporting for laboratory testing. Baseline venous blood sampling was collected for fasted glucose, insulin, and inflammatory cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-6 and IL-1 receptor antagonist (IL-1Ra). Participants were randomly allocated a 30 min cycling intervention of either high intensity interval training (HIT): 1:4 ratio of 60 s at 100% and 240 s at 50% VO_{2peak} , or moderate intensity continuous training (MICT); continuous cycling at 60% VO_{2peak} . Blood sampling was additionally drawn post intervention (0, 30, 60 min) before subsequent sleep opportunity was assessed via actigraphy. A main effect for time (immediately and 30 min post) was observed with increased IL-1Ra following HIT ($p < 0.05$). An effect for time was observed with decreased wake after sleep onset (WASO) for MICT ($p < 0.05$). No significant changes were observed for supplementary sleep variables, insulin sensitivity, IL-6 or TNF- α for either intervention group ($p > 0.05$). High- and moderate-intensity exercise elicit acute effects in inflammatory status and sleep quality among rotational shift workers, results associated with improved cardio-metabolic function. The independent effects observed in post-exercise inflammatory response and sleep quality additionally indicate intensity-based adaptations that may influence future exercise prescription.

Key Words: rotational shift work, acute exercise, actigraphy, inflammation.

Introduction

Cardio-metabolic function is regulated by sleep, circadian rhythms and the immune system to provide predictive responses to a dynamic environment (Kecklund & Axelsson, 2016; Lange et al., 2010). However, the integration of regulatory mechanisms, via shared anatomical structures and oscillatory interactions, allow for misaligned cues to be propagated across the individual systems and facilitate pathogenesis (Schilperoort et al., 2020). The occupational specific characteristics of shift work, including limited sleep opportunity and structured inversion of biological signals provide misaligned biological cues are associated with disturbed cardio-metabolic function (De Bacquer et al., 2009; Kecklund & Axelsson, 2016; Korsiak, Tranmer, Day, et al., 2018; Lange et al., 2010; Torquati et al., 2018). Acute bouts of simulated shift work elicit the individual components of cardio-metabolic dysfunction, including systemic inflammatory responses (Bescos et al., 2018; Leproult et al., 2014), while employment in rotational shift work is associated with an increased incidence of cardio-metabolic disorders (De Bacquer et al., 2009; Kecklund & Axelsson, 2016; Torquati et al., 2018) .

The adverse effect of shift work highlights the importance of interventions aimed at improving employee health and well-being. Exercise is a viable method with a myriad of research demonstrating primary and secondary benefits for general and cardio-metabolic health (Warburton et al., 2006). Specific to shift work, exercise has been demonstrated to improve sleep quality (Carandente et al., 2011; Morita, Sasai-Sakuma, & Inoue, 2017) and augment cardio-metabolic function via improved inflammatory status and insulin sensitivity (Bird & Hawley, 2012; Gebel et al., 2015). However, the diverse components of exercise including duration, intensity and timing are yet to be fully elucidated (Yao & Basner, 2019), especially among shift workers (Flahr et al., 2018). For example, acute bouts of exercise elicit a dynamic inflammatory response, with increased oxidative metabolism (Kasapis & Thompson, 2005) and tissue damage (Woods et al., 2012) capable of increasing pro-inflammatory circulation including increased interleukin (IL)-6 and tumour necrosis factor (TNF)- α . Alternatively,

muscle-contraction induced IL-6 and subsequent anti-inflammatory cytokines, including IL-1 receptor antagonist (IL-1Ra) can directly inhibit TNF- α action, and suppress the pro-inflammatory cascade (Brown et al., 2015). Collectively, given the potential pro-inflammatory status among shift workers and the dynamic inflammatory response to acute bouts of exercise, further work is warranted in this area (Flahr et al., 2018).

In addition to the limited research currently conducted among shift workers, occupational specific barriers to exercise need consideration. Limited time between consecutive shifts has been identified as a potential factor impacting total sleep duration (Korsiak, Tranmer, Day, et al., 2018; Shantha, 2013). Consequently, despite the positive effect of exercise on sleep parameters (Carandente et al., 2011; Morita et al., 2017), advocating an exercise intervention may further restrict total sleep time (TST) and exacerbate potential pathogenesis. The timing and intensity of exercise is an additional consideration, with exercise and increased physiological arousal close to sleep time potentially having a negative impact on sleep quality (Irish et al., 2015). Conversely, exercise-induced improvements in thermoregulation and acute elevations in core body temperature may improve sleep latency (Irish et al., 2015). Collectively, while exercise is a validated intervention to improve the sleep architecture among the general population, the effects among rotational shift workers are in need of further exploration (Flahr et al., 2018).

The limited time between consecutive shifts may also effect exercise adherence, with a lack of time cited as the primary reason for non-exercise participation (Atkinson et al., 2008; Nea et al., 2017). High intensity interval training (HIT) is a valid alternative to the classically prescribed moderate intensity continuous training (MICT), with comparable and in some cases superior adaptations associated with increased intensity and decreased total training time (Gibala et al., 2012). However, the concerns

regarding a potential pro-inflammatory response and negative effect on sleep architecture may be exacerbated by higher intensity exercise among previously sedentary shift workers.

Therefore, the current research project aimed to investigate the acute effect of exercise among rotational shift workers regarding objectively measured sleep quality, inflammatory response, and insulin sensitivity. Further the effect of HIT will be compared to MICT to investigate intensity-based differences in exercise interventions for shift worker health. It is hypothesised that both exercise modalities will improve the inflammatory status and sleep quality of rotational shift workers. Further a significant effect will be observed among exercise interventions with superior adaptations demonstrated by the HIT group.

Methods:

Participants:

Twenty-six sedentary but otherwise healthy male shift workers volunteered for this study (baseline characteristics in Table 5.1). Participants were required to be employed in rotational shift work (8 - 12 h clockwise rotating morning, afternoon, and night shifts) with occupations included emergency services, manufacturing, and health workers. Further, participants were required to report as sedentary (<60 min of planned physical activity per week) non-smoker and not diagnosed with any known cardio-metabolic and/or sleep disorders. All participants gave informed written and verbal consent before commencing the study, which was approved by the Institutional Human Ethics Committee.

Study Overview:

Following recruitment and pre-screening health and physical limitations, a familiarisation session was conducted where all procedures and protocols were explained and demonstrated before completion by participants. The session included an anthropometry assessment and a maximal exercise test to determine aerobic capacity (VO_{2peak}), before participants were issued a wrist-worn actigraphy device (Actiware 2, Philips Respironics, Andover, MA, USA) and sleep diary, recorded and completed over a 7 day (d) period. Participants returned for the experimental trial between 0600 and 0900 h, and included venous blood sampling following an overnight fast, completion of a randomly allocated acute exercise intervention and post-exercise venous blood sampling immediately, 30- and 60-min post exercise. Finally, the actigraphy device was worn and sleep diary completed during the night following the intervention.

Familiarisation and baseline testing:

Anthropometry

Participants completed an anthropometry assessment with body mass recorded wearing minimal clothing, using calibrated electronic scales (A and D HW-PW200, Thebarton, SA, Australia). Height was recorded using stadiometer in an upright position with head in the Frankfort plane. Waist circumference were measured at the mid-point between the costal arch and the iliac crest and hip circumference were measured in line with the greater trochanter (steel tape; EC P3 metric graduation, Sydney, NSW, Australia).

Graded Exercise Test (GXT)

Participants completed a ramped, maximal GXT on a stationary cycle-ergometer (Wattbike Trainer, Smeaton Grange, NSW, Australia) with 1 min stages, an initial power output of 50 W and incremental increases of $25 \text{ W}\cdot\text{min}^{-1}$ until volitional exhaustion was achieved. Expired gases and flow volumes were

collected during the GXT and analysed by a calibrated metabolic cart (TrueOne 2400, ParvoMedics, Salt Lake City, Utah) to provide peak oxygen uptake (VO_{2peak}).

Actigraphy

Participants were issued an actigraphy watch and sleep diary to record subjective at-home bedtime, sleep time, awake time and number of awakenings. Actigraphy was recorded in 30 s epochs over a 7 d period and analysed using Actiware v5.70 software (Actiware 2, Philips Respironics, Andover, MA, USA) in correspondence with participant sleep diaries recorded during the collection period. Variables obtained included bed and wake time, time in bed (TIB; period between bedtime and wake time), total sleep time (TST; time asleep during TIB), sleep latency (period between bedtime and sleep onset), sleep efficiency (percentage of time in bed spent sleeping), total time awake after sleep onset (WASO), and number of awakenings during total sleep period which were averaged across the 7 d. If the actigraphy device was removed (assessed via sleep diary and a zero-activity measurement), or technical difficulties were experienced within an hour of documented bed or rising time, data of the affected time and subsequent sleep period were manually excluded.

Experimental trials

Participants arrived at the laboratory between 0600 and 0900 h following a day off or day shift to limit the potential effect of sleep deprivation or circadian misalignment following night shift. The experimental trial was based on previous work by Sim, et al (2014), assessing the effect of differing intensity exercise bouts matched for total mechanical work (schematic overview Figure 5.1). Exercise protocols included HIT: 30 min alternating high and low intensity efforts performed at a ratio of 1:4 (60 s at 100% VO_{2peak} : 240 s at 50% VO_{2peak}) or MICT: 30 min performed at a continuous moderate intensity (60% VO_{2peak}) (Sim, Wallman, Fairchild, & Guelfi, 2014). Borg's 6-20 scale rating of perceived

exertion (RPE) (Borg, 1998) and heart rate (HR) were recorded every 5 min for MICT and at the end of each high intensity interval for HIT. The participants completed intravenous blood sampling pre- and post-intervention (0, 30 and 60 min) (Figure 5.1) and continued to wear actigraphy sleep watch to assess subsequent at-home sleep opportunity.

Venous Blood Collection and Analysis

Participants underwent venepuncture procedures in which a cannula (22G BD Medical, North Ryde, NSW, Sydney) was inserted into a medial antecubital vein and blood was manually drawn by syringe and ejected into pre-chilled tubes treated with ethylenediaminetetraacetic acid and serine protease inhibitor (Pefabloc SC, Sigma-Aldrich, Sydney, Australia) to prevent cytokine degradation. Drawn blood was also ejected into serum separator tubes and left to clot for ~20 min. The pre-chilled and clotted serum tubes were centrifuged (refrigerated) for 10 min at 4 °C, before being stored at -80 °C until analyses. Analyses was performed with commercially available enzyme-linked immunosorbent assays (ELISA kit). Serum was analysed for TNF- α (Human TNF-alpha ELISA kit/catalogue no. EK-0001; Crux Biolabs, Scoresby, VIC, Australia) and insulin (Human Insulin ELISA kit/catalogue no. ELH-Insulin-1; Raybiotech, Scoresby, VIC, Australia). Plasma was analysed for IL-6 (Human IL-6 ELISA kit/catalogue no. EK-0012; Crux Biolabs, Scoresby, VIC, Australia) and IL-1Ra (Human Insulin ELISA kit/catalogue no. ELH-IL1ra-1; Raybiotech, Scoresby, VIC, Australia). The manufacturer's specified assay and analytical methods were used throughout. All samples were completed in duplicate with a minimum detectable cytokine levels for each kit: IL-6 (<5pg.ml⁻¹), TNF- α (<5pg.ml⁻¹), IL-1ra (<100 pg.ml⁻¹) and Insulin (<4 μ IU.ml⁻¹) respectively. Inter-assay co-efficient of variation is <10%, duplicate readings for each standard, control and sample were averaged and the average zero standard optical density reading was subtracted. A standard curve was created using a 4-parameter logistic curve fit. Concentration values were then determined, using this curve for control and sample average optical densities per the ELISA kit instructions. The Pearson product-moment correlation coefficient (r) was determined by

comparing the known standard concentration with the curve fit; r was shown to be greater than 0.98 for all the assays. The homeostasis model for insulin (HOMA-IR) was calculated using the established formula, (fasting insulin [$\mu\text{IU}\cdot\text{ml}^{-1}$] x fasting glucose [$\text{mmol}\cdot\text{L}^{-1}$]/22.5) (Bonora et al., 2002).

Statistical Analysis

A *Priori* power analysis was conducted using GPower (G*Power v.3), for an *F Test*, with significance set at 0.05, power at 0.80, 2 groups (MICT and HIT), minimum of 4 measurements (pre, immediately post, 30 min and 60 min) and correlation among measures of 0.5 based on conservative intra-assay CV of ELISA plates. A total sample size of 24 participants was required for an effect size of 0.25. Data are reported as mean \pm standard deviation. Independent samples *t* test was used to evaluate difference in baseline characteristics and session variables (Watts (W), RPE and HR). Inflammation, glucose, and insulin data were analysed using a repeated within-between ANOVA with the between factor, group (2 levels) and the within subject factor, time (4 levels). Significant time and group x time interactions ($p < 0.05$) were analysed using Tukey's post hoc analysis. All statistical analyses were conducted with SPSS software (version 26.0 SPSS Inc, Chicago, IL) with significance set at $p < 0.05$.

Results

Descriptive Characteristics

Descriptive data are presented in Table 5.1. No significant differences were observed between groups for baseline descriptive characteristics, $\text{VO}_{2\text{peak}}$, BP or anthropometry measures (BMI and WHR) ($p > 0.05$) (Table 5.1).

Acute Intervention Measures

No significant difference was observed for the session mean power output (HIT 146 ± 40 W and MICT 131 ± 21 W; $p > 0.05$). No effect was observed for average RPE ($p > 0.05$). A significant main effect was observed for HR ($F(6, 17) = 114.887$, $p < 0.001$, partial eta = .976) with post analysis identified significantly higher HR for the HIT group at 20 min ($p = 0.016$), 25 ($p = 0.004$) and 30 min ($p = 0.011$) (Figure 5.2).

Inflammatory Profile and Insulin Sensitivity

Due to several participants reporting below detectable ranges for metabolite levels the total sample values for each group were (HIT $n = 8$, MICT $n = 7$). No effect was observed for blood glucose, insulin, or indexed insulin resistance (HOMA-IR) for either group ($p > 0.05$; Figure 5.3). A main effect for time ($F = 4.596$) $p = 0.023$, partial eta = .535 was observed for IL-1ra (Figure 5.3) with a post analysis identified a significant time effect for HIT at 0 ($p = 0.04$) and 30 min ($p = 0.025$) in comparison to baseline values. No effect was observed for IL-6 or TNF- α for HIT ($p > 0.05$) or MICT for all inflammatory and insulin variables ($p > 0.05$; Figure 4.4).

Objective Sleep Assessment

Several participants (HIT $n = 4$ and MICT $n = 3$) had actigraphy data excluded due to technical difficulties. As shown in Table 5.2, both a significant interaction for main effect of time $F(1, 17) = 5.496$, $p = 0.031$, partial eta squared = 0.244 and Group*Time interaction $F(1, 17) = 4.582$, $p = 0.047$, partial eta squared = 0.212 were observed for WASO, with WASO decreased in the MICT group. No significant effects were observed for either intervention for TST, TIB, efficiency, latency, or number of awakenings.

Discussion

The current study aimed to examine the effects of acute exercise interventions at varying intensities on markers of cardio-metabolic function and sleep among a shift work population. The major finding is the significant effect of an acute exercise interventions on anti-inflammatory mediators and objectively measured WASO with an apparent difference in outcomes according to exercise intensity. IL-1Ra was significantly increased post-HIT, without inducing a pro-inflammatory response (non-significant change in TNF- α), collectively indicating an anti-inflammatory response among participants, albeit with small sample sizes. Further, MICT significantly reduced WASO, an objective measure of sleep fragmentation and associated with improved cardio-metabolic function under chronic conditions (Shantha, 2013). The current research project supports the myriad of previous research advocating exercise as a health intervention (Bird & Hawley, 2012; Gebel et al., 2015). However, these findings are novel in that the current project adds to the limited body of research conducted among shift workers (Flahr et al., 2018). Further, the current results indicate a potential difference in intensity-based adaptations that may help shape future research and exercise prescription for shift workers. Collectively, the exercise (albeit at different intensities) induced improvements in inflammatory status and sleep quality support exercise as an acute intervention among rotational shift workers aimed at improving cardio-metabolic health.

Through shared anatomical structures and oscillatory mediators, sleep has an established regulatory role in cardio-metabolic function (Lange et al., 2010), perhaps most clearly demonstrated by the adverse effects of poor sleep. Reduced TST due to limited time between consecutive shifts and misaligned sleep opportunity due to inverted sleep-wake cycles, present labour specific risks to shift workers cardio-metabolic regulation. Consequently, the increased reported sleep disorders among shift workers, including reduced TST (Korsiak, Tranmer, Day, et al., 2018) and decreased continuity (fragmentation) (Shantha, 2013) are potential mechanistic links to the observed increased incidence

of cardio-metabolic disorders and a target for exercise interventions. Fragmentation of sleep, an inability to maintain effective sleep continuity, is an example of maladaptive sleep architecture, previously demonstrated to impair insulin sensitivity and glucose metabolism among health volunteers (Stamatakis & Punjabi, 2010). WASO is a commonly used actigraphic measure of sleep fragmentation, derived from polysomnography validated algorithms that reflects the time participants spent awake after sleep begins (Spira et al., 2017). Morning time MICT significantly decreased the WASO time, result which are supported by previous research (Carandente et al., 2011; Morita et al., 2017) and indicate a decrease in sleep fragmentation. Under chronic conditions where multiple acute exercise bouts improving subsequent sleep periods, the MICT induced improved sleep may indicate a plausible intervention for improving the cardio-metabolic regulation of shift workers.

In contradiction to the hypothesis, HIT had no effect on objectively measured sleep assessment. HIT protocols are associated with greater acute stimulus, including increased glycogen depletion and metabolite accumulation (Larsen et al., 2019b). As such, the research group hypothesised that deconditioned shift workers would require extended recovery time and subsequent sleep (Larsen et al., 2019b) compared to the MICT. A result hypothesised to be reflected in significantly increased TST, reduced latency and WASO. However, such an effect was not supported by the current results and may require further investigation to explain. A limitation of the current project and potential factor in the observed effect of exercise intensity on sleep was the timing of the exercise intervention. To avoid a potential circadian effect of exercise following night shift, the intervention and subsequent testing was administered in the morning, on consecutive rest days. While limiting co-factors for cardio-metabolic regulation, the research group potentially created an 'ideal exercise and sleep' opportunity that may not reflect the true effect of acute interventions during shift rotation periods. Further, widening the inclusion criteria to aid in recruitment of shift workers may have impacted inflammatory status due to occupational differences. Certain occupations such as manufacturing and mining may be

exposed to hazardous material, alternatively, emergency and health workers may be exposed to stressful stimuli at an increased rate, potentially impacting inflammatory status. Finally, participants were screened for current sleep disorders, resulting in the pre-exercise 7-day mean sleep data (Table 5.2), which acted as baseline sleep quality for the intervention, falling within the expected sleep ranges. Consequently, a ceiling effect may have been created in which there was not enough room for improvement to elicit a significant response by the HIT group.

Within the current project, IL-1Ra was increased post-HIT for rotational shift workers. IL-1Ra is considered an anti-inflammatory cytokine (Gleeson et al., 2011), while specifically targeting IL-1 β , the systemic effect of IL-1Ra upregulation blunts the production of TNF- α and subsequent pro-inflammatory cascade (Brown et al., 2015; Petersen & Pedersen, 2005). Low grade systemic inflammation is recognised to play a direct aetiological role in cardio-metabolic disorders (Libby et al., 2002), with TNF- α capable of moderating insulin resistance (Rehman & Akash, 2016) and pro-inflammatory markers facilitating all stages of atherosclerosis (Libby et al., 2002). Consequently, significant increases in IL-1Ra support the hypothesis that acute bouts of HIT may induce an anti-inflammatory effect among shift workers and improve cardio-metabolic function. Although the precise mechanisms remain unclear, HIT is hypothesised to induce a superior anti-inflammatory response compared to MICT (Brown et al., 2015) via increased muscle contraction. In support, our results align with previous research (Markovitch, Tyrrell, & Thompson, 2008) demonstrating a non-significant effect of MICT on anti-inflammatory mediators. However, if increases in training intensity reduce the total time required to elicit health effects (Gibala et al., 2012), MICT may induce comparable results, but would require an equivalent increase in total exercise time. In support, MICT protocols ranging from a minimum of 45 to 60 min have previously induced a significant anti-inflammatory response (Kaspar et al., 2016; Scott et al., 2011). Collectively, MICT may be a viable

intervention to improve inflammatory status, however the effect may require longer total exercise duration, further supporting HIT as a time effective alternative.

The inflammatory response to an acute bout of exercise is complex, with the variables of intensity, duration and condition of the participants interacting to potentially facilitate both a pro- and anti-inflammatory response (Brown et al., 2015; Cerqueira, Marinho, Neiva, & Lourenço, 2020). Exercise induced pro-inflammatory response may be associated with increased metabolic stress (Kasapis & Thompson, 2005) and the potentially tissue damage or injury associated with unaccustomed exercise (Woods et al., 2012). Consequently, HIT over an extended period (relative to participant conditioning) may induce a pro-inflammatory response. As such, an initial consideration of the research project was the possibility for unaccustomed exercise stimulus to induce a pro-inflammatory response among sedentary shift workers (Woods et al., 2012), exacerbating cardio-metabolic risk. However, exercise, regardless of intensity, did not induce a significant change in pro-inflammatory status (indicated via a lack of TNF- α response). A potential limitation of the current results is the limited sample size in post analysis assessment, which future projects should look to address. Therefore, HIT induced an anti-inflammatory response hypothesised to improve cardio-metabolic function in the absence of a pro-inflammatory response associated with unaccustomed exercise prescription. MICT did not induce significant changes in inflammatory status however longer duration acute bouts may be a viable mode in need of further investigation.

The current project demonstrated no effect of exercise, regardless of intensity, on post intervention insulin sensitivity as measured by the HOMA-IR index. HOMA-IR is the result of a computed model in which fasting glucose and insulin values are plotted to allow assessment of the expected β -cell response and insulin function (Antuna-Puente et al., 2011). As outlined in Figure 4.2, no significant difference was observed in the blood glucose or insulin values from pre to post-exercise intervention

regardless of intensity, translating to no observable change in indexed insulin sensitivity. Results which oppose our initial hypothesis but may be explained by different metabolic pathways activated during and immediately post-exercise. Specifically, contraction-mediated glucose transport-4 (GLUT4) is hypothesised to be the primary pathway responsible for post-exercise glucose metabolism, acting independently of insulin (Cartee, 2015). Consequently, the contraction-mediated pathway following exercise may have improved glucose metabolism, however indexing insulin sensitivity may not have reflected the post-exercise GLUT-4 translation effect. Further, a recent review of exercise-insulin pathway concluded that enhanced muscle and whole body insulin sensitivity may not be measurable for up to 4 h post exercise, and can persist for 24 - 48 h (Cartee, 2015). In support, previous research demonstrating acute bouts of exercise significantly improving insulin sensitivity were recorded 12-16 hours post exercise intervention (Brestoff et al., 2009), however, due to study methodology, collection of blood samples at these recovery time points were not possible in the current study. As such, it is plausible that the current exercise intervention may have provided some effect on insulin sensitivity among shift workers, however the sampling window did not capture this effect. Consequently, future research exploring the acute effect of exercise on shift workers insulin sensitivity may look to extend the testing window to include up to 24 h post assessment.

Conclusion

The current project aimed to explore the effect of different intensities of acute exercise bouts on cardio-metabolic function and sleep of rotational shift workers. The results support exercise as a viable intervention to improve moderators of cardio-metabolic health, with HIT eliciting an anti-inflammatory effect and MICT significantly reducing sleep fragmentation assessed by WASO. Further research is required to explore the mechanistic cause of the difference in intensity-based effect, but exercise may be prescribed as a valid intervention to improve shift worker health.

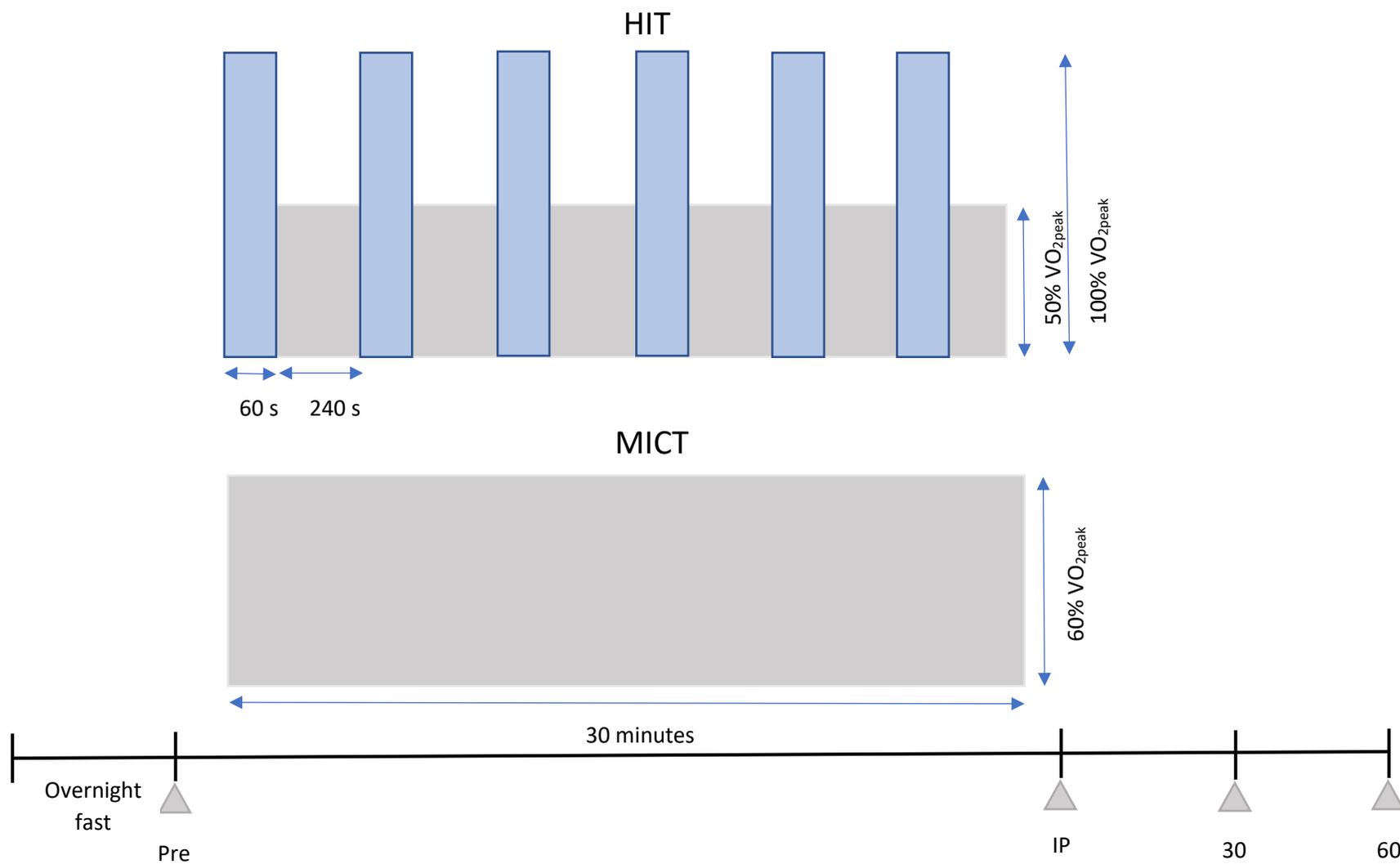


Figure 5.1: Training schematic of HIT and MICT matched for time and total energy expenditure. Pre: pre-exercise intervention. S; seconds. VO_{2peak} ; workload at peak measured oxygen consumption. IP; immediately post. 30; 30 min post. 60; 60 min post intervention. \blacktriangle ; collection of blood sample

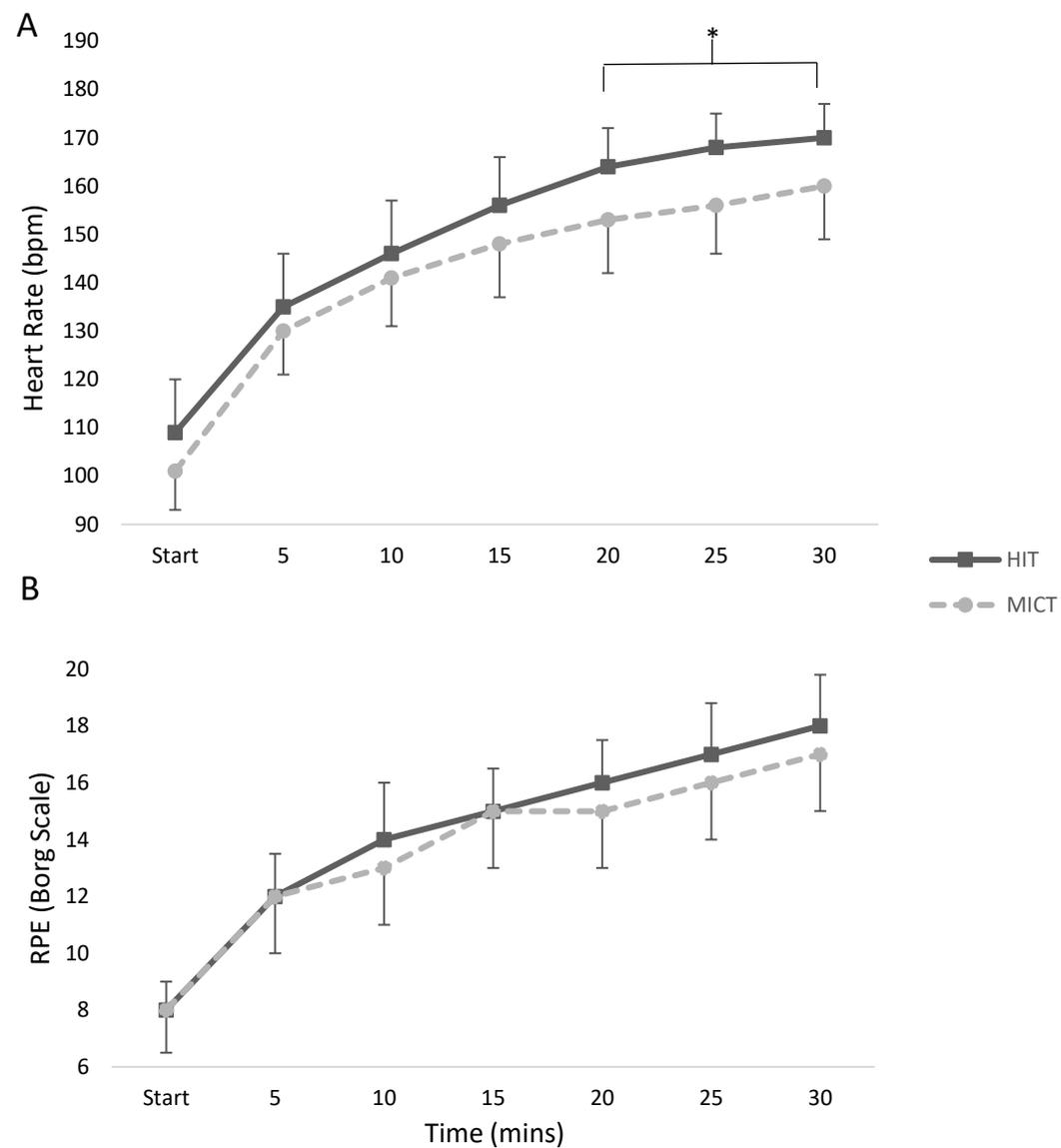


Figure 5.2 A: Sessional Heart Rate (HR); **B:** Rating of Perceived Exertion (RPE) in response to HIT or MICT. bpm; beats per minute, mins; minutes, * denotes significant difference between groups ($p < 0.05$)

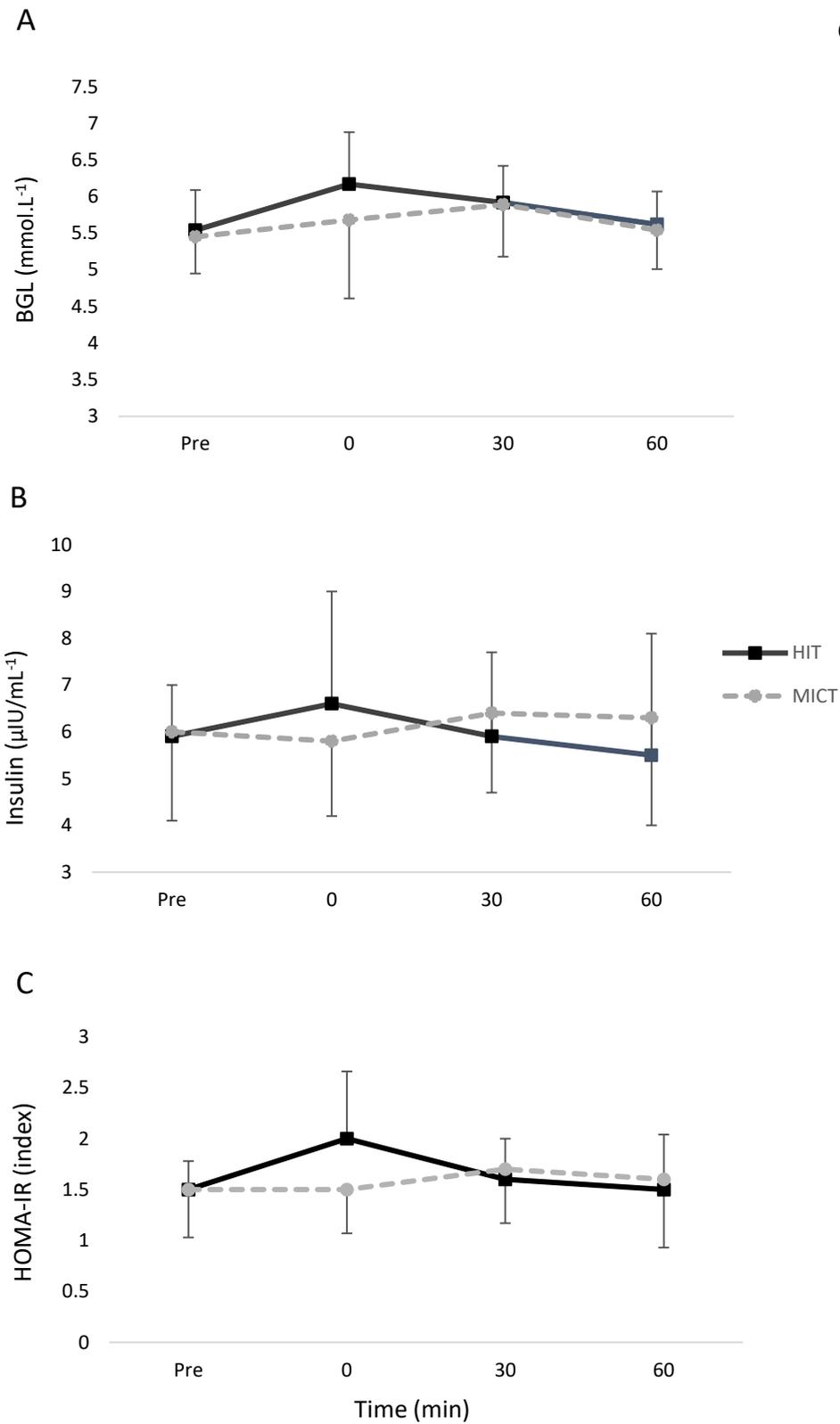


Figure 5.3 Acute pre-to-post intervention blood glucose, insulin and indexed insulin sensitivity (HOMA-IR). (A) Blood glucose (BGL) (B) Insulin (C) HOMA-IR. Pre; pre intervention, 0; immediately post intervention, 30; 30 min post, 60; 60 min post. Values are mean \pm SD.

No significant difference was observed ($p > 0.05$)

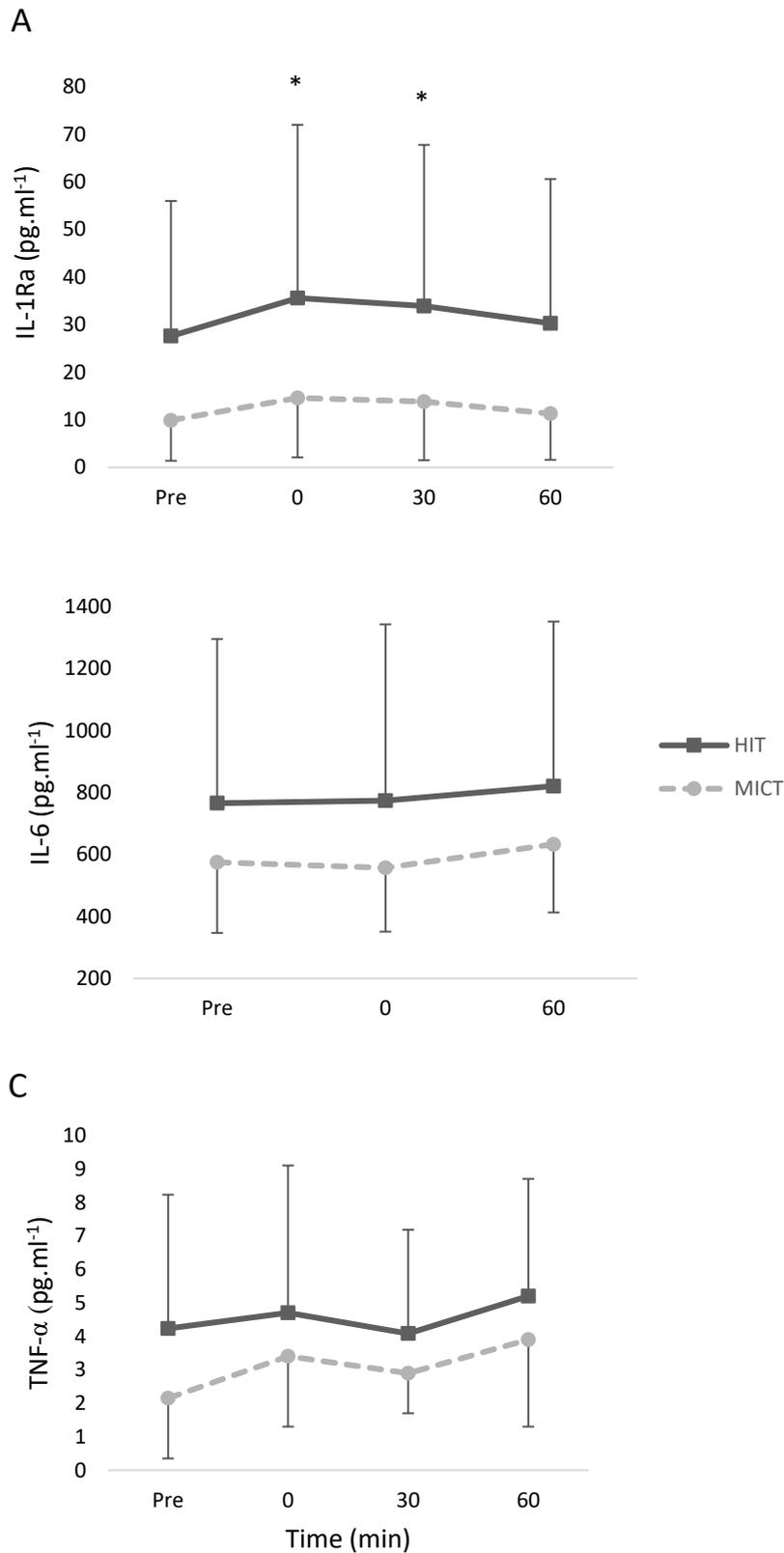


Figure 5.4 Acute pre-to-post inflammatory response to bouts of HIT and MICT. (A) IL-1Ra (B) IL-6, (C) TNF- α . Pre; pre intervention, 0; immediately post intervention, 30; 30 min post, 60; 60 min post. Values are mean \pm SD.

* denotes significant difference from pre ($p < 0.05$)

Table 5.1 Baseline Characteristics of rotational shift workers

Measure	Value	
	HIT (<i>n</i> = 13)	MICT (<i>n</i> = 13)
Age (years)	37 ± 7	40 ± 9
BMI (kg.m ²)	31.6 ± 5.2	32.8 ± 6.8
WHR (index)	0.96 ± 0.01	0.98 ± 0.12
Systolic BP (mmHg)	131 ± 8	134 ± 9
Diastolic BP (mmHg)	86 ± 9	88 ± 7
VO _{2peak} (ml.kg.min ⁻¹)	34.3 ± 6.3	30.9 ± 7.1
IL-1Ra (pg.ml ⁻¹)	27.59 ± 28.36	9.98 ± 8.55
IL-6 (pg.ml ⁻¹)	765.5 ± 530.7	575 ± 228.7
TNF-α (pg.ml ⁻¹)	4.23 ± 4.0	2.15 ± 1.8
Insulin (pg.ml ⁻¹)	5.9 ± 1.1	6.0 ± 1.9
Blood Glucose (mmol.L ⁻¹)	5.5 ± 0.6	5.5 ± 0.5
HOMA-IR (index)	1.45 ± .31	1.47 ± .47

Data are presented as mean ± SD. BMI; body mass index. BP; blood pressure. VO_{2peak}; peak aerobic capacity. WHR; waist-to-hip ratio. HOMA-IR; homeostatic model assessment indexed insulin resistance.

No significant differences observed at baseline (*p*>0.05)

Table 5.2 Pre and post exercise intervention values for actigraphy measured sleep quality.

Measure	Value			
	HIT (<i>n</i> = 9)		MICT (<i>n</i> = 10)	
	Pre	Post	Pre	Post
TIB (mins)	470 ± 39	475 ± 30	502 ± 65	523 ± 79
TST (mins)	409 ± 36	423 ± 24	421 ± 80	450 ± 90
Efficiency (%)	87.5 ± 2.5	88.7 ± 3.3	83.9 ± 7.9	86.4 ± 8.7
Latency (min)	9.5 ± 5.5	9.2 ± 8.1	10.8 ± 8.4	13.8 ± 18.9
WASO (mins)	28 ± 10.9	27 ± 12.9	53 ± 29.2	36 ± 17.1*
Awakenings (#)	31 ± 9.4	31 ± 12.7	47 ± 17.9	39 ± 18.3

Data are presented as mean ± SD. TIB; time in bed. TST; total sleep time. WASO; wake after sleep onset.
 * denotes statistically different pre to post value ($p < 0.05$).

Chapter Six:

Shift work and exercise adherence: the effect of a 12-week mixed-modality training intervention on cardio-metabolic health.

The research project presented in Chapter Four identified significant differences in cardio-metabolic function among male rotational shift workers. Chapter Five explored exercise as a potential intervention by investigating the effect of acute bouts of exercise on markers indicative of cardio-metabolic function. An acute bout of HIT significantly increased IL-1 α (anti-inflammatory mediator) while MICT significantly reduced WASO (marker of sleep fragmentation). Results indicating exercise may be a valid intervention and justifying exploration of the chronic training effect of exercise on cardio-metabolic function among shift workers.

Title: Shift work and exercise adherence: the effect of a 12-week mixed-modality training intervention on cardio-metabolic health.

Running Title: 12-week exercise intervention for shift workers

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Abstract

Purpose: To assess the adherence to and effect of a 12-week resistance or aerobic training intervention on markers of cardio-metabolic function and sleep among rotational shift workers.

Method: Thirty-eight sedentary, apparently healthy, rotational shift workers were recruited and randomly allocated to a non-exercise control (CON) group, 3 sessions/week of moderate intensity continuous (MICT), or resistance training (RT) for 12 weeks in a semi-supervised setting. Pre- and post-testing assessed markers of cardio-metabolic function including peak aerobic capacity (VO_{2peak}), glucose metabolism, insulin sensitivity, body composition, inflammatory markers and objective sleep assessment.

Results: Mean session attendance across the intervention was 25 (± 7) of a possible 36 sessions. A significant group by time interaction was observed for MICT, with lower c-reactive protein (CRP) values observed post-training ($p = 0.049$). A significant effect for time was observed for both MICT ($p = 0.04$) and RT ($p = 0.021$), increasing total sleep time (TST) following a night shift post-intervention. Data redistribution regarding exercise adherence: < 24 (N-ADHERE) or ≥ 24 (ADHERE) resulted in significant pre-to-post reduction in body fat ($p = 0.024$) and fat mass percentage ($p = 0.014$) among ADHERE, but no difference was observed for intervention on insulin sensitivity, glucose metabolism or aerobic capacity.

Conclusion: The results of the current study support exercise as a valid intervention to improve the cardio-metabolic health of rotational shift workers. Average sessional attendance suggests shift workers are exposed to labour specific barriers to exercise that may need to be addressed to improve health outcomes.

Key words: shift work, resistance training, moderate intensity, cardio-metabolic.

Introduction

Shift work as a labour structure is primarily concerned with the systematic extension of labour opportunity to meet the growing demands of modern society (Flahr et al., 2018; Nea et al., 2017). To achieve this, total labour time is divided into rotational or fixed shifts to extend productivity beyond the capabilities of an individual work period (Flahr et al., 2018; Puttonen et al., 2010). However, the rotational divisions, or fixed shifts in opposition to regulatory circadian cycles, misalign behavioural regulators and disrupt homeostatic function (Nea et al., 2017; Puttonen et al., 2010). Given the cardio-metabolic system is under the integrated control of several biological systems including circadian and sleep-wake cycles (Zimmet et al., 2019), homeostatic disruptions are associated with adverse effects on insulin sensitivity (Bescos et al., 2018; Leproult et al., 2014), sleep quality (Korsiak, Tranmer, Day, et al., 2018), body composition (Liu et al., 2018) and inflammatory status (Leproult et al., 2014; Puttonen et al., 2011). Consequently, shift work is associated with an increased relative risk of developing cardio-metabolic disorders (Flahr et al., 2018; Puttonen et al., 2010) and disturbed homeostatic regulation is hypothesised to play an aetiological role (Flahr et al., 2018; Puttonen et al., 2010).

Intervention strategies have been implemented to offset the hypothesised health effect of shift work, with exercise established as a viable method (Flahr et al., 2018; Warburton et al., 2006). Exercise exerts a broad range of salutary effects on health, including reduced all-cause mortality and established primary and secondary prevention mechanisms for cardio-metabolic conditions (Warburton et al., 2006). Specifically to shift work, exercise has been demonstrated to augment cardio-metabolic function via improved body composition (Lim et al., 2015), glucose metabolism (Abd El-Kader, 2011) and anti-inflammatory proliferation (Donges, Duffield, & Drinkwater, 2010). However, exercise is a broad term of organised physical activity (PA), with independent adaptations associated with specific physiological stimulus (Baar, 2009). Aerobic exercise has consistently been demonstrated

to improve cardiopulmonary fitness, oxidative capacity and vascular function (Park et al., 2020; Warburton et al., 2006), while resistance training (RT) is often associated with enhanced skeletal muscle mass (Park et al., 2020), and potential benefits in glycaemic control (Warburton et al., 2006). Exercise is therefore hypothesised to be a viable intervention to improve the cardio-metabolic health of shift workers, however a limited body of research currently existing to substantiate the hypothesis, or identify the most effective mode (s) (Flahr et al., 2018).

Despite the theoretical support for exercise as a health intervention, shift workers are reported to engage in lower levels of PA (Peplonska et al., 2014). Fundamental barriers to exercise participation appear to be exacerbated by organisational limitations of the labour structure and may explain decreased adherence (Nea et al., 2017). For example, limited break time between consecutive shifts is often prioritised for sleep and social obligations (Atkinson et al., 2008), and may fail to provide sufficient time to meet the recommended PA guidelines. Further, feelings of residual fatigue associated with both limited and/or circadian misaligned sleep opportunity may reduce the motivation to exercise (Hargens et al., 2013). Finally, shift rotations may provide exercise opportunities in opposition to team sport or gym facility scheduling (Blake et al., 2017; Nea et al., 2017), and prevent the development a set routine for exercise participation (Blake et al., 2017; Nea et al., 2017). Consequently, the validity of exercise as a health intervention may be difficult to investigate with substantial barriers preventing adherence among rotational shift workers

Therefore, the current study aims to assess the adherence to a semi-supervised exercise training intervention among shift workers. Further, an investigation of the potential effect of aerobic and resistance exercise modalities on cardio-metabolic function, inflammatory markers and sleep will be assessed. It is hypothesised that shift workers will not attain the recommended weekly PA levels, despite being in a semi-supervised setting. Further, while both training modalities will improve

markers of cardio-metabolic function, MICT will be more effective at improving objectively measured sleep, maximal aerobic capacity, body composition and inflammatory status of shift workers.

Methods

Participants:

Thirty-eight male rotational shift workers, currently employed in clockwise rotating 8-12 h morning, afternoon, and night shifts, volunteered for this study (baseline data in Table 5.1). Further inclusion criteria required participants to be sedentary (<60 min planned PA per week) and report as non-smokers with no known cardio-metabolic, sleep or inflammatory disorders. In alignment with the declaration of Helsinki, this study was approved by the Institutions Human Ethics Committee, with informed written and verbal consent attained from all participants.

Study Overview:

Following recruitment, participants attended a familiarisation session in which procedures and protocols were verbally outlined. Participants then completed baseline testing procedures including anthropometry assessments, maximal graded exercise test (GXT) and objective sleep assessment (actigraphy). Following a 14 d actigraphy and diary sleep assessment, participants returned for a laboratory testing session that included baseline fasted venous blood sampling for assessment of inflammatory markers, an oral glucose tolerance test (OGTT), dual x-ray absorptiometry (DEXA) scan for body composition and 5 min rested heart rate variability (HRV). Participants were then randomly assigned one of three groups, a non-exercising control (CON; n = 13), moderate-intensity continuous training (MICT; n = 13) or resistance training (RT; n = 12) 3 sessions/week. Upon completion of the 12-week intervention all participants returned for standardised post-intervention testing comprising of the same pre-test procedures.

Baseline Characteristics

Exercise Pre-screening and Anthropometry

Participants' baseline health characteristics were assessed during the familiarisation session via the Adult Pre-exercise Screening Tool (APSS). Anthropometric measures were obtained pre and post training including body mass, recorded wearing minimal clothing, using calibrated electronic scales (A and D HW-PW200, Thebarton, SA, Australia); height, recorded using a stadiometer in an upright position with head in the Frankfort plane; waist circumference, measured at the mid-point between the costal arch and the iliac crest; and hip circumference, measured in line with the greater trochanter (steel tape; EC P3 metric graduation, Sydney, NSW, Australia).

Graded Exercise Test (GXT)

All participants completed a ramped maximal graded exercise test (GXT) (Wattbike Trainer, Smeaton Grange, NSW, Australia) conducted with 1 min stages. An initial power output of 50 W was followed by incremental increase in power output of 25 W·min⁻¹ until volitional exhaustion was achieved. Expired gases and flow volumes were collected during the GXT and analysed by a calibrated metabolic cart (TrueOne 2400, ParvoMedics, Salt Lake City, Utah) to provide peak oxygen uptake (VO_{2peak}).

Actigraphy and Sleep Diaries

Participants were issued a wrist-worn actigraphy watch (Actiware 2, Philips Respironics, Andover, MA, USA) and sleep diary to record subjective bedtime, time of sleep initiation, awake time and the number of awakenings over a 14 d period. Actigraphy was recorded in 30 s epochs and analysed using Actiware v5.70 software (Philips Respironics) in combination with participant sleep diaries recorded during the collection period. Variables obtained included bed-time, wake time, time in bed (TIB; period between bedtime and wake time), total sleep time (TST; time asleep during TIB), sleep latency (period

between bedtime and sleep onset), sleep efficiency (percentage of time in bed spent sleeping), total time awake after sleep onset (WASO), and number of awakenings during total sleep period which were averaged across the collection period. If technical difficulties impacted data collection or the actigraphy device were removed for an extended period of time within an hour of documented bed or rising time, data of the subsequent sleep period was manually excluded.

Laboratory Testing Session

Following the 14 d actigraphy collection period, participants arrived at the laboratories between 0600 and 0900 h. Participants were instructed to avoid strenuous PA for 24-48 h and caffeine and food for ~12 h (fasted) prior to testing. As night shift is associated with acute sleep deprivation and circadian misalignment, testing occurred following either a day off or previous day shift to ensure adequate time for recovery and consistency between all participants. Participants underwent intravenous blood sampling, an OGTT, DEXA scan and five min rested HRV analyses.

Venous Blood Collection and Analysis

Venous blood was collected to determine baseline inflammatory markers pre- and post-intervention via a 22G catheter (22G BD Medical, North Ryde, NSW, Sydney) inserted into the medial antecubital vein. Samples were collected using ethylenediaminetetraacetic acid (EDTA) with a serine protease inhibitor (Pefabloc SC, Sigma-Aldrich, Sydney, Australia) or serum separator tubes (SST) which was left to clot for ~20 min before being centrifuged for 10 min at 4°C and stored at -80°C until analysis. Concentrations of IL-6, CRP, TNF- α and insulin were determined using commercially available enzyme-linked immunosorbent assays (ELISA) kit. Serum was analysed for C-reactive protein (CRP) (Human CRP ELISA kit/catalogue no. EK-0040; Crux Biolabs, Scoresby, VIC, Australia), TNF- α (Human TNF-alpha ELISA kit/catalogue no. EK-0001; Crux Biolabs, Scoresby, VIC, Australia) and insulin (Human Insulin

ELISA kit/catalogue no. ELH-Insulin-1; Raybiotech, Scoresby, VIC, Australia). Plasma was analysed for IL-6 (Human IL-6 ELISA kit/catalogue no. EK-0012; Crux Biolabs, Scoresby, VIC, Australia). The manufacturer's specified assay and analytical methods were used throughout. All samples were analysed in duplicate with minimum detectable cytokine levels for each kit: IL-6 ($<5 \text{ pg.ml}^{-1}$), TNF- α ($<5 \text{ pg.ml}^{-1}$), CRP ($<0.1 \text{ ng.ml}^{-1}$) and Insulin ($<4 \text{ }\mu\text{U.ml}^{-1}$) respectively. Duplicate readings for each standard, control and sample were averaged and the average zero standard optical density reading was subtracted. A standard curve was created using a 4-parameter logistic curve fit. Concentration values were then determined, using this curve for control and sample average optical densities per the ELISA kit instructions. The Pearson product-moment correlation coefficient (r) was determined by comparing the known standard concentration with the curve fit; r was shown to be greater than 0.98 for all the assays. The homeostasis model for insulin resistance (HOMA-IR) was calculated using the established formula, (fasting insulin [$\mu\text{U}/\text{mL}^{-1}$] x fasting glucose [mmol.L^{-1}]/22.5) (Bonora et al., 2002).

Oral Glucose Tolerance test

Following an overnight fast of at least 12 h, baseline blood glucose (BGL) levels were obtained via venepuncture, before participants consumed a 75 g oral glucose load dissolved in 300 ml carbonated water (Carbotest, Thermo Fisher Scientific, Australia) within 5 min. BGL was incrementally assessed every 30 min for a total of 120 min post-ingestion via Accu-Chek Performa 2 blood glucose monitoring system (Roche Diagnostics, Castle Hill, NSW, Australia). Testing procedures and blood glucose values were defined in accordance with American Diabetes Association (ADA) and World Health Organisation (WHO) Guidelines (American Diabetes Association, 2010). The blood glucose area under the curve (AUC) was calculated using the linear trapezoidal method: $\frac{1}{2} (C_1 + C_2) (T_2 - T_1)$ with the initial BGL value acting as a baseline.

Dual-energy x-ray Absorptiometry (DXA)

A supine DXA scan was undertaken to estimate whole-body composition (GE Lunar Prodigy, GE Healthcare, Madison, WI, USA). Participants assumed a supine position on the bench of the DXA machine and a whole-body scan was completed with a resolution of 4.5×9.0 mm and scanning speed of $130 \text{ mm}\cdot\text{s}^{-1}$. The scan was then analysed with customized software (Illuminatus DXA, version 4.2.0, Turnbull, CT, USA) for fat mass (FM) and fat free mass (FFM) which are reported in absolute (kg) and relative (%) terms.

Training Intervention

The training intervention was designed to meet the current exercise guidelines described by the World Health Organisation (Bull et al., 2020). Participants were required to exercise three days per week for 12 weeks in a semi-supervised setting. Each session was supervised by gym staff with a member of the research team supervising a minimum of one session per week. Both training groups completed a standardised 5 min warm up and cool down during the session. Outlined in Table 6.1, the program for the moderate-intensity continuous training (MICT) group consisted of 30 - 40 min of aerobic activity at 60 – 75 % of maximal heart rate (HR_{max}). Total session time could be accrued in a minimum of 10 min per aerobic mode including treadmill, stationary bike, or rower with mean sessional watts (W) or speed ($\text{km}\cdot\text{h}^{-1}$) recorded.

The resistance training (RT) group program comprised of two different sessions to be completed alternatively on pin-loaded weight machines targeting the major muscle groups. Participants completed 3 sets of 8 - 12 repetitions on all weight machine exercises with planks completed in sets of 3, progressing from 30 to 60 s. Progressive overload was provide to participants via increased weight incrementally over the 12 weeks when 12 repetitions were completed for each of the 3 sets. Further,

participants progressed from 3 sets per exercise to 4 sets for weeks 7-12. Repetitions and weight were recorded for each exercise in all sessions and used to assess training adaptation. Total session time including dynamic warm up and static stretch cool down was approximately 50 min, which when performed 3 days a week to meet the current PA guidelines (Bull et al., 2020).

Data Analysis

A *Priori* power analysis was conducted using GPower (G*Power v.3) (Faul, Erdfelder, Lang, & Buchner, 2007), for an repeated measures, within-between ANOVA with significance set at 0.05, power at 0.80, effect size at 0.25, groups 3 and measures 2. A total sample size of 42 participants was required. Data are reported as mean \pm standard deviation (SD) and statistical significance was accepted at $p \leq 0.05$. A one-way analysis of variance (ANOVA) was used to evaluate differences in baseline characteristics. A repeated within-between ANOVA, with the between factor, group (3 levels) and the within subject factor, time (2 levels) was used to assess training pre to post training effect. Significant time and group x time interactions ($p < 0.05$) were analysed using Tukey's post hoc analysis significant difference. All statistical analyses were conducted with SPSS software (version 26.0 SPSS Inc, Chicago, IL). Following statistical analyses data were regrouped for total sessions attended to assess the effect of exercise adherence (defined as equal to or greater than 2 sessions per week) regardless of training mode, on health outcomes. The same statistical approach was used for the regrouped data.

Results:

Baseline descriptive characteristics

As evident in Table 6.2, no significant differences were observed between intervention groups at baseline for age, anthropometry, blood pressure (BP) or aerobic capacity ($p > 0.05$).

Exercise adherence and performance measures

No significant difference was observed in total sessions attendance between the exercise groups ($p > 0.05$; Table 5.3). A significant pre-to-post training increase was observed in sessional workload (kg) for the three major RT exercises (chest press, lat pull down and leg press) ($p < 0.05$; Table 6.3). A significant pre-to-post increase was observed in the average sessional workload (W or km/h) for the aerobic exercise modes (treadmill, bike, and rower) ($p < 0.05$; Table 5.3).

Objective sleep assessment

Due to technical difficulties 8 (4 MICT, 2 RT and 2 CON) post-training actigraphy measures were not recorded and excluded from analysis, resulting in an $n = 30$. No significant differences were observed in pre-to-post training intervention for actigraphy variables, including TIB, TST, sleep efficiency, latency, WASO or number of awakenings (Table 6.4) ($p > 0.05$). However, comparison of sleep variables following a night shift revealed a significant main effect for time $F(1, 20) = 7.572$, $p = 0.012$, eta squared = 0.275. Post hoc analysis identified a significant pre-to-post increase in TST for MICT ($p = 0.04$) and RT ($p = 0.02$) (Table 6.4).

Inflammatory profile and glucose metabolism

Multiple participants reported metabolite levels below detectable ranges for both TNF- α ($n = 30$) and IL-6 ($n = 23$) resulting in the exclusion of analyses for those cytokines. A significant main effect for time was identified $F(1, 27) = 4.233$, $p = 0.049$, eta squared = 0.136 with post hoc analysis revealing a significant effect for MICT to reduce CRP from pre-to-post exercise ($p = 0.038$; Table 6.5). No significant difference was observed for insulin (MICT pre 5.8 ± 2.2 , post 5.0 ± 1.6 ; RT pre 6.4 ± 3.6 , post 6.4 ± 4.3 ; or CON pre 6.3 ± 2.9 , post $6.4 \pm 2.1 \mu\text{U}\cdot\text{ml}^{-1}$ $p > 0.05$). No significant time*group interaction as observed at any time point for the glucose response to an OGTT (Figure 6.2) or AUC for MICT pre 263.2 ± 115.1 ,

post 260.0 ± 224.6 , RT; pre 266.0 ± 102.5 , post 211.0 ± 164.9 , CON; 211.1 ± 121.0 , post 270.3 ± 124.6 $\mu\text{g}\cdot\text{ml}\cdot\text{min}^{-1}$. No significant difference was observed pre-to-post intervention for HOMA-IR ($p > 0.05$; Figure 5.1).

Body composition

A significant main effect for time was observed $F(1, 30) = 7.206$, $p = 0.012$, eta squared = 0.194 for WHR. Post hoc analysis identified a significant increase for the CON group WHR from pre-to-post ($p = 0.001$). No significant differences were observed for DXA measures of body composition including fat mass, fat free mass and fat mass as a percentage (%) ($p > 0.05$) (Table 6.5).

Re-grouped data relative to exercise adherence

Mean total session attendance for the ≥ 24 sessions (adherence; ADHERE; $n = 11$) group was 31 ± 4 , while the < 24 sessions (non-adherence; N-ADHERE; $n = 14$) group was 19 ± 2 sessions. No significant difference was observed in pre-to-post $\text{VO}_{2\text{peak}}$ for ADHERE (pre 32.7 ± 5.6 , post 34.6 ± 6.0 , N-ADHERE: pre 30.9 ± 5.7 , post 30.6 ± 5.2 $\text{ml}\cdot\text{kg}\cdot\text{min}^{-1}$). A significant group by time interaction was observed for body composition FM for ADHERE decreasing from pre (32.9 ± 4.7 to post 30.6 ± 3.6 kg $p = 0.013$) while no difference was observed for N-ADHERE (pre 36.7 ± 14.4 and post 37.1 ± 13.6 kg). A significant decrease in BF percentage was observed for ADHERE (pre 33.4 ± 2.9 and post 31.7 ± 3.2 %; $p = 0.009$); and no difference was observed for N-ADHERE (pre 35.3 ± 5.8 and post 35.7 ± 4.7 ; $p < 0.05$). No significant difference was observed for WHR (indexed) ADHERE (pre $0.95 \pm .06$, post 0.96 ± 0.07) and N-ADHERE (pre 0.99 ± 0.9 , post $1.0 \pm .09$; $p > 0.05$). No significant difference was observed for insulin ($\mu\text{U}\cdot\text{ml}^{-1}$) ADHERE; pre 6.4 ± 3.8 , post 5.2 ± 2.3 , N-ADHERE; pre 5.7 ± 1.6 , post 6.3 ± 4.0 ($p > 0.05$) or pre-to-post CRP or HOMA-IR ($p > 0.05$; Figure 5.1). No significant difference was observed for glucose response to OGTT ($p > 0.05$; Figure 5.2). No significant difference was observed for AUC ($\mu\text{g}\cdot\text{ml}\cdot\text{min}^{-1}$)

ADHERE; pre 254.7 ± 140.6 , post 209.6 ± 142.2 , N-ADHERE: pre 273.3 ± 114.5 , post 302.0 ± 177.4 ($p > 0.05$).

Discussion

The current project sought to investigate the pre-to-post effect of different exercise modes on markers of cardio-metabolic function among rotational shift workers. The key observation was 12 weeks of MICT significantly reduced CRP levels from pre-to-post interventions and increased TST following a night shift. In contradiction to the research hypothesis, no change was observed in the participants' average sleep characteristics, aerobic capacity, or glucose metabolism. Results which may be explained by total session attendance, with participants averaging $25 (\pm 7)$ of a possible 36, or 2.1 sessions per week. As exercise adaptations are dependent on stimulus, the reduced training adherence may have reduced the health benefits normally associated with exercise interventions. In support, re-grouping of participants regarding sessional adherence, rather than training modality, resulted in significantly improved body composition. Cumulatively, the findings suggest that the risk of developing cardio-metabolic disorders in apparently-healthy male shift workers may be reduced by engaging in at least 3 exercise sessions per week, particularly MICT, but several barriers exist to regular exercise participation in this population group.

Shift work is associated with an increased inflammatory state, including the increased circulation of CRP (Puttonen et al., 2011). The present findings indicate that 12 weeks of MICT significantly reduced pre-to-post levels of CRP among rotational shift workers (Figure 6.1). Regarding cardio-metabolic function, CRP is identified as a biomarker of systemic inflammation, and considered to be a strong univariate predictive of future adverse cardio-metabolic outcomes (Puttonen et al., 2011; Ridker, Hennekens, Buring, & Rifai, 2000). Consequently, the significant reduction in CRP levels induced by MICT may reduce the relative risk of future cardio-metabolic disorders among shift workers.

Conversely, RT did not influence CRP levels, and with no significant difference in group exercise adherence, the results suggest MICT may be a more effective exercise mode to improve inflammatory status among shift workers. A plausible explanation may be the differences in mode-based oxidative adaptations between exercise groups. Increased oxidative stress has previously been associated with shift work (Demir, Toker, Zengin, Laloglu, & Aksoy, 2016) and is hypothesised to facilitate increased levels of CRP (Abramson et al., 2005). Endurance exercise is associated with enhanced muscle metabolism, specifically increased oxidative capacity due to mitochondrial biogenesis (Hood, 2009; Wang, Mascher, Psilander, Blomstrand, & Sahlin, 2011). Consequently, MICT may have improved skeletal oxidative capacity and reduced a potential stimulant for CRP production. Alternatively, chronic training may have upregulated anti-inflammatory mediators including IL-6, capable of inhibiting the pro-inflammatory cascade and reducing systemic inflammatory markers (Del Giudice & Gangestad, 2018). Unfortunately, as the supplementary inflammatory markers (TNF- α and IL-6) were below detectable ranges within the current project, interpretations of potential mechanisms responsible for the divergent training adaptations are limited and require further elucidation.

Cardio-metabolic function is additionally moderated by sleep-wake cycles and poor sleep, particularly disturbed or reduced TST is hypothesised to facilitate pathogenesis (Kecklund & Axelsson, 2016). Employment in rotational shift work is associated with reductions in sleep quality (Niu et al., 2011; Shantha, 2013), with night shift specifically demonstrated to reduce TST of the subsequent sleep opportunity (Ferguson et al., 2019). When comparing the quality and quantity of sleep following a night shift, a significant increase in TST was observed following both MICT and RT. The MICT and RT intervention increased post night-shift TST by 31 and 35 mins respectively, results associated with decreased cardio-metabolic risk (Kecklund & Axelsson, 2016) despite no significant differences were observed for the pre-to-post intervention mean sleep assessment. Several factors may have contributed to the non-significant changes including screening for pre-existing sleep disorders.

Resultantly, the mean sleep variables, including TST, latency, and sleep efficiency of participants (Table 4), may have created a ceiling effect with little scope for improvement. Additionally, actigraphy and sleep diary assessment of sleep quality parameters were averaged over a 14-day period, including days on, off and different shift rotations such as night shift. Consequently, strategies including naps or extended sleep on days off may mask the differences in acute sleep bouts, or acute improvements in sleep quality (Wickwire et al., 2017).

Despite exercise interventions being associated with a range of health benefits (Warburton et al., 2006) no significantly improvements were observed in body composition, aerobic capacity or glucose metabolism post intervention. Lifestyle behaviours, including diet and alcohol intake have previously been demonstrated to effect body composition and glucose metabolism (Nea, Kearney, Livingstone, Pourshahidi, & Corish, 2015). To minimise the potential effect, participants were instructed to maintain their habitual dietary habits, however no food diary was recorded for analysis limiting interpretation of the current results. Exercise-based adaptations are additionally specific to the exercise stimulus and total dose (Baar, 2009). Shift workers face labour specific barriers to exercise adherence including limited break time, lack of specific exercise routine, and limited exercise opportunities in sync with biological rhythms (Atkinson et al., 2008; Blake et al., 2017; Nea et al., 2017). In addition to shift work specific barriers, the presence of undiagnosed obstructive sleep apnea may have impacted sessional attendance and physiological outcomes in participants. Screening for diagnosed sleep disorders were conducted by subjective health questionnaires, however given the inactive and overweight status of participants, disorders may have been present undiagnosed (Smith, Doyle, Pascoe, Douglas, & Jorgensen, 2007). Sleep apnea has previously been established as a barrier to exercise adherence with patients less inclined to increase exercise participation (Smith et al., 2007). While not assessed, it is likely that established barriers to exercise resulted in reduced adherence and insufficient stimulus to significantly improve body composition, aerobic capacity and glucose

metabolism. Re-distribution of interventions groups based on sessional attendance rather than training modality resulted in observable changes in body composition. A significant decrease in body fat (-2.3 ± 3.2 kg) and body fat percentage (-1.7 ± 2.0 %) for the ADHERE group. For additional context, the WHR significantly increased among CON group (Table 6.6) and while not reaching significance ($p > 0.05$), both the CON and N-ADHERE groups both gained fat mass over the course of the intervention.

Increased adiposity is classified as a risk factor for the development of cardio-metabolic disorders (Poirier et al., 2006) as hypertrophic adipocytes are hypothesised to have a direct mechanistic role due to molecular and cellular alterations (Berg & Scherer, 2005; Golbidi et al., 2012), and subsequent secretion of pro-inflammatory makers, increased leptin and decreased adiponectin levels and decreases in insulin sensitivity (Berg & Scherer, 2005; Golbidi et al., 2012; Greenberg & Obin, 2006). Consequently, the higher exercise adherence, regardless of modality, reduced adipose levels and may reduce the associated relative risk of developing cardio-metabolic disorders (Berg & Scherer, 2005; Wallberg-Henriksson & Zierath, 2015) among shift workers. Interestingly, the re-distribution of intervention groups based on sessional attendance resulted in a non-significant effect of training on CRP levels. A result which was not expected but may be partially explained by the combination of training modalities (MICT and RT) given RT did not elicit a significant response. Alternatively, the pre-testing CRP value (1.28 ± 0.7 ng/ml) may have been too low for the ADHERE session training group to elicit a statistically meaningful training response.

A mechanistic link underlying the association of obesity with cardio-metabolic conditions, is the observed effect of adipose tissue and inflammation on decreasing insulin sensitivity (Berg & Scherer, 2005; Greenberg & Obin, 2006). Further, exercise induced improvements in insulin sensitivity (HOMA-IR) have been associated with reduced adiposity (Shih & Kwok, 2018). However, both the normally

and redistributed data sets demonstrated no significant effect on the current markers of glucose metabolism, assessed by fasted blood glucose, insulin, and AUC in response to an OGTT. Results which may again be explained by the pre-intervention screening process excluding participants with diagnosed cardio-metabolic disorders. Resultantly, the mean baseline variables were within healthy ranges and potentially created a ceiling effect with minimal room for significant improvements. Further, the reduced exercise adherence may be a limitation in interpreting the current observations. Previous interventions, conducted with similar methodology among comparable participants have resulted in significant changes in body composition, fasting glucose and HOMA-IR (Jorge et al., 2011; Saremi, Asghari, & Ghorbani, 2010). However the protocols prescribed increased intensity and volume throughout the interventions, and participants reported higher adherence rates, completing 94 % of exercise sessions (Saremi et al., 2010). Resultantly, the current exercise intervention may not have provided sufficient stimulus to elicit metabolic improvements, however decreased adherence to the program impeded such interpretations.

Cumulatively, exercise presents as a viable intervention method to improve cardio-metabolic health among male shift workers. Sessional attendance and an observed dose-response effect on body composition indicates further considerations are required to address exercise adherence. The structure of shift work is theorised to exacerbate general barriers to exercise including a lack of time, lack of motivation and feeling too tired to participate (Hargens et al., 2013; 2017). The current research project attempted to increase exercise adherence via providing a semi-autonomous (regarding cardio mode), structured and flexible training intervention. For example the provision of a semi-supervised training intervention was theorised to provide a flexible training schedule and potentially address the issue of exercise opportunities in opposition to gym scheduling (Blake et al., 2017; Nea et al., 2017). However, the mean session attendance over the 12 weeks (25 ± 7) and observation that less than half of the participants (44%) completed more than 2 sessions per week,

indicate that more research is required to facilitate shift work exercise adherence. Several factors may have contributed to reduced exercise adherence within the current project including labour specific restrictions or a preference for the training modality not prescribed to participants. Alternatively, the sessional attendance may reflect current trends of non-adherence among the general population (Burnet, Higgins, Kelsch, Moore, & Stoner, 2020). Therefore, the inclusion of a general population control group may have strengthened the current study design and provided context for exercise adherence. Regardless, previous research among shift workers suggest that both supervised training and incorporating worksite interventions (Matsugaki et al., 2017) may be a more effective strategy, however the limited body of exercise based interventions among shift workers limits the current interpretations.

Conclusion

The results support exercise as a viable intervention to minimise the relative risk of future disease development for male rotational shift workers. A limitation of the current project is the recruitment of male participants, potentially impacting the application of results to the general shift work population and female employees. Further, as indicated by the adherence to the training protocol, several barriers exist to participating in exercise interventions. Cumulatively, future research needs to both substantiate the health effect of exercise among general shift work population, while exploring the most effective method increase adherence.

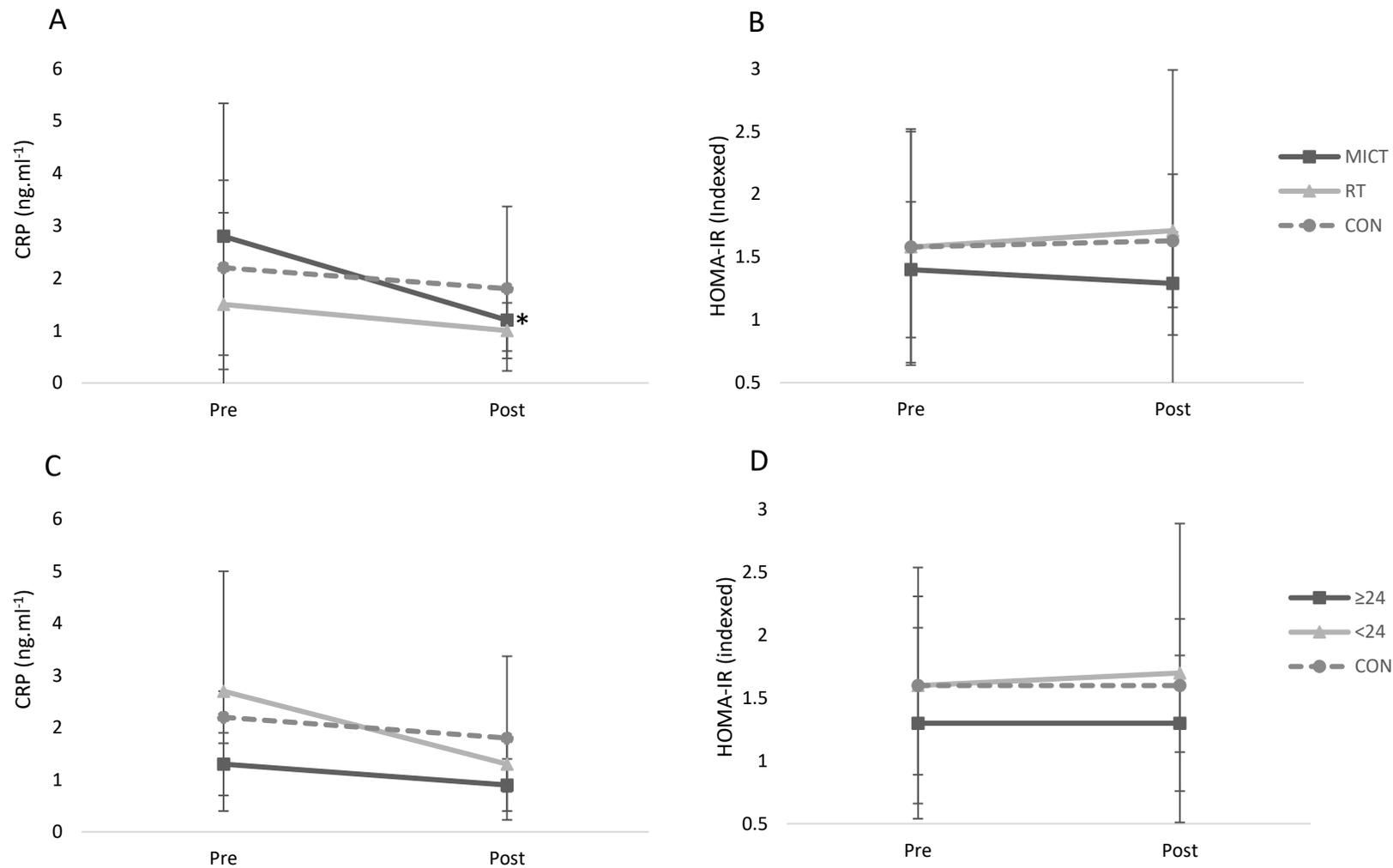


Figure 6.1 Pre-to-post 12 week training response for CRP and insulin resistance (HOMA-IR index). (A) Pre-to-post CRP values (B) Pre-to-post insulin resistance as calculated by HOMA-IR (C) Redistributed pre-to-post CRP values (D) Redistributed Pre-to-post insulin resistance as calculated by HOMA-IR. Pre; pre intervention, post; post intervention. Values are calculated mean \pm SD. * denotes significant difference from pre ($p < 0.05$)

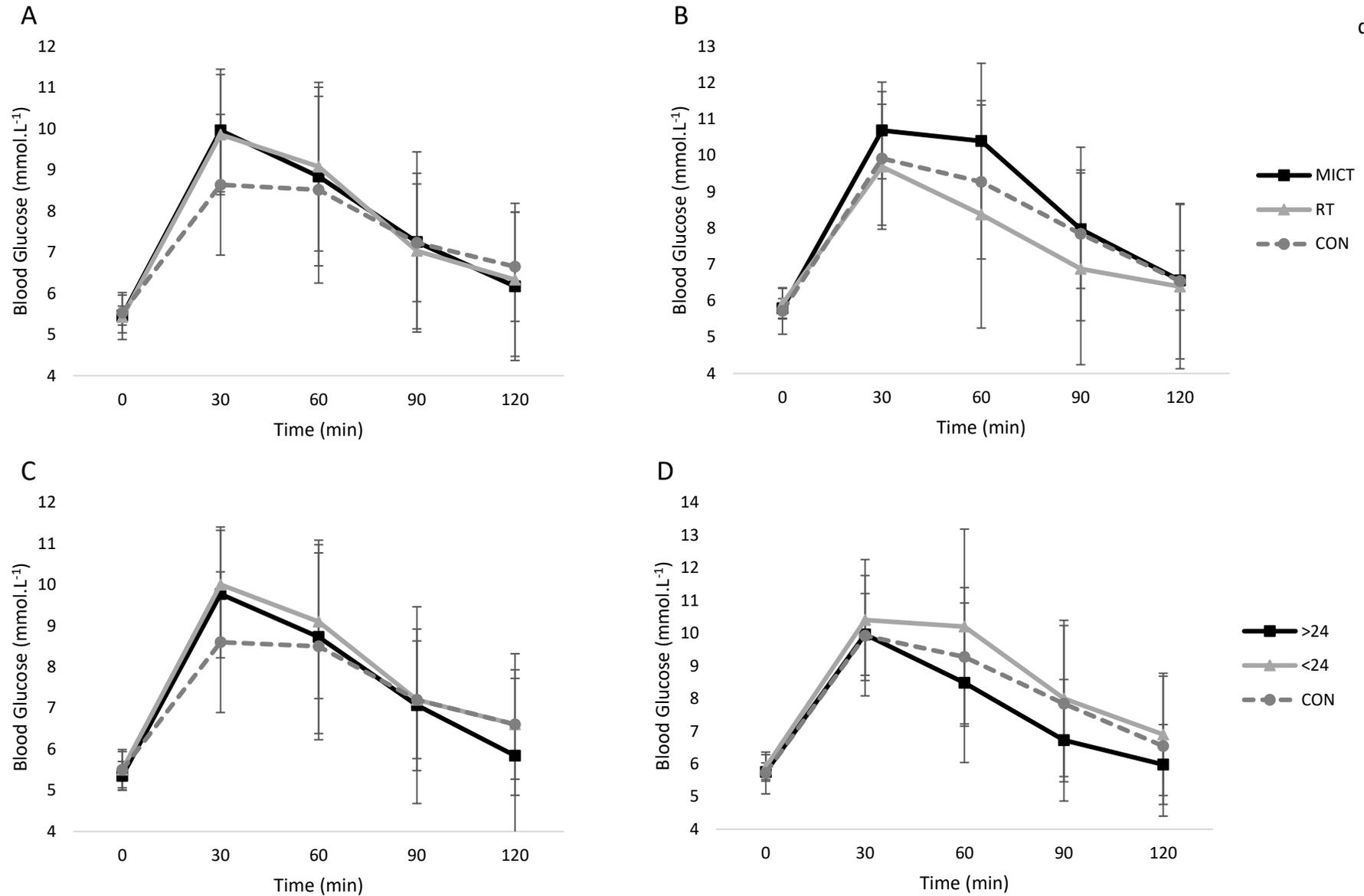


Figure 6.2 Pre-to-post 12 week training blood glucose response to OGTT. (A) Pre OGTT response (B) Post training OGTT (C) Redistributed pre training OGTT (D) Redistributed post training OGTT. 0; pre ingestion, 30; 30 mins, 60; 60 mins, 90; 90 mins. 120; 120 mins post. Values are calculated mean \pm SD. No significant differences were observed ($p > 0.05$)

Table 6.1 Training intervention program overview including exercise, mode, sets and repetitions

RT	Weeks 1-6	Weeks 7-12
<i>Session 1</i>		
Chest Press	3 S x 8-12 R	4 S x 8-12 R
Lat Pull Down	3 S x 8-12 R	4 S x 8-12 R
Leg Press	3 S x 8-12 R	4 S x 8-12 R
Shoulder Press	3 S x 8-12 R	4 S x 8-12 R
Bicep Curl	3 S x 8-12 R	4 S x 8-12 R
Plank	3 S x 30-60 s	4 S x 30-60 s
<i>Session 2</i>		
Chest Press	3 S x 8-12 R	4 S x 8-12 R
Seated Row	3 S x 8-12 R	4 S x 8-12 R
Hamstring Curl	3 S x 8-12 R	4 S x 8-12 R
Seated Leg Extension	3 S x 8-12 R	4 S x 8-12 R
Triceps Extension	3 S x 8-12 R	4 S x 8-12 R
Plank	3 S x 30-60 s	4 S x 30-60 s
MICT	Weeks 1-6	Weeks 7-12
Rower	Min 10 min @ 60-65% HR _{max}	Min 10 min @ 65-75% HR _{max}
Stationary Bike	Min 10 min @ 60-65% HR _{max}	Min 10 min @ 65-75% HR _{max}
Treadmill	Min 10 min @ 60-65% HR _{max}	Min 10 min @ 65-75% HR _{max}
Session Total	30-35 min @ 60-65% HR _{max}	40 min @ 65-75% HR _{max}

HR_{max}; measured maximum heart rate. MICT; moderate intensity continuous training. R; repetitions of the exercise. RT; resistance training. S; sets of the exercise. S; seconds. @; at. %; percent.

Table 6.2 Anthropometric and baseline characteristics of rotational shift workers presented by intervention group

Measure	MICT	RT	CON
Age (years)	41 ± 8	37 ± 8	38 ± 8
BMI (kg.m ²)	33.2 ± 5.9	32.0 ± 5.2	30.5 ± 4.6
WHR (index)	0.98 ± 0.1	0.97 ± 0.1	0.93 ± 0.1
Systolic BP (mmHg)	132 ± 7	129 ± 10	130 ± 10
Diastolic BP (mmHg)	87 ± 7	84 ± 7	85 ± 8
VO _{2peak} (ml.kg.min ⁻¹)	29.7 ± 4.3	33.8 ± 5.9	33.6 ± 5.5

BMI; body mass index. BP; blood pressure. CON; control. MICT; moderate intensity continuous training. RT; resistance training. WHR; waist-to-hip ratio.

No significant differences observed at baseline ($p > 0.05$)

Table 6.3 Training intervention sessional attendance and pre-to-post training adaptation

	MICT		RT		CON	
Performance Measure	Pre	Post	Pre	Post	Pre	Post
Total Sessions	26 ± 8		22 ± 5		-	
VO _{2peak} (ml.kg.min ⁻¹)	29.7 ± 4.4	31.1 ± 4.4	33.8 ± 6.2	33.8 ± 6.9	33.6 ± 5.7	33.9 ± 5.9
Chest Press (kg)	-	-	49 ± 8	72 ± 11 *	-	-
Lat Pull Down (kg)	-	-	41 ± 6	46 ± 5 *	-	-
Leg Press (kg)	-	-	80 ± 18	120 ± 24 *	-	-
Rower (W)	99 ± 24	132 ± 16 *	-	-	-	-
Bike (W)	118 ± 20	144 ± 29 *	-	-	-	-
Treadmill (km.h ⁻¹)	5 ± 1	6 ± 2 *	-	-	-	-

CON; control. kg; kilograms. MICT; moderate intensity continuous training. RT; resistance training. W; watts. km.h⁻¹; kilometres per hour. ml.kg.min⁻¹; oxygen consumption expressed in millilitres per kilogram per minute.

* denotes significant difference compared to baseline ($p < 0.05$)

Table 6.4 Training intervention pre-to-post training effect on actigraphy based sleep assessment

	MICT		RT		CON	
Actigraphy – Averaged assessment	Pre	Post	Pre	Post	Pre	Post
TIB (min)	452 ± 40	449 ± 31	464 ± 65	440 ± 38	440 ± 28	446 ± 33
TST (min)	397.5 ± 35.8	381.6 ± 42.5	409.8 ± 79.5	363.87 ± 40.9	372.6 ± 48.6	398.4 ± 34.9
Efficiency (%)	88.4 ± 3.8	85.1 ± 9.8	85.6 ± 6.4	82.7 ± 5.8	83.2 ± 11.4	88.2 ± 2.2
Latency (min)	7.5 ± 2.8	14.2 ± 13.8	14.7 ± 8.8	23.4 ± 13.8	19.5 ± 22.2	11.2 ± 6.2
WASO (min)	32.8 ± 13.2	32.2 ± 22.5	34.7 ± 16.1	33.8 ± 14.7	32.4 ± 9.8	28.6 ± 7.9
Awakenings (#)	32.2 ± 7.9	27 ± 10.3	39.9 ± 14.3	33.1 ± 13.6	32.1 ± 8.9	33.1 ± 8.1
Actigraphy - Night Shift	Pre	Post	Pre	Post	Pre	Post
TIB (min)	419 ± 59	463 ± 26	423 ± 35	455 ± 35	-	-
TST (min)	355 ± 41	386 ± 44 *	350 ± 40	385 ± 40 *	-	-
Efficiency (%)	85.8 ± 9.2	83.6 ± 8.7	82.7 ± 4.8	84.7 ± 5.9	-	-
Latency (min)	10.8 ± 9.3	13.2 ± 9.2	22.3 ± 7.3	18.6 ± 19.1	-	-
WASO (min)	33.0 ± 21.9	31.0 ± 20.9	34.5 ± 17.2	36.4 ± 14.2	-	-
Awakenings (#)	27.8 ± 10.5	26.7 ± 9.3	35.6 ± 14.1	38.0 ± 16.1	-	-

CON; control. MICT; moderate intensity continuous training. RT; resistance training. TIB; time in bed. TST; total sleep time. WASO; wake after sleep onset. #; number. %; percent. * denotes significant difference compared to baseline ($p < 0.05$)

Table 6.5 Pre-to-post training intervention effect on Dual-energy x-ray absorptiometry body composition and anthropometry

Measure	MICT		RT		CON	
	Pre	Post	Pre	Post	Pre	Post
Weight (kg)	102.4 ± 17.9	101.5 ± 17.6	103.4 ± 17.1	103.6 ± 17.32	94.3 ± 15.6	94.7 ± 15.9
LM (kg)	63.2 ± 7.1	63.4 ± 7.7	66.7 ± 8.2	66.9 ± 8.2	60.8 ± 8.4	61.1 ± 8.3
FM (kg)	36.1 ± 11.3	34.9 ± 10.9	33.9 ± 10.5	33.6 ± 10.1	30.4 ± 8.3	31.3 ± 8.1
BF (%)	35.7 ± 4.4	34.9 ± 4.4	33.0 ± 4.8	32.9 ± 4.5	32.8 ± 4.8	33.4 ± 4.3
BMI	32.1 ± 3.3	31.4 ± 3.2	32.9 ± 7.1	33.1 ± 7.2	30.5 ± 4.7	30.8 ± 4.6
WHR	0.95 ± .06	0.96 ± 0.07	.99 ± 0.9	1.0 ± .09	.95 ± .07	.98 ± .08*

BF; body fat. BMI; body mass index. CON; control. FM; fat mass. LM; lean mass. MICT; moderate intensity continuous training. RT; resistance training. WHR; waist-to-hip ratio. %; percent.

* denotes significant difference compared to baseline ($p < 0.05$)

Chapter Seven:

Discussion

7.1 Overview of the thesis

This thesis examined the potential effect of shift work on cardio-metabolic function, inflammation, and sleep among male employees, while assessing the validity of exercise as an intervention to mitigate the potentially negative effects of shift work and improve employee health. The specific aims of the thesis were:

1. Examine the effect of employment in rotational shift work on markers of cardio-metabolic function including sleep quality, body composition, metabolic efficiency, inflammatory status and autonomic modulation;
2. Examine the effect of exercise, including variations in mode, intensity, and chronicity (acute vs training), on markers of cardio-metabolic function and sleep among rotational shift workers.

The thesis aims were achieved by comparing the cardio-metabolic health of rotational shift workers to age-matched, fixed day, counterparts (Chapter Three). In relation to thesis aim two; both the acute (Chapter Four); and chronic training effect (Chapter Five), of exercise were investigated regarding pre-to-post changes in markers of cardio-metabolic function among shift workers. The acute and chronic training interventions further explored the potential effect of exercise intensity and training modality by comparing moderate continuous and high intensity intervals (Study Two); and investigating the specific effects of aerobic and resistance training (Study Three) on cardio-metabolic function. This chapter will discuss the main findings and interpretations collectively in relation to the thesis aims and the existing literature.

7.2 Summary of the Major Findings

7.2.1 Chapter Four - Shift work and cardio-metabolic function

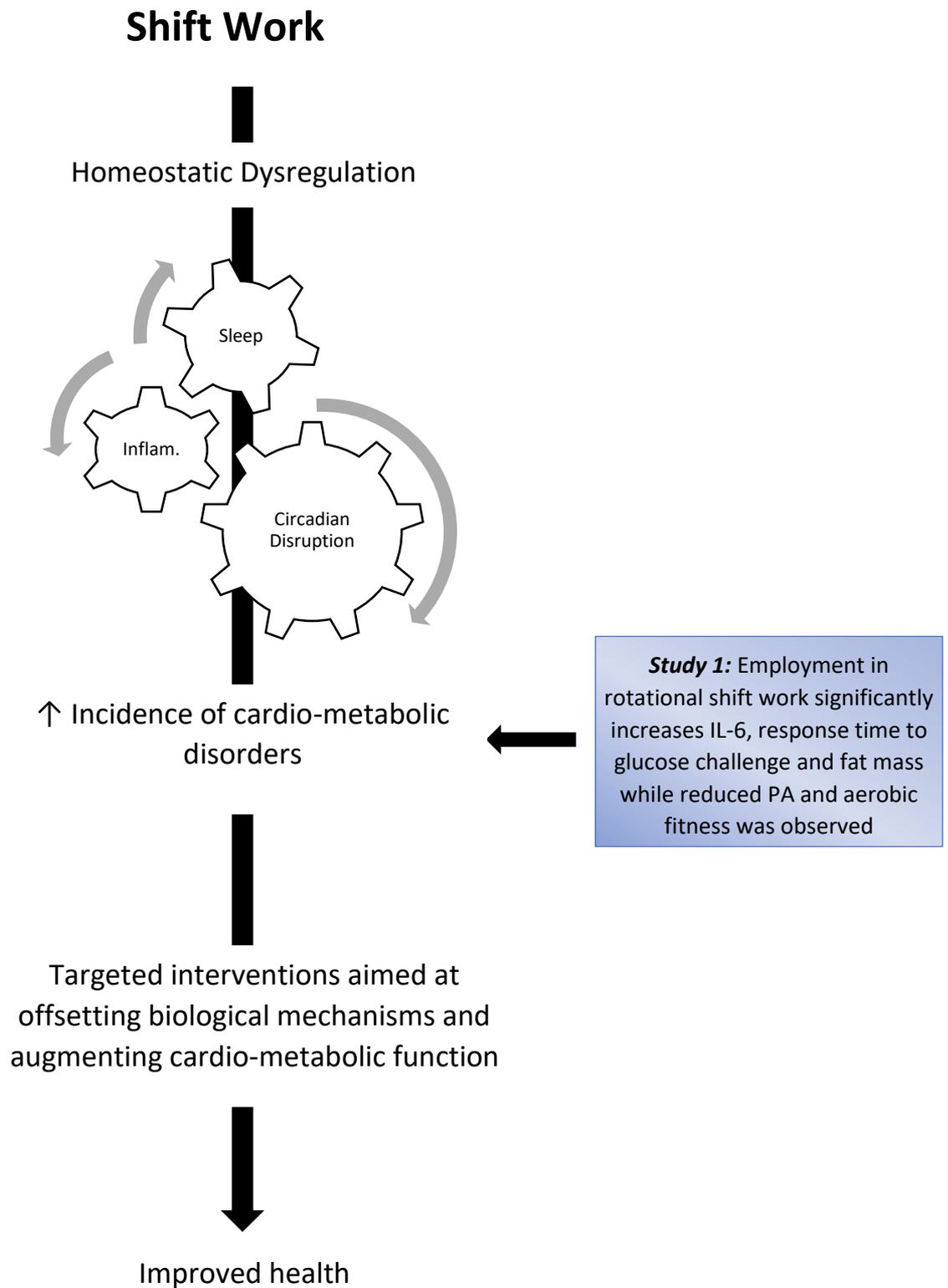
The theoretical approach to designing this thesis centred on two fundamental questions regarding the relationship between shift work and cardio-metabolic health. Does shift work adversely affect cardio-

metabolic function? And, if so, what are the primary mechanisms responsible for pathogenesis? Therefore, the first study in this thesis (Chapter Four) examined the physiological effect of rotational shift work on markers of cardio-metabolic function. Male participants were recruited and matched for lifestyle behaviours including physical activity (PA) levels and non-smoking status, before being screened (and excluded from the study) for any current cardio-metabolic or sleep disorders. The dependant variables included measures of cardio-metabolic function, body composition, sleep quality, metabolic efficiency (fasted and in response to a glucose challenge) and inflammatory status. The independent variable was occupational status, with men, currently employed in rotational shift work (8-12 h morning, afternoon, and night rotations) compared to fixed day-work counterparts. The findings support the study hypothesis and previous research (Puttonen, Viitasalo, & Härmä, 2012), associating rotational shift work with an increased risk of future cardio-metabolic disorders, and suggest a role of the work structure in pathogenesis. Specifically, shift workers had an increased response time to a glucose challenge (OGTT), higher levels of adiposity (fat mass and fat mass as a %) and increased levels of circulating inflammatory markers (IL-6). While these results may be associated with a range of both lifestyle and physiological factors, given all participants were considered inactive (<150 mins/week), currently health non-smokers, the results indicate an independent pathogenic role of employment in rotational shift work. One of the potential pathogenic mechanisms is the significant reduction in TST following a night shift compared to non-shift workers. While this difference, when averaged across the 14-d collection period, become non-significant, the acute bouts of 'sleep deprivation' following night shift have previously been demonstrated to adversely affect metabolism and inflammatory status (Donga et al., 2010; Saner et al., 2018).

Shift work employees did report a significant reduction in subjectively reported weekly PA, which may also have contributed to the observed increase in cardio-metabolic risk factors. Alternatively, the shift structure limiting exercise opportunity may have facilitated both reduced weekly PA and increased

cardio-metabolic disease risk. An additional novel observation of the project were the non-significant differences in HRV and supplementary inflammatory markers (CRP and TNF- α). Previous acute intervention projects have demonstrated adverse effects on the aforementioned measures, which may suggest a divergence in the acute and chronic effect of shift work. *Figure 7.1* provides an update on the theoretical model proposed in the introduction chapter (Chapter One) with the inclusion of key observations from Study One (Chapter Four).

Figure 7.1. Updated theoretical model investigating shift work pathogenesis of cardio-metabolic disorders to include the observations of Study One



7.2.2 Chapter Five & Six - Exercise as an intervention

Acute cardio-metabolic effect of exercise:

Exercise is a valid intervention strategy with a myriad of research demonstrating wide ranging salutary effects on cardio-metabolic health (Myers et al., 2004; Warburton et al., 2006). However, exercise is a broad term of PA, varying in mode, intensity, and volume with independent adaptations specific to the stimulus (Baar, 2009). Consequently, research projects may look to delineate between specific stimuli to investigate potential adaptations among selected population groups. However, there is currently a limited body of research conducted among rotational shift workers regarding exercise interventions and specific adaptations (Flahr et al., 2018). Therefore, Study Two (Chapter Five), aimed to assess the acute effect of exercise at different intensities on cardio-metabolic health among male rotational shift workers. The study included pre- and post-sampling of cardio-metabolic markers following two acute training interventions, i) moderate intensity (MICT) and ii) high intensity interval training (HIT).

The acute exercise project demonstrated significant increases in an anti-inflammatory mediator, IL-1Ra, following HIT, and decreased wake after sleep onset (WASO), indicative of reduced sleep fragmentation following MICT. The primary findings support the cogent body of previous research advocating the acute effect of exercise for improving cardio-metabolic function (Bird & Hawley, 2012; Gebel et al., 2015), while adding to the scarce research conducted among shift workers (Flahr et al., 2018). Specifically, as cardio-metabolic function is mediated, in part, by sleep and integrated inflammatory responses, the acute improvements in anti-inflammatory proliferation (IL-1Ra) and reduced sleep fragmentation, may decrease the relative risk of developing cardio-metabolic disorders. Consequently, acute bouts of HIT and MICT appear to be a viable intervention strategy to improve the future health outcomes of shift workers. Further, the apparent difference in intensity mediated results (HIT induced an anti-inflammatory response while MICT improved objectively measured sleep),

warrant further investigation to explore the mechanistic differences and develop the most effective intervention methods to elicit improvements in health and wellbeing.

One consideration moving forward is the timing of exercise intervention for rotational shift workers. Chapter Two (Literature Review) highlighted the potential role exercise may have in shifting circadian phases and effecting objectively measured sleep stages. Collectively, exercise is a viable intervention to improve circadian regulation and sleep quality, alternatively poorly timed exercise (too close to subsequent sleep opportunity or in opposition to individual chronotypes) may exacerbate the homeostatic desynchronisation among shift workers. To limit research co-factors, including the effect of circadian misalignment on cardio-metabolic health measures, testing was conducted on consecutive days off. However, such a restriction may have created an ‘ideal test and sleep window’ that does not explore the potential negative effect of poorly timed exercise interventions or interaction with circadian misalignment. A potential limitation that may not reflect the effect of exercise under less conducive conditions. *Figure 2* provides an update on the theoretical approach outlined in the thesis introduction (Chapter 1) to include the key findings from Study Two, and the contribution made to overall thesis.

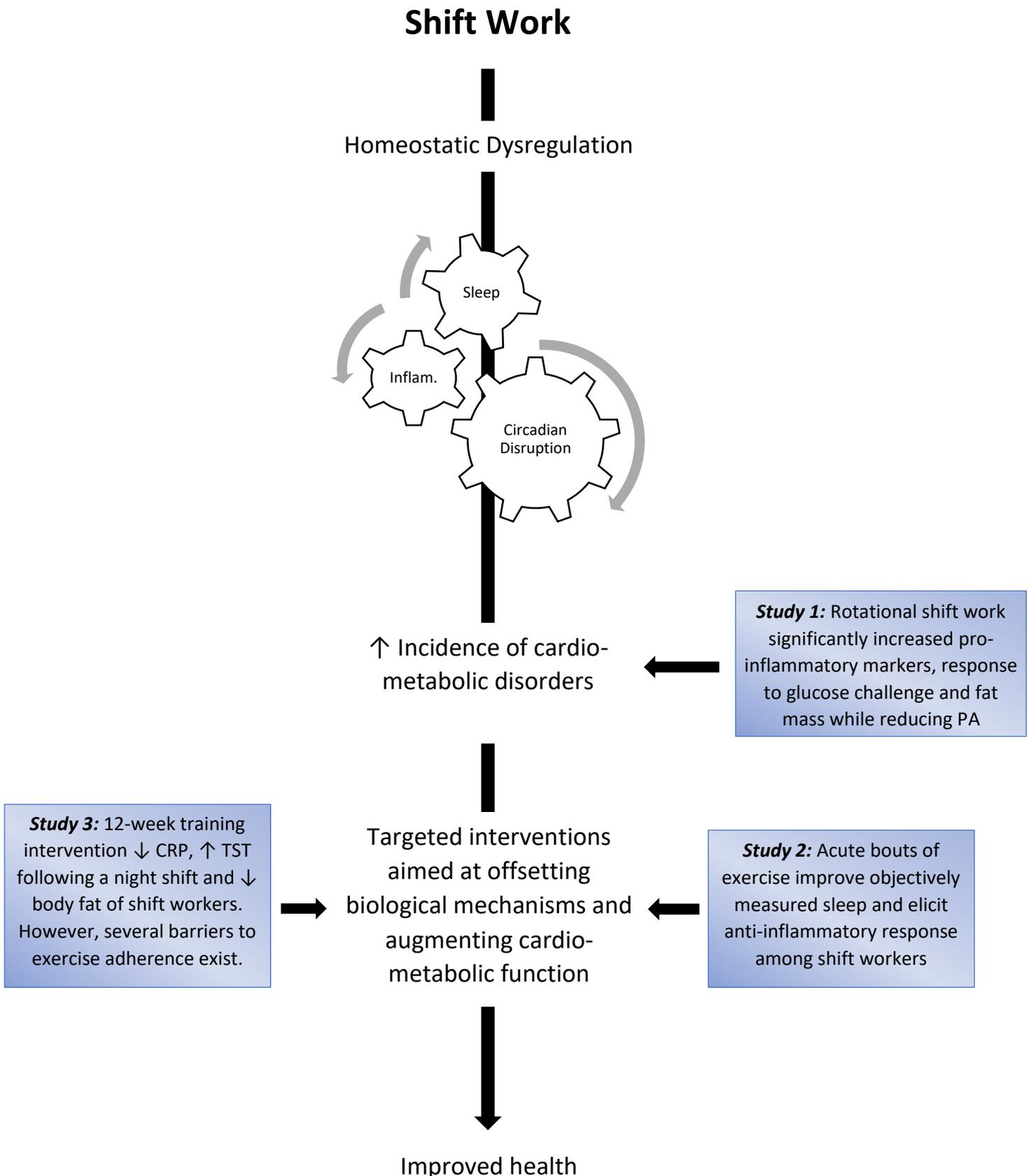
Chronic effect of exercise:

The third study (Chapter Six) examined the chronic effect of exercise on cardio-metabolic function, inflammation, and sleep among rotational shift workers. Current shift workers volunteered to complete a 12-week exercise training intervention with pre and post cardio-metabolic testing. Participants were divided into three intervention groups: moderate-intensity continuous training (MICT), resistance training (RT) or a non-exercise control (CON). In relation to the second thesis aim, the novel finding of Study Three is the exercise induced improvement in markers of cardio-metabolic

function and reduced the relative risk of future adverse cardio-metabolic conditions. As the cardio-metabolic system is moderated, in part, by sleep and inflammatory status, inflammatory markers such as CRP, TNF- α and IL-6 are commonly used to assess cardio-metabolic health and future disease risk. Following a 12-week training intervention, shift workers demonstrated a significant reduction in CRP concentration following MICT, and increased TST following night shift for both MICT and RT groups. Further, re-distribution of training data based on sessional attendance resulted in a significant reduction in body composition (reduced fat mass and fat mass %) in the ADHERE (>24 sessions) group.

Collectively, the results from the final project support exercise as a viable intervention to both improve cardio-metabolic function and potentially decrease the future disease risk among rotational shift workers. However, the necessity to re-distribute data to further investigate the salutary effect of exercise is indicative of the barriers to exercise adherence experienced by shift workers. The mean sessional attendance was 25 of a possible 36 sessions, equating to only 2.1 sessions per week. Attendance rates which did not meet the prescribed requirements over the 12 weeks, despite the provision of free, semi-autonomous and pre-programmed training within a semi-supervised setting. Low attendance of the intervention has two influential outcomes on future exercise prescription. Firstly, low adherence limits the research group's interpretations of the potential effectiveness of the prescribed training interventions. For example, Chapter 5 reported no significant changes in aerobic capacity, a key marker of cardiorespiratory fitness and predictive of future cardio-metabolic conditions (Swainson, Ingle, & Carroll, 2019). However, it is plausible that the current project design (3 sessions per week), which is in accordance with exercise prescription guidelines, was sufficient to improve aerobic capacity, but low adherence prevented the observation of significant results. Secondly, the adherence rates indicate shift workers face substantial barriers to exercise participation, consequently future interventions should target increased adherence to assess the health effects of exercise.

Figure 7.2 Updated theoretical model investigating the effect of shift work, acute and chronic exercise interventions on cardio-metabolic function.



7.2.3 Homeostatic dysregulation

Collectively, the thesis explored the concept of homeostatic dysregulation on pathophysiology and the potential risk of systemic inflammation, autonomic regulation and cardio-metabolic disease. While there are a plethora of physiological perturbations associated with shift work, these variables were of particular focus as shift work has been shown to have a clear association with an increased incidence of cardio-metabolic disorders (Flahr et al., 2018; Kecklund & Axelsson, 2016). However, there are discrepancies concerning the etiological contribution to pathology due to methodological issues and disease co-factors. For example, the research projects identifying an increase in disease risk associated with occupation suggest multiple pathogenic mechanisms, including lifestyle factors such as engaging in smoking and drinking alcohol at higher rates than non-shift colleagues (Dorrian et al., 2017; García-Díaz et al., 2015; Schilperoort et al., 2020). Further established cardio-metabolic risk factors including increased adiposity and decreased aerobic capacity have been explained by structural limitations of shift work including the availability of non-nutritious food during night shifts or inability to maintain an active lifestyle (Han, Trinkoff, Storr, & Geiger-Brown, 2011; Kervezee et al., 2018; Schilperoort et al., 2020). Alternatively, this thesis posits that the primary mechanisms responsible for cardio-metabolic pathogenesis among rotational shift workers is disruption of the regulatory systems that mediate cardio-metabolic function.

The first aim of this thesis focused on the potential role homeostatic desynchronisation plays in the development and progression of cardio-metabolic disorders to investigate pathogenic mechanisms and provide context for targeted interventions. Cardio-metabolic function is moderated by integrated biological systems to ensure proficient function within a dynamic environment (Flahr et al., 2018; Saper et al., 2010). Circadian rhythms, with a hierarchical structure and integration of exo- and endogenous signaling provides the temporal regulation of homeostatic responses (Antunes et al., 2010). Sleep plays a role in calibrating circadian rhythms and provides a designated time for the

partitioning of resources, growth and repair (Carskadon & Dement, 2005). Finally, the immune system has a central role in somatic maintenance and defense, including coordinating metabolic function and responses to stressful stimuli (Lange et al., 2010; Medzhitov, 2008). The aforementioned systems not only interact to mediate cardio-metabolic function but share anatomical structures, neural projections and humoral mediators which are regulated by both central and peripheral clocks. Consequently, the cardio-metabolic system has the distinct advantage of predictive, rather than reactive homeostatic mediation, however, during circumstances such as shift work, exo- and endogenous disruptions are propagated across multiple systems and under chronic conditions, induce pathogenesis (Leproult et al., 2014; Schilperoort et al., 2020). Common disruptions for shift workers include disturbed sleep or engaging in entraining behavior such as feeding, sleep or activity out-of-sync with circadian timing. Consequently, the structure of shift work, with rotating or fixed shifts in opposition to biological rhythms, facilitates homeostatic disruption, which under chronic conditions, increases the risk of developing cardio-metabolic disorders.

In support, Thesis Chapter Three (Study One) compared markers of cardio-metabolic function between employees of shift and fixed day work. Attempts were made to limit the effect of behavioral factors including PA, smoking status, current cardio-metabolic or sleep disorders, and the acute effect of circadian disruption via testing on consecutive days off. Following these delimitations, the results supported previous observational and simulated shift work projects demonstrating an adverse effect of chronic homeostatic desynchronisation (long term employment in shift work) on objectively measured sleep following a night shift (reduced TST), body composition, inflammatory status, and metabolic efficiency (Morris et al., 2017; Puttonen et al., 2012). Interestingly, significant differences were not observed in HRV, objective sleep assessment (averaged over the 14 d period) or fasted blood glucose and insulin levels. Previous research projects simulating shift work under laboratory conditions have demonstrated that an acute bout of circadian disruption can significantly reduce HRV,

TST and efficiency, as well as insulin sensitivity (Leprout et al., 2014; Morris et al., 2016; Wright Jr et al., 2015). However, at the time of testing (following a day off or day shift) no such differences were observed. The results indicate that shift work may acutely affect autonomic function, sleep quality, and insulin sensitivity, but this effect may be masked by subsequent recovery sleep on days off. However, under chronic conditions, the cumulatively acute disruptions translate to the observable differences in metabolic efficiency, increased adiposity and circulating IL-6. While markers of circadian rhythmicity were not specifically measured, the results support an etiological role of homeostatic desynchronisation in shift work pathogenesis and address the first thesis aim.

7.2.4 Exercise as an intervention

In addition to the general health benefits of exercise, including primary and secondary prevention of cardio-metabolic disorders, exercise-induced adaptations share biological pathways with shift work pathogenesis (Saner et al., 2018; Warburton et al., 2006). Previous research interventions have reported subjective and objective improvements in a variety of sleep parameters including continuity and total length (Chennaoui et al., 2015; Reid et al., 2010; Yang et al., 2012). Given the role of sleep in moderating cardio-metabolic function and pathogenic implications of reduced sleep, exercise is therefore proposed as a tool to offset the established detrimental effects of shift work on sleep and improve health outcomes. Similarly, exercise has established anti-inflammatory effects associated with both acute myokine secretion (Brown et al., 2015) and chronic secondary effects on body composition and interrelated inflammatory status (adipose tissue is recognized as active endocrine and pro-inflammatory tissue) (Lavie et al., 2011). However, the adaptations are dose and stimulus-specific, while exercise refers to a broad spectrum of structured PA, with variations in mode, intensity, duration, and volume (Yao & Basner, 2019). To date, there is a distinct lack of evidence to substantiate such observations among rotational shift workers specifically or explore the independent effect of exercise variations. Flahr and colleagues recently published a systematic review evaluating

randomised control trials with shift workers as the target population and PA listed as the primary intervention (Flahr et al., 2018). Between 1988 and 2017 only seven studies were identified, and while standardised exercise modality and workloads have significantly improved cardio-metabolic markers (Kim et al., 2015; Lim et al., 2015), more research is needed.

The thesis, while acknowledging some limitations, addresses the second thesis aim by investigating the acute and chronic effect of exercise on cardio-metabolic function among shift workers. Specifically, Study Two investigated the effect of an acute bout of exercise on cardio-metabolic function among male rotational shift workers. HIT significantly increased IL-1Ra, an anti-inflammatory mediator previously demonstrated to inhibit the pro-inflammatory cascade (Brown et al., 2015; Petersen & Pedersen, 2005). A novel observation given the identified link between shift work and pro-inflammation as well as the adverse effect of systemic inflammation on cardio-metabolic function. Study Three further explored the primary anti-inflammatory effect of exercise training on the inflammatory status of rotational shift workers. MICT significantly decreased CRP values from pre-to-post training, improving inflammatory status and decreasing future disease risk of participants. Results from Study Three and re-distributed data sets based on exercise adherence add further support to secondary anti-inflammatory effects of exercise. Adipose tissue is recognized as active endocrine tissue, with hypertrophic adipocytes upregulating pro-inflammatory cytokines associated with cardio-metabolic pathogenesis (Lavie et al., 2011). Attending an average of ≥ 24 sessions over the 12 weeks significantly reduced adipose tissue from pre-to-post intervention. While not directly associated with inflammatory results, decreased adipose tissue provides secondary prevention of pro-inflammatory markers and adverse cardio-metabolic function with potential long term health benefits (Poirier et al., 2006).

Exercise interventions further demonstrated significant improvements in objectively assessed sleep quality among rotational shift workers. Within Study Two, MICT was shown to significantly decrease sleep fragmentation (decreased WASO). Again, considered a potentially novel finding given the structural limitations shift work places on sleep (limited break times and biologically misaligned opportunity) and the detrimental effect fragmented sleep has on cardio-metabolic health (Stamatakis & Punjabi, 2010). One theoretical approach to describing the chronic benefits of exercise is consistently providing acute adaptations. For example, the current exercise guidelines advocate exercising for a minimum of 30 minutes per day on 5 days per week. Consequently, individuals that meet the exercise recommendations would rarely have more than 24 h between successive exercise bouts and subsequent physiological adaptations. Therefore, if the current exercise bouts, providing acute improvements in inflammatory status and sleep, were performed consistently, the acute adaptations may translate into chronic improvements in key modulators of cardio-metabolic function among shift workers.

Both MICT and RT additionally improved TST following night shift. A novel result given the established detrimental effect of night shift on subsequent sleep opportunity and the potential pathogenic role that it may play. Study One demonstrated that despite no significant differences in the 14-day average sleep quality between shift and non-shift workers, a significant difference was observed following night shift in comparison to the daily averages. Results which suggest that acute differences in sleep quality may be masked by subsequent recovery sleep or sleep recovery methods on day shift or days off. Further, the observations of Study One highlight a potential target area for improvement (TST following a night shift) and demonstrate the novelty of sleep improvements in Study Three. Of interest, but beyond the scope of the current research projects, is the physiological mechanisms responsible for exercise induced improvements in sleep. Predominant theories highlight sleep's role in energy conservation, anabolic processes, improvements in thermoregulation and circadian

entrainment as potential mechanisms (Driver & Taylor, 2000), however no measures were collected to explore such responses in this thesis.

The collective results not only support exercise as valid intervention and contribute to the scarce body of current research but may influence future exercise prescription. Acute bouts of MICT improved sleep quality (reduced WASO), while a 12-week training intervention reduced CRP and improved TST following night shifts. Collectively, while HIT and RT contributed to significant changes in measures of sleep and cardio-metabolic function, MICT appears to be the most effective training mode among rotational shift workers, and while greater adaptation is associated with increased exercise dose, a minimum of 2 sessions a week may elicit meaningful results.

7.2.5 Barriers to exercise participation among shift workers

The thesis has contributed to the current body of research supporting a direct etiological role of shift work in the development and progression of cardio-metabolic disorders among apparently healthy employees. Further, this thesis added to the literature investigating and supporting the health effects of acute and chronic exercise interventions in offsetting shift work pathology. However, the main contribution in terms of shaping future research, may be the identification of barriers to exercise adherence in this population group. Despite the provision of free, programed and periodised training with apparently motivated participants (freely volunteered), the average sessional attendance did not meet the prescribed exercise guidelines to elicit meaningful health benefits. The labor characteristics of shift work appears to exacerbate the commonly cited barriers to exercise, namely a lack of time, lack of motivation and difficulty in establishing a routine. While no subjective data was collected, several participants commented on the difficulty of attending sessions immediately before or after a 12 h shift due to travel time to and from home and work. Further remarking they found exercise on

their days off easier but depending on the roster, could limit them to two sessions per week. Comments that are supported by previous research highlighting a limited break time in which to exercise and exercise opportunities out-of-sync with biological mediators effecting both motivation and the development of a set exercise routine as established barriers to adherence (Blake et al., 2017; Nea et al., 2017). Consequently, future research looking to expand on the current findings must first maximize exercise engagement before investigating the physiological effectiveness. Previously hypothesized strategies include the provision of fully supervised training interventions, using alternate ‘time-efficient modes’ including HIT and incorporating worksite interventions to encourage employee participation.

7.3 Strengths and Limitations

A collective strength of the research projects is the use of established scientific approaches to maximise the validity of the research projects. The thesis incorporated both experimental (Study Two & Three) and observational research (Study One) to explore the potential effect of shift work and exercise on cardio-metabolic function. Each project included a control group to minimise the effect of variables not being tested (outside of the independent variable). For example, Study One was designed to minimise potential co-factors for disease progression including pre-established disease states, low PA, and lifestyle behaviors such as smoking to increase the internal validity (the observable outcomes are due to the independent variable/experimental manipulations). Further, testing was conducted on consecutive days-off to minimise the acute effects of sleep deprivation and circadian misalignment associated with night shift. Both Study Two & Three were randomised to offer each participant equal opportunity to be assigned to a given group and minimise the effect of potential researcher bias. Finally, a strength of the current thesis is the novelty of results reported in the study chapters and the potential applications these may have in future research and clinical application. Study One has contributed to the exploration of specific pathogenic mechanisms among rotational

shift workers. While the results of Study Two & Three contribute to the scarce body of exercise intervention research that currently exists with highly applicable, and externally valid results. Despite the variations in shift work (direction of rotation, length of shifts and subsequent rest periods) and potential independent physiological effects, the predominant mechanisms (homeostatic desynchronisation) are ubiquitous. Resultantly, the exercise-based improvements in cardio-metabolic health reported within this thesis may be applicable to a variety of shift structures.

Despite these strengths, there are several limitations which need to be acknowledged to both give context to the results and assist the direction of future research. Firstly, while a clear rationale is provided in Chapter Three (Methodology) for the recruitment of male participants, excluding female shift workers from the research projects is a limitation. Females make up a large percentage of the shift work population and may have independent barriers to exercise participation or physiological responses to interventions. For example, the menstrual cycle has previously been demonstrated to independently effect the serum concentrations of inflammatory markers (CRP) in both normal-weight and overweight subjects (Blum et al., 2005). Further, both night and rotational shift work can disrupt normal physiological response of the menstrual cycle, with shift workers reporting a higher frequency and increased severity of menstrual cycle disorder (Attarchi et al., 2013; Su et al., 2008). Cumulatively, the adverse effects observed in Study One (increased levels of inflammatory markers) may be exacerbated among female employees. Future research should look to replicate the current results among female shift work volunteers. Further, priori power analysis was conducted for all research projects, however due to difficulty in recruiting and testing shift workers both Study One & Three were underpowered by fifteen and four participants, respectively. As such it is pertinent for future research to replicate the thesis findings with a larger cohort of participants to ensure appropriate statistical power within the observations. A potential strategy to help with recruitment

may include engaging a specific company rather than individual workers or data collection to be completed on site to reduce the total commitment time for participants.

For Study One, the potential mechanisms responsible for shift work mediated cardio-metabolic disorders were investigated, however no assessment of circadian rhythmicity was conducted. This limitation was primarily a budgetary issue, but future research should incorporate assessment of melatonin, core temperature or cortisol to provide circadian context of pathogenesis. Further, participants were matched for PA (categorised as sedentary for not attaining the recommended 150 min/week of exercise), however this approach resulted in a large range of activity levels. Using a subjective assessment of PA may also be erroneous if participants do not have an adequate understanding of PA intensities (Freene, Waddington, Chesworth, Davey, & Cochrane, 2014). Finally, the literature review (Chapter Two) highlighted the potential effect of diverse shift structures on cardio-metabolic function and the necessity of reporting distinct shift characteristics. However, due to difficulties with recruitment several shift structures had to be combined under the sub-category of 8-12 h clockwise rotational shifts. Future research may explore recruiting through one jobsite with an established rostering system to assist in standardizing rostering and recruitment.

Study Two assessed the acute effect of exercise among shift workers, however, to minimise the acute effect of circadian disruption and aid recruitment, testing was conducted on consecutive days off. The result of which was the potential creation of an 'ideal' testing window that may not reflect the potential role of circadian disruption on the impact of acute exercise interventions. Future research should therefore aim to assess exercise before or following night shift to address this limitation. An additional limitation of Study Two is the pre-post study protocol. Structuring the research project as a randomised cross-over design with all participants completing both protocols (HIT and MICT) would have increased statistical power. Unfortunately, limitations on resources preventing this research

structure, but future projects should look to replicate the observations within as acute cross-over intervention. While Chapter Six is a novel study due to the scarce amount of research currently investigating exercise as an intervention, several barriers to exercise participation among shift workers resulted in reduced adherence to the exercise intervention. This limitation allowed for further organisation and exploration of the data to examine the effects of exercise adherence on outcomes related to sleep, health and cardio-metabolic function. However, the study may have benefitted from a subjective assessment (questionnaire) of the main barriers to exercise to inform future research. The study would also have benefitted from recording participant exercise times (along with sessional attendance). Chapter Two (literature review) highlighted the potential effect of individual chronotype and time of exercise on measures of cardio-metabolic function including inflammatory status and metabolism. Therefore, while incorporating flexible training times was deliberate to increase exercise adherence, recording exercise times would have allowed for additional analysis. Regardless, future research projects should aim to identify and remove barriers to exercise, increasing adherence and further investigating exercise as an intervention among shift workers. Finally, Chapter Two establishes the potential role of lifestyle factors including diet and alcohol intake on cardio-metabolic function (Nea et al., 2015) which may have impacted the effectiveness of the prescribed exercise interventions. Participants were instructed to maintain their habitual diet, however as no subjective recording was kept, analysis of diet and alcohol intake cannot be performed. Future research projects should incorporate subjective assessment of lifestyle behaviours to provide additional context for interpreting intervention success.

7.4 Conclusion

The findings presented address the thesis aims and add to the current understanding of both the risk of cardio-metabolic disease and exercise as an intervention for the shift work population. Specifically, the projects collectively demonstrate the adverse effect rotational shift work has on cardio-metabolic

function with preliminary assessment supporting the hypothesis of a direct pathogenic role of the labour structure. Homeostatic desynchronisation, facilitated by misaligned entraining cues, impairs physiological function, and increases the risk of adverse health conditions. Conversely, acute, and chronic exercise are viable intervention methods with primary and secondary benefits for the pathogenic pathways observed within shift work disorders. Specifically, exercise improves both cardio-metabolic function and the individual risk factors for the development of disorders including poor sleep, increased body fat and systemic inflammation. As such, exercise was hypothesised to be an effective intervention strategy for shift workers, however limited research had substantiated the assumption. In conclusion, shift work is an important labour structure that by design adversely effects physiological function. While this may be an unavoidable by-product, exercise can be utilised to offset the physiological risk factors and improve shift workers overall health.

Chapter Eight:

Summary and Conclusion.

8.1 Overview

The main purpose of this thesis was to examine the physiological effect of rotational shift work on cardio-metabolic function to explore potential pathogenic mechanisms. In addition, this thesis examined the effectiveness of acute and chronic exercise interventions in improving the cardio-metabolic function of shift workers.

8.2 Research Aims – Study One:

1. *The aim of this study was to assess the effect of rotational shift work on biological risk factors for future development of cardiovascular, metabolic, and inflammatory disorders among currently healthy shift workers.*

The findings reported in Chapter Three (Study One) highlight the role homeostatic desynchronisation plays in the adverse health effects associated with shift work. Participants were screened for current cardio-metabolic or sleep disorders, matched for age and physical activity (PA) levels and reported no measurable differences in average sleep quality. Consequently, the significant differences in cardio-metabolic function (increased fat mass, increased response time to a glucose challenge and increased circulating IL-6) indicate a direct pathogenic role of the work structure. Further, the absence of significant differences in measures previously associated with acute bouts of shift work (decreased HRV, sleep and insulin sensitivity) may indicate an independent acute and chronic effect of rotational shift work.

8.3 Research Aims – Study Two:

1. *The primary aim of the study was to investigate the acute effect of exercise among rotational shift workers regarding objectively measured sleep quality, inflammatory response and insulin sensitivity.*

Study two demonstrated that acute bouts of HIT significantly increase anti-inflammatory mediators and while MICT improved subsequent sleep quality (as indicated by reduced WASO). Further, neither intervention

resulted in an increase in pro-inflammatory markers or decreased sleep parameters. Collectively, these findings suggest that acute bouts of exercise improve the cardio-metabolic function of rotational shift workers.

- 2. The secondary aim of this study was to compare the effect of HIT to MICT to investigate intensity-based differences in exercise interventions for shift worker health.*

Study two (Chapter Four) concluded that the physiological effect of acute exercise on cardio-metabolic function among shift workers is dependent on intensity. Specifically HIT elicited a significant increase in IL-1Ra, an anti-inflammatory cytokine capable of mediating the pro-inflammatory cascade, an established risk factor for cardio-metabolic disorders. An effect not observed within the MICT group. Conversely, only MICT significantly improved objectively measured sleep quality the subsequent sleep opportunity following the exercise intervention. This study suggests intensity-dependent mechanisms may be responsible for the difference in observed effects on inflammation and sleep.

8.4 Research Aims – Study Three

- 1. The primary aim of this study was to assess the effect of a semi-supervised exercise training intervention on cardio-metabolic risk factors among shift workers.*

Study Three demonstrated that a 12-week intervention incorporating different aerobic modes of exercise is a viable intervention for improving the cardio-metabolic risk profile of shift workers, and both MICT and RT can increase TST following a night shift. Further, when the intervention groups were re-distributed regarding exercise dose (attendance) rather than mode, attending a minimum of 2 sessions per week was sufficient training stimulus to improve body composition through reduced fat mass. Results which decrease the risk of developing future cardio-metabolic disorders, and support exercise as a health intervention among shift workers, of which scarce evidence currently exists. An additional novel finding of Study Three is the effect of shift work mediated barriers for exercise adherence. The shift structure appears to exacerbate the

conventionally cited reasons for non-exercise adherence and future research project should investigate reducing said barriers.

2. *The secondary aim of this study was to investigate a potential difference in effect on cardio-metabolic function between aerobic and resistance training.*

Study three (Chapter Six) additionally investigated the difference in exercise modality for cardio-metabolic effects between aerobic and resistance training. As stated, MICT significantly improved inflammatory status (decreased CRP) while both training modes increased TST following a night shift. The results indicate that MICT is a more effective mode of improving cardio-metabolic function among rotational shift workers however the specific mechanisms responsible warrant further investigation. As the results stand, MICT may be preferably prescribed for shift workers aiming to reduce the risk of future adverse cardio-metabolic health outcomes.

8.5 Summary and Conclusion

The studies in this thesis were designed to investigate the role rotational shift work plays in the development of cardio-metabolic disorders while assessing the legitimacy of exercise as an intervention to improve health markers of future disease states. Based on the results presented in Study One, shift work plays a mechanistic role in cardio-metabolic pathogenesis with homeostatic dysregulation identified as a potential cause. Circadian rhythms, sleep and the immune system provide integrated regulation over cardio-metabolic function with shift work systematically disrupting regulation through mistimed and inverted exogenous signaling.

Given the important role shift work plays in the current globalised and expanded labor structures, interventions must be considered to improve the overall health of employees. Results in Study Two and Three support exercise as an intervention to improve cardio-metabolic function and reduce future disease risk

development among shift workers. Acute bouts of exercise increase anti-inflammatory profile and improve sleep while chronic bouts of exercise improve inflammatory profile, TST and body composition. Of interest is the observation that shift work further exacerbates barriers to exercise, with rotating work reducing the regulation of exercise routines, while 12 h shifts provide exercise opportunity in opposition to biological regulation and/or exercise facilities. As a result, shift workers have difficulty adhering to exercise intervention and future research and exercise programs implemented within shift work industries must priorities exercise adherence to elicit the full benefit of exercise interventions.

8.6 Practical Applications

- Findings from Study One provide preliminary results investigating the specific mechanisms responsible for increased incidence of cardio-metabolic disorders among rotational shift workers. Results which may help inform companies currently employing rotational shift work about the hypothesised health risks and potential need for interventions;
- Acute bouts of exercise improve the cardio-metabolic profile of shift workers and may be implemented as an intervention strategy to reduce the impact of acute disturbances including night shift, with no likely disruption to subsequent sleep;
- Chronic exercise intervention improves the cardio-metabolic risk profile of shift workers and reduce the likelihood of future adverse conditions. Therefore, industries utilising rotational shift work may implement exercise interventions to improve the health of employees and reduce future absenteeism associated with illness.

8.7 Recommendations for Future Research

- While it is evident that homeostatic disruption plays a mechanistic role in cardio-metabolic pathogenesis, future research should include markers of circadian disruption (melatonin, core temperature and cortisol levels) to further investigate the individual components of cardio-metabolic regulation;
- Study Two demonstrated the effectiveness of an acute bout of exercise to improve the cardio-metabolic function. However, the research project assessed this effect on consecutive days off, conducted within accurate but perhaps ‘ideal’ testing window that may not reflect the effect on disturbed homeostatic regulation. Consequently, future research should look to replicate the findings when conducted before or after a night shift;
- Study Three demonstrated that a 12-week exercise intervention induces cardio-metabolic adaptations associated with reduced risk of future disease states. However, the average sessional attendance (in line with exercise guidelines) did not meet the required exercise amount. Future research should therefore investigate methods of increases exercise adherence, some ideas include: incorporating onsite (workplace) interventions, provision of fully supervised sessions, access to 24 h gymnasiums, prescribing HIT with shorter total exercise time and incorporating individual factors including chronotype to personalise exercise prescription.
- Collectively, due to budgetary restraints, the three studies used wrist work actigraphy devices and sleep diaries to provide objectively scored sleep quality. However, actigraphy sleep assessment does not provide sleep staging information that may provide additional mechanistic information for cardio-metabolic regulation. Future research projects may look to incorporate PSG sleep assessment to rectify this limitation;
- The concept of integrated regulatory mechanisms and homeostatic dysregulation facilitating disease progression may also be applicable for mechanistic research relating to other disease states among shift workers including increased incidence of cancer, however further research will be required to substantiate this hypothesis.

Chapter Nine:

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Appendix A

Movement as medicine: an exploration of exercise to mitigate negative effects of shift work on cardio-metabolic health.

Additional manuscript independent to the thesis that has been prepared during the PhD Candidature.

Manuscript is being prepared to submit to the *Preventative journal Reports*.

Title: Movement as medicine: an exploration of exercise to mitigate negative effects of shift work on cardio-metabolic health.

Running Title: Exercise as a shift work health intervention

Article type: Narrative Review

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Abstract

Shift work is a division of labour associated with an increased incidence of cardio-metabolic disorders. Compared to non-shift colleagues, the relative risk of developing cardiovascular disease (CVD) and metabolic syndrome (MetS) increases by 40 and 50 %, respectively. The mechanisms responsible for this elevated risk require further investigation, however maladaptive sleep architecture, circadian disruption, and physical inactivity are implicated. Systemic inflammation may also play a pathogenic role, in addition to serving as an indicator of disease development and progression. Regardless, shift work remains a crucial labour structure, utilised in 20-25 % of the modern workforce. As such, intervention strategies have been theorised to offset some of the negative health implications. Exercise is a validated intervention, with broad health benefits inversely related to all-cause mortality. Specific to shift work, exercise improves sleep architecture, regulates biological rhythms, and augments biological pathways disrupted in the aforementioned pathological conditions. Despite this, reported physical activity (PA) and exercise levels among shift workers remain low, and a limited number of exercise intervention studies exist targeting shift work employees. As such, assessment of the effectiveness and suitability of an exercise-based intervention aimed at improving shift work health requires further elucidation.

Keywords: Shift work, physical activity, circadian rhythm, sleep disruption, inflammation

Introduction

Shift work incorporates a broad spectrum of labour organisations associated with potentially adverse health outcomes including an increased incidence of cardio-metabolic conditions (Esquirol et al., 2011; Flahr et al., 2018). Utilised across a broad range of industries including health, emergency services, hospitality and manufacturing, numerous variations in shift structuring exist to meet the unique demands of these specific industries. The common characteristic however, is the extension of conventional work hours through subdividing total labour opportunity into consecutive rotational and/or fixed shifts (Esquirol et al., 2011; Flahr et al., 2018). Mounting evidence suggests that the rotating operating systems facilitates the development and progressions of non-communicable disease states with circadian misalignment, sleep restriction and physical inactivity being implicated in pathogenesis (Flahr et al., 2018). Systemic inflammation is also proposed as an additional risk factor, conventionally used as a marker for disease risk and progression (Puttonen et al., 2011), now theorised as a common pathogenic pathway for cardio-metabolic disorders (Dandona et al., 2004).

Despite the potential adverse health association, shift work remains an essential work structure, utilised within 20-25 % of the modern workforce (Flahr et al., 2018; Shantha, 2013). As such, exercise, with physiological health adaptations associated with decreased morbidity and mortality rates (Warburton et al., 2006), may be a viable therapeutic intervention. Exercise is additionally associated with improved sleep (Atkinson et al., 2008; Driver & Taylor, 2000), biological rhythm regulation (Atkinson et al., 2008; Driver & Taylor, 2000) and augmentation of biological pathways implicated in cardio-metabolic pathologies (Flahr et al., 2018). This review will investigate the potential aetiological relationship between rotational shift work and cardio-metabolic disorders, while evaluating prescribed exercise as an intervention strategy.

Homeostatic desynchronisation and cardio-metabolic pathogenesis

The cardio-metabolic system is under homeostatic control, with circadian rhythms, sleep and the immune system partitioning and moderating metabolic resources in response to a dynamic environment (Lange et al., 2010; Logan & Sarkar, 2012). The circadian system is a temporal biological regulator, aimed at optimising human physiology and performance through cyclic moderation of cardio-metabolic function (Atkinson et al., 2007; Levi & Schibler, 2007; Luyster et al., 2012). Sleep, among other functions, serves as a time of reduced metabolic requirement and specified period of growth and repair (Luyster et al., 2012). Finally, the immune system is responsible for maintaining normal tissue function, and is capable of directly mediating metabolic processes due to the high metabolic requirements of the system (Del Giudice & Gangestad, 2018; Libby, 2007). While often discussed separately, these biological regulators share anatomical structures, humoral mediators and oscillatory interactions (Borbély et al., 2016; Fuller et al., 2006; Lange et al., 2010). The behaviour of sleep manipulates the light-dark cycle (closed eyes), while facilitating physical rest and fasting, which entrain the circadian rhythm (Lange et al., 2010) and regulate sleep opportunity. Sleep regulatory regions also share anatomical structures with the immune system and influence the immune response through direct innervation of the autonomic nervous system (ANS), as well as the signalling of hormones and neurotransmitters (Besedovsky et al., 2012). Immune cells also contain molecular clock components and are entrained by circadian regulation through neural and endocrine signals (Logan & Sarkar, 2012). An overview of the shared cardio-metabolic system is provided in *Figure 1*.

Figure 1 about here

Regulation of cardio-metabolic function, therefore, involves integration of multiple systems as well as endo- and exogenous signals (Fuller et al., 2006; Lange et al., 2010). The distinct advantage is the provision of predictive, instead of reactive, metabolic and cardiovascular processes to maximise homeostatic regulation (Atkinson et al., 2007; Levi & Schibler, 2007; Luyster et al., 2012). However, conflicting or misaligned cues are capable of disrupting the regulatory systems, eliciting a stress response and upregulated immune function

(Irwin & Cole, 2011); a state not only predictive of future pathogenesis, but hypothesised to play an aetiological role in the development of cardio-metabolic disorders (Dandona et al., 2004; Leproult et al., 2014). Central to shift work is the subdivision of labour into consecutive rotational or fixed shifts to provide extended labour opportunity and maximise productivity. However, the rotational division simultaneously impede cardio-metabolic function through manipulation or inversion of key regulators including the sleep-wake phase, feed-fasting and activity cycles (Bøggild & Knutsson, 1999; Haus & Smolensky, 2006). Cumulatively, increasing the risk of cardio-metabolic disorders and justifying the investigation of intervention methods.

Exercise as an intervention

Exercise is a structured component of physical activity (PA) that elicits physiological adaptation and augments biological function (Myers et al., 2004). The general adaptations of exercise are inversely related to all-cause mortality in a dose-response manner and predictive of future pathological development (Myers et al., 2004). Specific to shift work, the physiological functions ameliorated by exercise share biological pathways with the pathological cardio-metabolic conditions commonly associated with the work structure (Saner et al., 2018; Warburton et al., 2006). Furthermore, traditional theories of sleep function indicate exercise may have potent sleep-promoting and circadian entraining effects (Driver & Taylor, 2000). As such, exercise may both improve physiological function and offset the hypothesised mechanisms responsible for shift work mediated pathology (Warburton et al., 2006) .

However, the implementation and maintenance of an active lifestyle among shift workers must address several labour specific factors including increased sedentary behaviour and limited opportunity for exercise. In opposition to the physiological effects of exercise, increased sedentary behaviour is associated with pathogenesis, and is considered an underlying factor for shift workers' adverse health outcomes (Hulsege et al., 2017). Meta-analysis and review articles have concluded that occupation status is influential in work-

related PA levels (Prince et al., 2019), which considering the time spent at work, may mitigate health outcomes. Hulsegge et al, (2017), when comparing leisure time and occupational PA of shift and non-shift workers, concluded that shift workers are more sedentary at work and engage in less occupational light and moderate-to-vigorous PA when working at night (Hulsegge et al., 2017). An observation which may be further exacerbated between various industries with vast differences in required occupational PA. While not specifically differentiating between shift and non-shift work, healthcare, protective services, call centres and drivers had varying occupational PA levels (Prince et al., 2019). Collectively, exercise interventions aimed at shift workers may look to involve work place programs, however the individual characteristics of shift work industries and potential impact on health requires further research.

Increased occupational sedentary behaviour may further impact the prescription of PA guidelines for shift workers in comparison to the general public. Currently, PA guidelines advocate the accumulation of 150 minutes of structured PA per week to achieve significant health benefits. However, investigations assessing shift and non-shift leisure time PA have concluded that irregular shift workers report comparable or higher levels of leisure time PA (Loef et al., 2017; Marqueze et al., 2014), despite recording significantly higher body mass index (Marqueze et al., 2014). The research suggest that shift workers may engage in equivalent PA levels, yet remain more prone to cardio-metabolic disease states. Consequently, the current PA guidelines may be a conservative prescription for shift workers and not enough to offset the biological risks associated with the work structure.

An additional barrier to exercise specific to shift workers is the availability of exercise opportunity, impacted by structural restrictions or out-of-sync opportunities. A key determinant in exercise participation is the total break time afforded between consecutive shifts, which may be affected by factors including commute time, social obligations and prioritisation of sleep (Vedaa et al., 2016). Cumulatively a lack of leisure time available between shifts is cited as a barrier to exercise adherence (Atkinson et al., 2008) and impacts exercise

prescription. Advancement in research regarding high-intensity interval training (HIT) and superior adaptations associated with increased intensity (Gibala et al., 2012) may provide both a greater adaptation stimulus and time-efficient alternative. However, HIT has a number of shift work specific considerations. Expanded upon in later sections, HIT may potentially increase arousal, impacting sleep quality (Irish et al., 2015), as well as inducing a pro-inflammatory response associated with intense or unaccustomed exercise bouts (Woods et al., 2012). HIT may therefore be an attractive time-effective alternative for shift workers however limited research exists exploring the potential physiological effects (Flahr et al., 2018).

Finally, the effect of excessive daytime sleepiness and exercise opportunities out-of-sync with biological rhythms may impact the effectiveness of an exercise intervention per se or the specific exercise delivery components (Chennaoui et al., 2015; Hargens et al., 2013). Feelings of residual fatigue associated with both limited and/or circadian misaligned sleep opportunity reduce motivation to exercise (Hargens et al., 2013). A factor impacted by the specific characteristics of the shift structure and potentially varying between workplaces. Shift scheduling additionally impacts participation through providing exercise opportunities in opposition to team sport or gym facility scheduling (Blake et al., 2017; Nea et al., 2017). Finally, the irregular rotation structures utilised in some industries are identified as a barrier to developing a set routine for exercise participation (Blake et al., 2017; Nea et al., 2017). Therefore, shift workers report reduced leisure time PA (Peplonska et al., 2014; Peplowska et al., 2014) and while exercise is a validated intervention method, the effect of the aforementioned adherence barriers require further investigation.

Exercise and sleep promotion for shift workers

Sleep complaints among shift workers can be broadly attributed to two specific labour characteristics; a transient inability to sustain sufficient-quality sleep due to circadian misaligned sleep opportunity (Gonnissen et al., 2013; Shaker et al., 2018). Or the inability to achieve sufficient total sleep time (TST) due to limited total

sleep opportunity between consecutive work periods (Heath et al., 2019). Therefore shift workers commonly report sleep onset and maintenance difficulties, higher incidence of sleep disorders (Shantha, 2013), and shorter sleep duration in comparison to their non-shift counterparts (Wolk, Gami, Garcia-Touchard, & Somers, 2005). Conversely, epidemiological research suggests that exercise hours per week are inversely related to the severity of sleep disorders (Peppard & Young, 2004); while exercise intervention studies have significantly improved subjective and objective measures of sleep (Chennaoui et al., 2015; Reid et al., 2010).

Several factors influence the success of an exercise intervention aimed at improving sleep among shift workers. Advocating exercise during periods where shift scheduling restricts the time for sleep opportunity may further restrict TST and exacerbate the adverse health effects (Atkinson et al., 2008). Conversely, while further impacting sleep opportunity is a valid concern, exercise interventions may improve the quality of available sleep time (Atkinson et al., 2008). Chronic exercise training (6 months 3 days/week) has previously been shown to improve sleep quality among middle-aged sedentary participants with chronic primary insomnia (Passos et al., 2011). The exercise intervention significantly decreased sleep onset latency and significantly increased sleep efficiency measured via polysomnography (Passos et al., 2011). Given the lack of time to attain sufficient sleep and inability to extend sleep opportunity, improvements in sleep latency and quality may be of greater importance and a more realistic outcome. However, exercise induced improvements in sleep are yet to be effectively demonstrated among shift workers.

The timing of exercise directly before sleep is of additional concern with increased physiological arousal hypothesised to affect sleep architecture (Irish et al., 2015). However, evening (Larsen et al., 2019a) and late-night (Myllymäki et al., 2011) exercise among middle-aged and fit young participants, respectively, had little to no adverse effects on sleep quality. Furthermore, exercise-induced improvements in thermoregulation and acute elevations in core body temperature may improve sleep latency (Irish et al., 2015). Supported by the observation that acute aerobic exercise, performed within 3 hours of sleep opportunity, decreased objectively

measured sleep onset latency among middle-aged (44.4 ± 8 years) individuals with difficulty initiating sleep (Passos et al., 2010). Consequently, the timing of exercise before bed does not appear to negatively impact sleep quality (Larsen et al., 2019a; Myllymäki et al., 2011), however additional factors such as desynchronised sleep-wake cycle may impact this observation among shift workers (Thomas et al., 2020).

Traditional sleep theories support exercise as a viable intervention to improve sleep architecture among shift workers. In general, sleep is hypothesised to reduce metabolic requirements and facilitate peripheral repair (Driver & Taylor, 2000). As exercise acutely increases energy expenditure (Miles, 2007) a proportional increase in sleep propensity would be required to fulfil this role (Driver & Taylor, 2000). Exercise is additionally associated with improved modulation of core temperature, a key regulator of sleep propensity. Therefore, exercise-induced improvements in thermoregulation may improve sleep latency, total sleep time and overall quality (Driver & Taylor, 2000).

Specific to shift work, exercise may improve sleep architecture via circadian entrainment (Driver & Taylor, 2000). Exercise modulates core temperature, facilitates phase shifts in melatonin, and provides light exposure, with exercise commonly taking place in well-lit areas (Atkinson et al., 2007). Further, review articles assessing the effect of PA on circadian rhythmicity have concluded that acute bouts of exercise facilitate circadian re-entrainment (Yamanaka et al., 2006), with the overall shape of phase-response curves (PRC) differing from photic PRC's (Mistlberger & Skene, 2005). In summary, exercise is considered a potent regulator of the circadian system (Driver & Taylor, 2000). However, worthy of consideration is the potential for poorly timed exercise to exacerbate circadian desynchronization. An observation supported by Thomas, et al., (2020) when demonstrating the timing of exercise in regards to individual chronotypes induced phase shifts capable of improving or exacerbating circadian misalignment (Thomas et al., 2020). Therefore the timing of exercise, aimed at improving sleep among shift workers is important and requires further exploration.

A limitation in the prescription of exercise to improve sleep among shift workers is the scarcity of interventions (Atkinson et al., 2008; Flahr et al., 2018); and of this limited research, only subjective sleep measures have been assessed (Atlantis, Chow, Kirby, & Singh, 2006; Härmä et al., 1988). Harma et al, (1998) reported improved subjective measures of sleep time and fatigue following evening shifts during 4 months of physical training. Additionally, Atlantis, et al (2006) identified improved subjective sleep quality following a randomised and controlled 24 week exercise intervention (Atlantis, Chow, Kirby, & Singh, 2006). Both projects used varying interventions, altering the modality and amount of exercise sessions per week, depending on availability and fitness levels. Harma et al (1998), utilised a program that varied from 2-6 sessions per week depending on the physical conditioning of participants (Härmä et al., 1988), while Atlantis et al (2006), incorporated both aerobic and resistance training 3 or 4 days per week (Atlantis, Chow, Kirby, & Singh, 2006). The inconsistent methods and lack of objective measures create difficulties to assess the effectiveness of an exercise intervention or explore the mechanistic links between exercise and sleep among shift workers. Cumulatively, current research does not inform the guidelines with which future intervention should be implemented. Nor does it investigate the mechanisms and causal links between sleep and exercise among shift workers.

Collectively, sleep theories and methodical assessment using similar population groups independent of occupation, support exercise as a plausible intervention for the shift worker; however, the mechanisms responsible for these sleep improvements, appropriateness of exercise timing, and the impact on overall health and wellbeing are currently lacking definitive assessment.

Systemic inflammation in shift workers and effects of exercise

Chronic elevation of inflammatory markers and acute-phase proteins including interleukin-6 (IL-6), tumour necrosis factor-alpha (TNF- α), and C-reactive protein (CRP), compromise tissue function and are implicated in

future pathogenesis of cardio-metabolic disorders (Kasapis & Thompson, 2005; Mathur & Pedersen, 2008). Atherosclerosis, formerly considered a lipid storage disease, involves an ongoing inflammatory response mediating all stages of disease from initiation, progression and thrombotic complications (Libby et al., 2002). Further, pro-inflammatory mediators are involved in the pathogenesis of tissue-specific insulin resistance (Rehman & Akash, 2016). Differentiated monocytes including TNF- α and IL-6 directly impact beta cell function within the pancreas, disturbing insulin signalling and sensitivity (Baker, Hayden, & Ghosh, 2011).

Simulated shift work has previously demonstrated an inflammatory response, significantly increase pro-inflammatory markers (Leprout et al., 2014; Morris et al., 2016). Specifically, a two 8-day laboratory protocols assessed the effect of 12 h inverted behavioural and environmental cycles (observed in shift work). The intervention reported increased pro-inflammatory markers including CRP and TNF- α (Morris et al., 2016). Puttonen et al. (2011) further supports the notion that shift work associated with low-grade systemic inflammation with a cross-sectional analyses of 1877 airline company employees who utilised varying rotational and fixed shifts (Puttonen et al., 2011). Rotating morning, afternoon and night shifts were associated with significantly higher levels of CRP in comparison to other work schemes (Puttonen et al., 2011). Consequently, the structure of shift work may propagate chronic systemic inflammation and pathological conditions.

Both the immune system and exercise exert a wide-ranging repertoire of homeostatic regulation and defence (Del Giudice & Gangestad, 2018; Warburton et al., 2006). However, the interaction between exercise and inflammation is complex and in need of consideration before advocating an intervention among shift workers. Acutely, exercise is associated with increased oxidative metabolism and stress, leading to oxidation of cell components and inducing a pro-inflammatory cascade (Kasapis & Thompson, 2005). Additionally, acute muscle and connective tissue damage caused by unaccustomed or prolonged exercise may induce inflammation (Woods et al., 2012). Conversely, the acute and chronic anti-inflammatory effect of exercise may

reduce prevalent disease states observed among shift workers (Lavie et al., 2011; Petersen & Pedersen, 2005; Starkie et al., 2003). Starkie et al. (2003), demonstrated that moderate-intensity cycling could blunt the pro-inflammatory cascade (Starkie et al., 2003). Attributed to muscle contraction stimulated release of myokines, including IL-6 (Petersen & Pedersen, 2005), not only propagating the production of further anti-inflammatory mediators but also directly inhibiting TNF- α and the pro-inflammatory cascade (Mathur & Pedersen, 2008). Additionally, exercise may indirectly suppress TNF- α through increased production of epinephrine, previously demonstrated to blunt the pro-inflammatory signaling of TNF- α *in vivo* (Petersen & Pedersen, 2005). Cumulatively, individual bouts of exercise may provide an anti-inflammatory effect, acutely mediating the pro-inflammatory state associated with shift work.

Regular exercise likewise exhibits an anti-inflammatory effect, with higher physical fitness levels associated with reduced basal and post-exercise pro-inflammatory markers (Kleiven et al., 2018). This chronic effect of exercise may be attributed to several direct and indirect factors, including modulation of the ANS (Warburton et al., 2006). Exercise augments parasympathetic tone and vagus nerve secretion of acetylcholine (ACh), theorised to suppress pro-inflammatory cytokines (Papathanassoglou et al., 2015). Furthermore, fat cells are recognised as active endocrine organs that produce adipokines capable of initiating the pro-inflammatory cascade (Lavie et al., 2011). Regular exercise alters body composition through reducing fat cell tissue, suppressing adipokine activity and subsequent inflammation (Woods et al., 2009). In addition, myokine production in response to exercise directly remodels adipose tissue, improving metabolism and decreasing pro-inflammatory markers (Schnyder & Handschin, 2015; Wallberg-Henriksson & Zierath, 2015). Exercise may also mitigate inflammation via ameliorated endothelial function (Kasapis & Thompson, 2005), with reports that regular exercise enhances antioxidant capacity of cells, upregulates antioxidant enzymes and increases the bioavailability of nitric oxide (NO) (Beavers et al., 2010; Kasapis & Thompson, 2005). The combination of which decreases the susceptibility of low-density lipoprotein (LDL) oxidation, preventing endothelial injury and associated inflammation response (Kasapis & Thompson, 2005). Theoretical and experimental data support

exercise as a plausible intervention method to downregulate systemic inflammation and offset pathological conditions. However, this hypothesis is in need of empirical research among the shift work population.

Cardiovascular disease risk in shift workers and the effects of exercise

Homeostatic desynchronisation associated with shift work, adversely effects various structures and functions of the cardiovascular system and associated structures including autonomic modulation (Morris et al., 2016; Tobaldini et al., 2013), insulin sensitivity (Leproult et al., 2014), and inflammatory response (Morris et al., 2016; Tobaldini et al., 2013), aetiologically involved in the development and progression of atherosclerosis (Libby et al., 2002). Homeostatic dysregulation is further associated with impaired endothelial function (Suessenbacher et al., 2011), indicative of atherosclerotic development and predictive of future CV events (Inaba, Chen, & Bergmann, 2010). Cumulatively, shift workers appear to be at an increased relative risk of developing cardiac pathology with an observed 40 % higher relative risk compared to day worker colleagues (Bøggild & Knutsson, 1999; Esquirol et al., 2011); a risk that demonstrated a dose-response with that incrementally increases with continued involvement (Torquati et al., 2018).

The general benefits of exercise include an established inverse relationship between PA and CV events (Miles, 2007). Moreover, exercise intervention studies have consistently improved CV function and decreased pathological risk among a variety of population groups (Warburton et al., 2006). In regards to the specific pathological mechanisms facilitated by shift work, exercise downregulates sympathetic drive (Miles, 2007), suppresses the pro-inflammatory cascade (Starkie et al., 2003) and increases insulin sensitivity (Miles, 2007). The chronic effect of exercise further augments vagal cardiac modulation (Hallman et al., 2017), decreases inflammatory markers and adhesion molecules (Lim et al., 2015) and ameliorates endothelial function (Miles, 2007). Collectively, exercise presents as an intervention method with general health benefits and specific adaptations for rotational shift workers.

The physiological effect of exercise on CV outcomes has previously been investigated among permanent night shift workers, with the results further supporting the use of exercise interventions (Lim et al., 2015). Lim et al., (2015) concluded that a 10 week randomised and controlled exercise protocol among middle-aged male night shift workers decreased levels of the cathepsins proteas group and arteriosclerosis adhesion molecules, independent biomarkers of future CV risk (Lim et al., 2015). Additionally, Faria and Faria (1991), reported 30 min combination of aerobic activity and circuit training three days/week for 32 weeks improved coronary heart disease risk among firefighters working in rotational shifts (Faria & Faria, 1991). The intervention increased aerobic capacity, including both anaerobic threshold and maximum oxygen consumption (VO_{2max}) while improving cholesterol profile through increased high-density lipoproteins (HDL) (Faria & Faria, 1991). Despite inconsistent exercise modes, the results support aerobic activity as a valid intervention, improving key physiological mechanisms associated with shift work pathology and reducing the risk of future CV events (Lim et al., 2015).

Metabolic Syndrome risk in shift workers and the effects of exercise

Similar to CVD, homeostatic dysregulation is implicated in the pathogenesis of metabolic syndrome (MetS) (Leproult et al., 2014). The term MetS refers to the clustering of interrelated metabolic disturbances including obesity, insulin resistance, hypertension and dyslipidaemia (Tasali & Ip, 2008) which is 50 % more likely to occur among shift workers in comparison to day work counterparts (Sookoian et al., 2007). Further, in comparison to healthy individuals, MetS is associated with an approximate two-fold and five-fold increased relative risk for CV events and the development of type II diabetes mellitus, respectively (Wolk & Somers, 2007). Physical inactivity has classically been associated with the development of MetS (Golbidi et al., 2012), and mounting evidence suggests that the circadian disruption and sleep curtailment may play a role in the individual disturbances and MetS pathogenesis (Leproult et al., 2014). Additionally, systemic inflammation,

both directly and indirectly facilitates insulin resistance and has been described as the common mechanisms linking the individual components of MetS (Berg & Scherer, 2005; Welty, Alfaddagh, & Elajami, 2016).

The curtailment of sleep is compared to family history of diabetes and physical inactivity, for the relative risk of developing insulin resistance (Anothaisintawee et al., 2016). Acute and sub-chronic episodes of sleep restriction, like those experienced in shift work, have impaired glucose tolerance among healthy volunteers (Donga et al., 2010; Herzog et al., 2013). Circadian misalignment is likewise associated with impaired metabolic function, with significantly reduced insulin sensitivity observed following four consecutive night shifts despite no significant change in sleep duration (Bescos et al., 2018). The combination of circadian misalignment and potential short sleep duration experienced among shift workers has also demonstrated an adverse metabolic effect with Leproult et al., (2014) simulating shift work conditions to assess the independent effect of sleep loss and circadian misalignment on metabolic function. The results indicated that both sleep restriction and circadian misalignment independently decrease insulin sensitivity and increased levels of CRP (Leproult et al., 2014). Consequently, simulated shift work under laboratory conditions demonstrates the independent ability of homeostatic desynchronisation to impact metabolic function and increase the risk of future metabolic disorders.

Longitudinal and cross-sectional research support laboratory findings, associating shift work with both the development of MetS and the separate metabolic abnormalities (Sookoian et al., 2007). Assessment of over 7000 male day and night shift workers over a 14 year period concluded that alternating shift patterns is an independent risk factor for both body weight gain (Suwazono et al., 2008) and impaired glucose metabolism (Suwazono et al., 2009). Additionally, when 738 nurses, free from any component of MetS at baseline were annually evaluated over a four year period, the development of MetS was observed to be significantly higher among rotational shift workers in comparison to non-shift colleagues (Pietrojusti et al., 2010). Cumulatively,

in terms of developing both the independent components and clinical diagnosis of MetS, a one and half fold increase in relative risk exists among rotational shift workers (Sookoian et al., 2007).

As with CVD and systemic inflammation, a plethora of observational and intervention-based research supports exercise as an intervention to mediate the development of MetS, independent of occupation (Church, 2011). Exercise not only has an observed inverse relationship with the development of MetS, but exercise based interventions offset the individual pathophysiological characteristics. One such example is the salutary effect of exercise on the metabolic risk factor of excessive adipose tissue (Golbidi et al., 2012). Reported to have a higher incidence among shift workers (Liu et al., 2018), obese hypertrophic adipocytes and stromal cells within adipose tissue directly augment systemic inflammation, mediating multiple pathogenic mechanisms associated with MetS (Berg & Scherer, 2005). Exercise remodels subcutaneous fat tissue (Wallberg-Henriksson & Zierath, 2015), augments the oxidative capacity of skeletal muscles, improves lipid metabolism (Golbidi et al., 2012) and supplements weight loss while maintaining lean body mass, essential for fat metabolism and glucose transport (Golbidi et al., 2012). Acute and chronic exercise additionally augments endothelial function, including reducing BP and minimises the risk of developing the hypertensive MetS risk factor (Miles, 2007).

Substantial evidence also validates exercise as an effective method of enhancing insulin sensitivity and upregulating glucose transport (de Souza et al., 2017; Golbidi et al., 2012; Miles, 2007), which is particularly pertinent to shift workers given the increased risk of MetS throughout the progression of their employment. Specifically, de Souza et al., (2017) demonstrated that two weeks of HIT (total of six sessions), increased insulin sensitivity among healthy males despite 24 hours of total sleep deprivation (de Souza et al., 2017). The intervention concluded that while sleep curtailment had previously induced insulin resistance, the HIT intervention group minimised this deleterious effect (de Souza et al., 2017). While not conducted among shift work, it is demonstrated that exercise can be an effective intervention among voluntary sleep curtailment, like that observed among shift workers. The mechanisms associating exercise with increased insulin sensitivity

are likely both acute and chronic adaptations. Acute exercise facilitates muscle contraction, activating glucose transport independent of insulin-stimulated pathways, resulting in increased rates of glucose uptake and utilisation (Henriksen, 2002); while the chronic effect of exercise increases insulin sensitivity through enhanced glycogen synthesis, increase mitochondrial biogenesis, enhanced Beta(β)-oxidation and increased expression and translocation of the muscle glucose transporter four (GLUT-4) to cell surface.

Exercise may also be beneficial in reducing the risk of MetS through favourable changes in plasma lipids and lipoproteins profiles (Golbidi et al., 2012). As such, exercise augments oxidative capacity of skeletal muscle, improving fat oxidation capacity and removal of plasma free fatty acids (Golbidi et al., 2012). Furthermore, HDL-cholesterol (HDL-C), associated with decreased risk of CVD (Boden, 2000) is generally responsive to aerobic exercise and increases in a dose-dependent manner with increased energy expenditure (Miles, 2007). In support, Kim et al. (2015) reported physical intervention of exercise or combined exercise and deep abdominal ultrasound therapy was sufficient stimulus to improve body composition and augment blood lipid profile among shift workers (Kim et al., 2015). Consequently, exercise ameliorates lipid metabolism (Kim et al., 2015), reduces BP, positively affects body composition (Lim et al., 2015), increases insulin sensitivity and glucose transport as well as exerting an anti-inflammatory effect within the body. As such, a critical observation in support of exercise as a strategy to improve the health of rotational shift workers is addressing the individual characteristics of MetS.

Limitations of current shift work intervention

Despite the strong theoretical and evidence-based research, many shift workers fail to achieve the recommended PA guidelines (Flahr et al., 2018). Additionally, a limited number of exercise-based interventions have been implemented among the shift work population group to substantiate the hypothesised benefits among shift workers (Flahr et al., 2018). The one systematic review of shift work and

exercise based interventions identified seven studies between 1988 and 2017 (Flahr et al., 2018). The inclusion criteria were comprised of the use of randomised control trials or protocols, shift workers as the target population and PA listed as the primary intervention component (Flahr et al., 2018). Of these interventions, standardised exercise modality and workloads, as in the case of both Kim et al., (2015) and Lim et al., (2015) have resulted in improved body composition (Kim et al., 2015; Lim et al., 2015), blood lipid profiles (Kim et al., 2015) and decrease biomarkers of CVD (Lim et al., 2015). Additionally, improvements in CV fitness, BP and subjective measures of sleep quality and fatigue among male (Atlantis, Chow, Kirby, & Fiatarone Singh, 2006), female (Härmä et al., 1988), healthy (Atlantis et al., 2004) and overweight (Morgan et al., 2011) shift workers have supported exercise as an intervention. However, the inconsistent use of interventions including mixed exercise modalities, different workloads, incorporation of a variety of behavioural interventions including health tutorials and seminars used concurrently with exercise and a high dependence on subjective sleep measures are limitations. As such, while exercise may be effective to mitigate the intermediate risk factors associated with shift work, limited interventions studies, comprised of inconsistent measures prevent the findings informing exercise intervention guidelines (Flahr et al., 2018). Further research is required to investigate the success and potential mechanism responsible for exercise as an intervention among shift workers.

Conclusion

In summary, shift work represents an essential labour structure within the current workforce that facilitates cardio-metabolic conditions. While circadian disruption and maladaptive sleep architecture represent inexorable risk factors for disease progression, augmented physiological function to offset modifiable risk factors including systemic inflammation are feasible. As such, further investigation of the pathophysiological mechanisms and plausible intervention strategies have become significant considerations within current research. Exercise represents a feasible strategy, ameliorating physiological adaptations implicated in shift work pathology and considered a potent sleep promoting and circadian entraining factor. Despite the

overwhelming evidence supporting exercise as a therapeutic intervention, currently research is limited among shift work populations. Further, research is required to identify the pathophysiological mechanisms and to assess the effectiveness of exercise in improving health outcomes of shift workers.

Appendix B

Study 1 documentation including Institutional Ethics Approval, Participate Information, Informed Consent, General Health Questionnaire, Participant Booklet and Sleep and Wellness Questionnaires



ETHICS AND COMPLIANCE UNIT

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Boorooma Street
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12 July 2018

Mr Blake Collins
Email: blcollins@csu.edu.au

Dear Mr Collins,

Thank you for providing further information in response to a request from the Charles Sturt University Human Research Ethics Committee relating to your research proposal.

The Charles Sturt University Human Research Ethics Committee is constituted and operates in accordance with the National Health and Medical Research Council's [National Statement on Ethical Conduct in Human Research](#) (*National Statement*).

Based on the guidelines in the *National Statement* the Committee has approved your research proposal. Please see below details of your approved research project:

Project Title: Rotating shift work, circadian desynchronisation and associated health concern
Approved until: 20 March 2019(subject to annual progress reports being submitted)
Protocol Number: H18142 (to be included in all correspondence to the Committee)
Progress Report due by: 20 March 2019

Could you please confirm that when results are forwarded to the GPs, they will be accompanied by a copy of the Information Sheet so that the GPs know the background of the research.

You must report to the Committee at least annually, and as soon as possible in relation to the following, by completing the 'Report on Research Project' form:

- any serious and/or unexpected adverse events or outcomes which occur associated with the research project that might affect participants, therefore, the ethical acceptability of the project;
- amendments to the research design and/or any changes to the project (Committee approval required);
- extensions to the approval period (Committee approval required); and
- notification of project completion.

This approval constitutes ethical approval in relation to humans only. If your research involves the use of radiation, biochemical materials, chemicals or animals, separate approval is required by the appropriate University Committee.

Please contact the Governance Officer on (02) 69334213 or ethics@csu.edu.au if you have any queries.

The Committee wishes you well with your research.

Sincerely,

Ms Ellen Hannigan
Governance Officer
on behalf of Associate Professor Catherine Allan
Presiding Officer, HREC

cc: Dr Melissa Skein, Dr Frank Marino, Dr Tegan Kastelein

Research Project: Understanding the relationship between sleep, circadian disruption and the potential role of exercise in physical and mental wellbeing.

Contact: Mr Blake Collins (PhD Candidate)
School of Exercise Science, Sport and Health
blcollins@csu.edu.au
0409598135

Project Supervisor: Dr Melissa Skein
School of Exercise Science, Sport and Health
mskein@csu.edu.au

Background:

Sleep is a key physiological function, evidenced by our instinctive and rhythmic sleeping behaviours and the pathological state of its absence. The biological act of sleep is regulated by two synergistic but potentially opposing systems, our homeostatic drive for sleep and circadian rhythm regulating wakefulness. Under normal conditions circadian rhythm, our 'biological clock' is synchronised with our internal drive for sleep exerting a regulatory influence over sleep and other key physiological functions including the cardiovascular system. However a concerning trend associated with modern '24-hour', 'round-the-clock' lifestyles is a desynchronization between these key physiological function leading to the development and progression of disease states. Shift work, while a key style of rotational labour, is an example of the artificial desynchronization of our physiological function associated with the development and progression of several disease states including a 40% increase in the development of cardiovascular disease and 50% increase in the likelihood of developing the metabolic syndrome. Therefore we aim to assess and compare the health status of different population groups to better understand the mechanism potentially responsible for the increase in disease states and develop feasible intervention strategies.

Recruitment:

- Currently employed in rotation style shift work (minimum 2 years including day and night shift splits) or lifestyle matched alternative in either labour intensive work (tradesmen) or sedentary (office work) alternatives.
- Males aged between 25 and 50 years of age
- Never been diagnosed with a sleep disorder such sleep apnoea, insomnia, restless leg syndrome
- No known immune or inflammatory conditions (including influenza in the preceding 4 weeks)
- Achieve a minimum of 5.5 hours of sleep most nights
- Not currently meeting the Australian guidelines of >150 minutes of structured exercise per week
- Free from any condition which may be exacerbated by exercise
- Be available for testing during September 2018 and June 2019

Study Overview:

September – November 2018

A comparative study to assess the different risk factors of disease development associated with altered sleep architecture and reduced sleep duration. The participants will undergo a variety of prognostic health tests, involving assessment of their body composition (fat vs fat-free mass), cardiovascular, arterial and inflammatory health. The participants will receive a full report providing an in-depth explanation of important measures and their implication for future health outcomes.

Measures:*Actigraphy*

Prior to attending the Charles Sturt University laboratories an actigraphy watch will be supplied to assess sleep architecture including total sleep duration and number of awakenings over a 14-day period. The watch is approximately the same size as a fitness watch and can be worn at all times excluding swimming, baths or showers.

Heart Rate Variability:

Recording and analyses of the beat-to-beat neural variations of consecutive heart beats. This measures involves wearing a chest strap and heart rate monitor which provides an assessment of the controlling neural influence of your cardiovascular system and is predictive of future negative cardiovascular events.

Dual-Energy X-Ray Absorptiometry:

Full body scan to assess body composition including mass, total fat mass and total fat free mass. The scan involves laying supine on the DEXA machine for approximately 10 minutes as the scan is completed.

Oral Glucose Tolerance Test:

Considered the gold standard assessment of metabolic health and is used clinically as a diagnostic tool for insulin resistance and non-insulin dependent diabetes. The test involves being fasted overnight, taking baseline blood sample, drinking a 300ml carbohydrate drink than taking incremental blood samples to assess your body's ability to absorb and use glucose.

Inflammation Status:

When baseline blood samples are collected for the oral glucose tolerance test, additional samples will be collected to assess inflammation status. Low grade systemic inflammation is associated with the development and progression of a variety of pathological states including cardiovascular disease and diabetes.

Maximal oxygen consumption (VO_{2max})

Considered the gold standard of cardiovascular fitness, a maximal oxygen consumption test will be conducted on a bike to assess current fitness levels, predictive of future cardiovascular disease. The test involves cycling on an exercise bike until exhaustion and typically takes 8-12 minutes.

Cerebral Oxygenation:

Finally an assessment of cerebral oxygenation will be conducted to assess the amount of oxygen used by the brain under resting conditions. The test involves placing a probe on the forehead and wearing a headband to secure it in place for 5 minutes of data collection.

Time commitments:

This study is an acute comparative study comprising of two sessions. A familiarisation session to obtain informed consent, fill out health and sleep questionnaires and complete the exercise test (totalling approximately 1 hour). The second session will involve the prognostic health assessments mentioned about (total of approximately 2 and ½ hours). Additionally wearing the actigraphy watches for 14 days between the two sessions.

Additional Research:

A further two studies have been proposed to investigate the role of exercise in improving the aforementioned health measures and will include both an acute (one of exercise session) and training intervention (3 days/week for 12 weeks). Further information can be provided regarding participation in these studies which would provide additional health assessment and free, supervised exercise training.

Thank you for your time if you have any further question please contact myself via the email address included,

Sincerely,

A handwritten signature in black ink, appearing to read 'Blake Collins', written in a cursive style.

Mr Blake Collins
PhD Student
School of Exercise Science, Sport and Health
Charles Sturt University
blcollins@csu.edu.au

Rotating shift, circadian desynchronization and associated prognostic outcomes.

Primary Investigator

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Co-Investigator

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PURPOSE OF THE STUDY

The study aims to assess the effect of rotating shift work on circadian rhythm and explore the possible mechanistic links between this disruption and clinically significant disease progression. Additionally this relationship will be explored independent of confounding lifestyle factors including diet and physical activity levels to delineate potential mechanisms and develop appropriate intervention strategies.

I, _____ have read the information contained in the participant information sheet provided and this consent form and any questions we have asked have been answered to my satisfaction.

I agree to participate in this project, realising I am free to withdraw my participation at any time without being subject to any penalty or discriminatory treatment from the study.

I agree that the purpose of this research and potential risks or discomforts involved with the testing have been sufficiently explained to me, with the opportunity to ask questions.

I understand that any information or personal details gathered during this research about me is confidential and that neither my name nor any other identifying information will be used or published without my written permission. Additionally, test results may be passed on to my specified GP in the event further testing or medical intervention is required.

General Practitioners Contact Name: _____ Number: _____

While unlikely to be needed, in the case of an emergency, please provide a name and contact number of a next of kin.

Name: _____ Phone: _____ Relationship: _____

Charles Sturt University's Human Research Ethics Committee has approved this study. I understand that if I have any complaints or concerns about this research I can contact:

Executive Officer
Human Research Ethics Committee, Office of Academic Governance
Charles Sturt University
Panorama Avenue, Bathurst NSW 2795
Ph: (02) 63384628 Fax: (02) 63384194

Signature of consent

Date

Signature of investigator

Date

GENERAL HEALTH QUESTIONNAIRE

Name: _____

Age: _____ yr

Weight: _____ kg Height: _____ cm

Do you have a sleep disorder? YES NO Is English your first language? YES NO Do you smoke? YES NODo you take illicit drugs (e.g., Marijuana)? YES NO**Section One: General**1. Have you travelled through time zones in the last 3 months? YES NO**If yes**, please provide trip details, including when you arrived here2. Are you, or have you ever been involved in shift work? YES NO**If so**: how long ago? _____ yr _____ months

for how long? _____ yr _____ months

Please provide details about the shift schedule:

_____ (e.g., times, durations)

3. If **NOT** involved in rotational shift work what would be the average start time for your

working day? : _____

4. How would you rate your physical activity levels during your average workday?

Sedentary

Light

Moderate

Vigorous

5. Please list the average amount of caffeine you consume per day (*e.g. cups of tea/coffee, cans of caffeinated soft drink and chocolate bars*)

6. Do you have any children living with you at home? YES NO**If so**, how many and their ages? _____

Section Two: Health

1. Have you had any serious accidents, head injuries, or concussion? YES NO
If yes, please give details:

2. Are you currently on any medication? YES NO
If yes, please give details:

3. What exercise do you do? _____
4. How much exercise do you do, on average per week? _____ hours
5. On average, how much alcohol do you drink per week? _____
6. Have you ever experienced any of the following medical conditions, and if so, when?

Don't know = 0 No = 1 Yes in the past = 2 Yes, sometimes = 3 Yes, at present = 4

- | | |
|--------------------------------------|------------------------------------|
| (a) Asthma_____ | (b) Hay fever_____ |
| (c) Eczema_____ | (d) Allergies (Food or other)_____ |
| (e) Thyroid Problems_____ | (f) Undue anxiety_____ |
| (g) Sleepwalking_____ | (h) Loud snoring_____ |
| (i) Nightmares_____ | (j) Bruxism (grinding teeth)_____ |
| (k) Difficulty reading/writing_____ | (l) Arthritis/Rheumatism_____ |
| (m) Depression_____ | (n) Heart problems_____ |
| (o) Stomach problems_____ | (p) Waking with a jolt_____ |
| (q) Waking up excessively early_____ | (r) Difficulty falling asleep_____ |
| (s) Stress/anxiety at home/work_____ | (t) Epilepsy_____ |
| (u) Migraine_____ | (v) Colour blindness_____ |
| (w) STD / STI _____ | (x) Ulcerative colitis_____ |
| (y) Periodontal disease_____ | (z) Recent Influenza/Gout_____ |
| (aa) HIV/AIDS _____ | (bb) Hepatitis_____ |

Section Three: Sleep

1. (a) **Shift Work** What time do you normally wake up?

Days On (NIGHT) _____ Days Off _____ Days On (DAY) _____ Days Off _____

1. (b) **Shift Work** What time do you normally go to sleep?

Days On (NIGHT) _____ Days Off _____ Days On (DAY) _____ Days Off _____

2. (a) **Non-Shift** What time do you normally wake up?

Work day _____ Weekend _____

2. (b) **Non-Shift** What time do you normally go to sleep?

Work day _____ Weekend _____

3. Do you normally nap during the day?

YES NO

If so, how often does this occur? _____

4. How well do you usually sleep (circle)?

very poorly _____ very well
 1 2 3 4 5

5. On average, how many times per night do you wake up?

never hardly ever 1 or 2 < 5 5 – 10

>10 don't know

Section Four: Stress and Workload

1. Do you have exams/assignments due/other tests scheduled for one week before or during the study? YES NO

Comments: _____
 —

2. Are you currently experiencing a greater than your normal amount of stress? (e.g. sick relative, relationship break-up, getting married, moving

house) YES NO

Comments: _____

Cohen's Perceived Stress Scale

The questions in this next scale ask you about your feelings and thoughts **during the last month**. In each case, you will be asked to indicate by circling *how often* you felt or thought a certain way.

1. In the last month, how often have you been upset because of something that happened unexpectedly?

never almost never sometimes fairly often very often
2. In the last month, how often have you felt that you were unable to control the important things in your life?

never almost never sometimes fairly often very often
3. In the last month, how often have you felt nervous and "stressed"?

never almost never sometimes fairly often very often
4. In the last month, how often have you felt confident about your ability to handle your personal problems?

never almost never sometimes fairly often very often
5. In the last month, how often have you felt that things were going your way?

never almost never sometimes fairly often very often
6. In the last month, how often have you found that you could not cope with all the things that you had to do?

never almost never sometimes fairly often very often
7. In the last month, how often have you been able to control irritations in your life?

never almost never sometimes fairly often very often
8. In the last month, how often have you felt that you were on top of things?

never almost never sometimes fairly often very often
9. In the last month, how often have you been angered because of things that were outside of your control?

never almost never sometimes fairly often very often
10. In the last month, how often have you felt difficulties were piling up so high that you could not

overcome them?

never almost never sometimes fairly often very often

Arousal Predisposition Scale

These next questions deal with a number of common behaviours and self-perceptions. For each question you should select the response, which best describes you and your behaviours.

1) I am a calm person

never
(or almost never)
always) seldom occasionally frequently always
(or almost

2) I get flustered if I have several things to do at once

never
(or almost never)
always) seldom occasionally frequently always
(or almost

3) Sudden changes of any kind produce an immediate emotional effect on me

never
(or almost never)
always) seldom occasionally frequently always
(or almost

4) Strong emotions carry over for one or two hours after I leave the situation which caused them

never
(or almost never)
always) seldom occasionally frequently always
(or almost

5) I am restless and fidgety

never
(or almost never)
always) seldom occasionally frequently always
(or almost

6) My mood is quickly influenced by entering new places

never
(or almost never)
always) seldom occasionally frequently always
(or almost

7) I get excited easily

never
(or almost never
always) seldom occasionally frequently always
(or almost
always)

8) I find that my heart keep beating fast for a while after I have been 'stirred up'

never
(or almost never
always) seldom occasionally frequently always
(or almost
always)

9) I can be emotionally moved by what other people consider to be simple things

never
(or almost never
always) seldom occasionally frequently always
(or almost
always)

10) I startle easily

never
(or almost never
always) seldom occasionally frequently always
(or almost
always)

11) I am easily frustrated

never
(or almost never
always) seldom occasionally frequently always
(or almost
always)

12) I tend to remain excited or moved for along period of time after seeing a good movie

never
(or almost never
always) seldom occasionally frequently always
(or almost
always)



The effect of circadian rhythm and sleep architecture on health outcomes amongst contrasting work roster

Participant: _____

Group: _____

Purpose of this Study

Sleep is a key physiological function, evidenced by our instinctive and rhythmic sleeping behaviours and the pathological state of its absence. The biological act of sleep is regulated by two synergistic but potentially opposing systems, our homeostatic drive for sleep and circadian rhythm regulating wakefulness. Under normal conditions circadian rhythm, our 'biological clock' is synchronised with our internal drive for sleep exerting a regulatory influence over sleep and other key physiological functions including the cardiovascular, metabolic and immune system. However a concerning trend associated with modern '24-hour', 'round-the-clock' lifestyles is a desynchronization between these key physiological function leading to the development and progression of disease states. Shift work, while a key style of rotational labour, is an example of the artificial desynchronization of our physiological function associated with the development and progression of several disease states including a 40% increase in the development of cardiovascular disease and 50% increase in the likelihood of developing the metabolic syndrome. Therefore we aim to assess and compare the health status of different population groups to better understand the mechanism potentially responsible for the increase in disease states and develop feasible intervention strategies.

Participant Inclusion Criteria

To be eligible for this study, you must meet the following criteria:

- Currently employed in labour intensive rotation style shift work (minimum 2 years including day and night shift splits) or lifestyle matched alternative in either labour intensive work or sedentary alternatives.
- Aged between 25y and 50y
- Never been diagnosed with a sleep disorder such sleep apnoea, insomnia, restless leg syndrome
- No known immunological irregularities or inflammatory conditions (including no diagnosis of influenza in the preceding 4 weeks)
- Achieve a minimum of 5.5 hours of sleep most nights
- Classified as physically inactive according to the Australian guideline (<150 mins of structured exercise/week)
- Free from any condition which may be exacerbated by exercise
- Current non-smoker

Illness or injury:

If you become ill or suffer an injury while participating in this study, please notify Blake as soon as possible to cancel upcoming laboratory trials and reschedule when you are well again.

Important Contacts:

Any questions that you may have about the study and the procedures implemented for this study should be firstly addressed to Blake using the following details:

Principal Investigator: Blake Collins

Email: bcollins@csu.edu.au

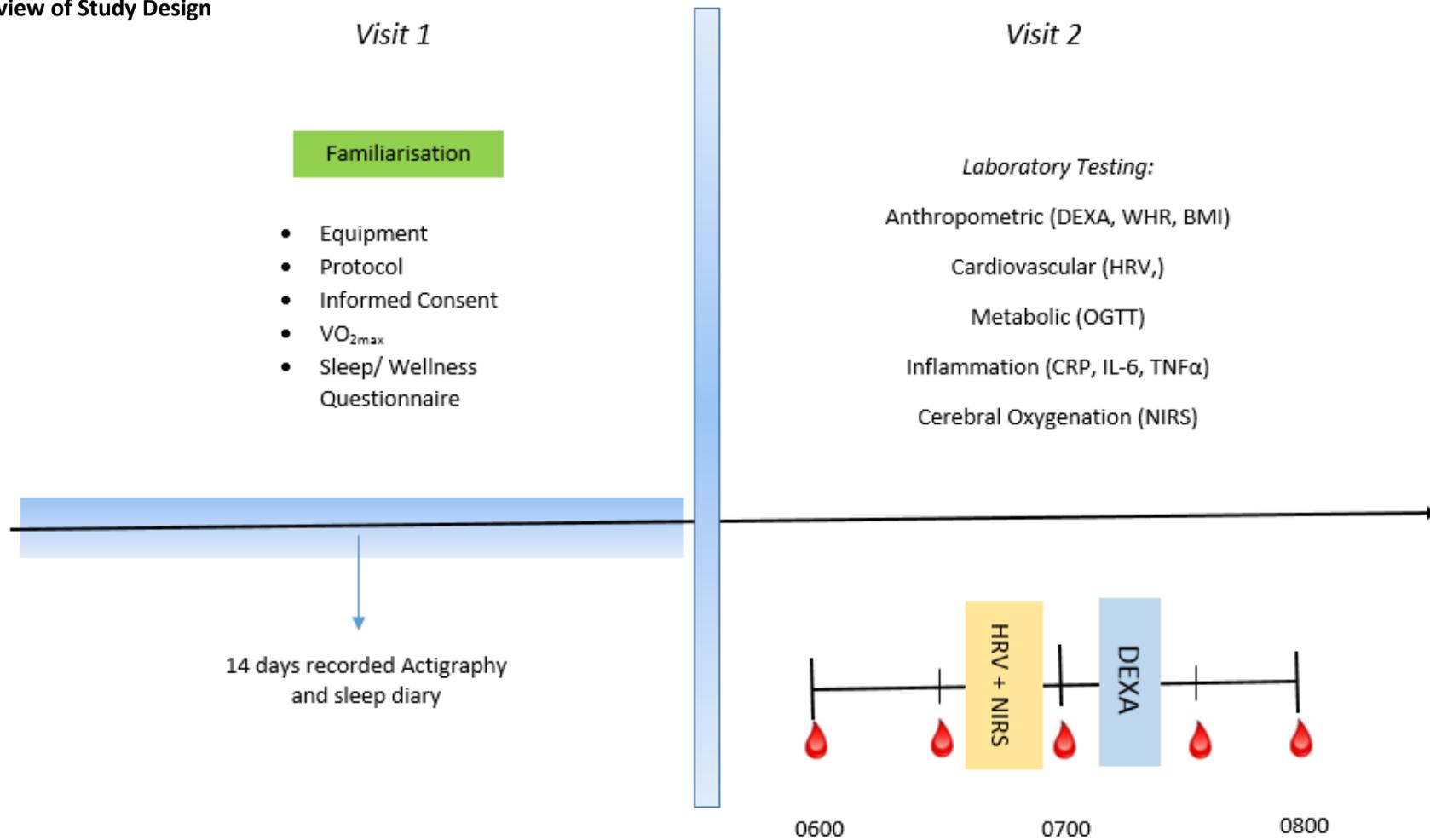
Mobile: 0409 598 135

If you need to reschedule or running late for a trial, please phone Blake as soon as possible.

Should you have any concerns regarding the study or Blake cannot answer your question please refer to the following contact list:

<p>Dr Melissa Skein School of Exercise Science, Sport & Health Charles Sturt University Panorama Ave, Bathurst Tel: 6338 4430 Email: mskein@csu.edu.au</p>	<p>Dr Skein is Blake's principal supervisor and will oversee this study. Any questions that Blake may not be able to answer can be directed to Dr Skein.</p>
<p>Dr Tegan Kastelein School of Exercise Science, Sport & Health Charles Sturt University Panorama Ave, Bathurst Tel: 6338 4268 Email: tkastelein@csu.edu.au</p>	<p>Dr Kastelein is one of Blake's co-supervisor and will be actively involved in the research process.</p>
<p>The Executive Officer Human Research Ethics Committee Office of Governance and Corporate Affairs Charles Sturt University Private Mail Bag 29 Panorama Avenue Bathurst NSW 2795 Tel: 6338 4628 Email: ethics@csu.edu.au</p>	<p>If you have any complaints or concerns about the ethical conduct of this study, you may contact the Ethics Committee.</p>

Overview of Study Design



DEXA; Dual-energy x-ray absorption, WHR; waist-hip ratio, BMI; Body Mass Index, HRV; Heart Rate Variability, FMD; Flow Mediated Dilatation, OGTT; oral glucose tolerance test, NIRS; Near-infrared spectrometry, CRP; C-reactive protein, IL-6; Interleukin-6, PSG; polysomnography

In laboratory Testing overview

Familiarisation

The familiarisation session will take approximately 1 hour to complete. During this time a number of pre-screening questionnaires describing your current health and sleep status will be completed, informed consent will be obtained and a graded exercise test to exhaustion will be performed on a stationary bike. As such, it is advised that you wear loose-fitted clothing and footwear which is appropriate to exercise in. You will also be supplied with an Actigraphy watch and sleep diary to be worn/completed for 14 days prior to the laboratory testing session.

Laboratory Testing Session

Following 14 days of Actigraphy monitoring you will need to arrive in a fasted state (no food for 8 hours, caffeine for 12 hours). Baseline blood samples are collected for both **fasted** blood glucose and baseline inflammatory measures before completing an oral glucose tolerance test (ingestion of a 75g glucose solution) and continuing periodic blood samples draws (every 30 minutes) for 2 hours. In between your blood samples we will complete anthropometric assessments and dual-energy x-ray absorptiometry (DEXA) scan, heart rate variability and near-infrared spectroscopy measures are recorded. The final test will be an assessment of flow mediated dilation using a blood pressure cuff and ultrasound. **We ask that you refrain from strenuous physical activity in the 24 hours previous to testing as high levels of physical exertion may affect some of the measures and that you arrive in a faster state.**

Baseline Testing: 14 days Actigraphy

Actiwatch ID: _____

Sleep Diary:

- 1. Complete a single line of the sleep diary for every sleep period** (i.e. major sleeps including naps longer than 20 min).
- 2. Date – the date that you go to bed.**
- 3. Sleep Location – where the sleep occurs. To identify whether you are in a different sleeping environment** (i.e. if you travel for work and sleep in a hotel instead of in your own bed).
- 4. How many cups (and quantity) of caffeine did you have today?** (Beverages include: coffee, energy drinks, coke, tea, etc.)
- 5. What time (24h) did you consume your last cup of caffeine for the day/night?**
- 6. Bed Time – the time you go into bed** (24h time).
- 7. Time you fell asleep - time you actively tried to go to sleep** (24h time).
- 8. Awake Time - time you woke up** (24h time).
- 9. Out of Bed – time you got out of bed to start your day** (24h time).
- 10. Number of Awakenings - the number of times you recall waking during the sleep period.**
- 11. Screen time before sleep: Indicate time spent watch television, or using laptop, computer, phone, etc. immediately before going to sleep. Be sure to include screen time within any area of the house (not just the bedroom)**
- 12. Sleep Quality – your perception of your quality of sleep for that period compared to a ‘normal’ sleep period.**

Date dd/mm	Sleep Location Own bed or alternative?	How many cups of caffeine have you had today? What time was your last cup?		Bed time hh:mm	Time you fell asleep hh:mm	Awake Time hh:mm	Out of Bed hh:mm	Number of awakenings	Did you have 'screen time' immediately before going to sleep & for how long?	Sleep Quality				
										very good	I good	I average	I poor	I very poor
15/09	Alternative (hotel)	large latte	16:00	22:30	23:00	06:30	07:00	4	Y 50 mins	<input type="checkbox"/>				
										<input type="checkbox"/>				
										<input type="checkbox"/>				
										<input type="checkbox"/>				
										<input type="checkbox"/>				
										<input type="checkbox"/>				
										<input type="checkbox"/>				

B

Date dd/mm	Sleep Location Own bed or alternative?	How many cups of caffeine have you had today? What time was your last cup?		Bed time hh:mm	Time you fell asleep hh:mm	Awake Time hh:mm	Out of Bed hh:mm	Number of awakenings	Did you have 'screen time' immediately before going to sleep & for how long?		Sleep Quality								
											very good	I good	I average	I poor	I very poor				
											<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
											<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
											<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
											<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
											<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
											<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>
											<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>		<input type="checkbox"/>

Baseline Testing

Physical Activity, Exercise and Work Diary

- 1. Complete a single column for each day you record activity**
- 2. Record the date of each day**
- 3. Record the time (24h), type (i.e cycling – interval; slow walk) and duration (for the entire session) (be as precise as possible). Following any exercise that you do, rate how hard you think you worked in reference to the scale on the final page of your diary (Rating of Perceived Exertion).**
- 4. Additional Information – only use if there are unexpected changes to diet such as falling ill or you attended a major social event (i.e. wedding, birthday party, etc.)**

Date dd/mm	Time hh/mm	Type	Duration / Distance hh/mm / (kms)	R.P.E (1-10 scale)	Work Hours	Additional Information
15/09	07:00	Walked the dog	00:40 / 4 kms	3	10 Hours	N/A

Date dd/mm	Time hh/mm	Type	Duration / Distance hh/mm / (kms)	R.P.E (1-10 scale)	Work Hours	Additional Information

rating	description
0	NOTHING AT ALL
0.5	VERY, VERY LIGHT
1	VERY LIGHT
2	FAIRLY LIGHT
3	MODERATE
4	SOMEWHAT HARD
5	HARD
6	
7	VERY HARD
8	
9	
10	VERY VERY HARD (MAXIMAL)

DASS21

Name:

Date:

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you over the past week. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

- 0 Did not apply to me at all
 1 Applied to me to some degree, or some of the time
 2 Applied to me to a considerable degree or a good part of time
 3 Applied to me very much or most of the time

1 (s)	I found it hard to wind down	0	1	2	3
2 (a)	I was aware of dryness of my mouth	0	1	2	3
3 (d)	I couldn't seem to experience any positive feeling at all	0	1	2	3
4 (a)	I experienced breathing difficulty (e.g. excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5 (d)	I found it difficult to work up the initiative to do things	0	1	2	3
6 (s)	I tended to over-react to situations	0	1	2	3
7 (a)	I experienced trembling (e.g. in the hands)	0	1	2	3
8 (s)	I felt that I was using a lot of nervous energy	0	1	2	3
9 (a)	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10 (d)	I felt that I had nothing to look forward to	0	1	2	3
11 (s)	I found myself getting agitated	0	1	2	3
12 (s)	I found it difficult to relax	0	1	2	3
13 (d)	I felt down-hearted and blue	0	1	2	3
14 (s)	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15 (a)	I felt I was close to panic	0	1	2	3
16 (d)	I was unable to become enthusiastic about anything	0	1	2	3
17 (d)	I felt I wasn't worth much as a person	0	1	2	3
18 (s)	I felt that I was rather touchy	0	1	2	3
19 (a)	I was aware of the action of my heart in the absence of physical exertion (e.g. sense of heart rate increase, heart missing a beat)	0	1	2	3
20 (a)	I felt scared without any good reason	0	1	2	3
21 (d)	I felt that life was meaningless	0	1	2	3

DASS-21 Scoring Instructions

The DASS-21 should not be used to replace a face to face clinical interview. If you are experiencing significant emotional difficulties you should contact your GP for a referral to a qualified professional.

Depression, Anxiety and Stress Scale - 21 Items (DASS-21)

The Depression, Anxiety and Stress Scale - 21 Items (DASS-21) is a set of three self-report scales designed to measure the emotional states of depression, anxiety and stress.

Each of the three DASS-21 scales contains 7 items, divided into subscales with similar content. The depression scale assesses dysphoria, hopelessness, devaluation of life, self-deprecation, lack of interest / involvement, anhedonia and inertia. The anxiety scale assesses autonomic arousal, skeletal muscle effects, situational anxiety, and subjective experience of anxious affect. The stress scale is sensitive to levels of chronic non-specific arousal. It assesses difficulty relaxing, nervous arousal, and being easily upset / agitated, irritable / over-reactive and impatient. Scores for depression, anxiety and stress are calculated by summing the scores for the relevant items.

The DASS-21 is based on a dimensional rather than a categorical conception of psychological disorder. The assumption on which the DASS-21 development was based (and which was confirmed by the research data) is that the differences between the depression, anxiety and the stress experienced by normal subjects and clinical populations are essentially differences of degree. The DASS-21 therefore has no direct implications for the allocation of patients to discrete diagnostic categories postulated in classificatory systems such as the DSM and ICD.

Recommended cut-off scores for conventional severity labels (normal, moderate, severe) are as follows:

NB Scores on the DASS-21 will need to be multiplied by 2 to calculate the final score.

	Depression	Anxiety	Stress
Normal	0-9	0-7	0-14
Mild	10-13	8-9	15-18
Moderate	14-20	10-14	19-25
Severe	21-27	15-19	26-33
Extremely Severe	28+	20+	34+

Epworth Sleepiness Scale

Name: _____ Today's date: _____

Your age (Yrs): _____ Your sex (Male = M, Female = F): _____

How likely are you to doze off or fall asleep in the following situations, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the **most appropriate number** for each situation:

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

It is important that you answer each question as best you can.

Situation	Chance of Dozing (0-3)
Sitting and reading _____	_____
Watching TV _____	_____
Sitting, inactive in a public place (e.g. a theatre or a meeting) _____	_____
As a passenger in a car for an hour without a break _____	_____
Lying down to rest in the afternoon when circumstances permit _____	_____
Sitting and talking to someone _____	_____
Sitting quietly after a lunch without alcohol _____	_____
In a car, while stopped for a few minutes in the traffic _____	_____

THANK YOU FOR YOUR COOPERATION

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Name _____

Date _____

Sleep Quality Assessment (PSQI)

What is PSQI, and what is it measuring?

The Pittsburgh Sleep Quality Index (PSQI) is an effective instrument used to measure the quality and patterns of sleep in adults. It differentiates "poor" from "good" sleep quality by measuring seven areas (components): subjective sleep quality, sleep latency, sleep duration, habitual sleep efficiency, sleep disturbances, use of sleeping medications, and daytime dysfunction over the last month.

INSTRUCTIONS:

The following questions relate to your usual sleep habits during the past month only. Your answers should indicate the most accurate reply for the majority of days and nights in the past month. Please answer all questions.

During the past month,

1. When have you usually gone to bed? _____
2. How long (in minutes) has it taken you to fall asleep each night? _____
3. What time have you usually gotten up in the morning? _____
4. A. How many hours of actual sleep did you get at night? _____
B. How many hours were you in bed? _____

5. During the past month, how often have you had trouble sleeping because you	Not during the past month (0)	Less than once a week (1)	Once or twice a week (2)	Three or more times a week (3)
A. Cannot get to sleep within 30 minutes				
B. Wake up in the middle of the night or early morning				
C. Have to get up to use the bathroom				
D. Cannot breathe comfortably				
E. Cough or snore loudly				
F. Feel too cold				
G. Feel too hot				
H. Have bad dreams				
I. Have pain				
J. Other reason (s), please describe, including how often you have had trouble sleeping because of this reason (s):				
6. During the past month, how often have you taken medicine (prescribed or "over the counter") to help you sleep?				
7. During the past month, how often have you had trouble staying awake while driving, eating meals, or engaging in social activity?				
8. During the past month, how much of a problem has it been for you to keep up enthusiasm to get things done?				
9. During the past month, how would you rate your sleep quality overall?	Very good (0)	Fairly good (1)	Fairly bad (2)	Very bad (3)

Scoring

Component 1	#9 Score	C1 _____
Component 2	#2 Score (<15min (0), 16-30min (1), 31-60 min (2), >60min (3)) + #5a Score (if sum is equal 0=0; 1-2=1; 3-4=2; 5-6=3)	C2 _____
Component 3	#4 Score (>7(0), 6-7 (1), 5-6 (2), <5 (3))	C3 _____
Component 4	(total # of hours asleep) / (total # of hours in bed) x 100 >85%=0, 75%-84%=1, 65%-74%=2, <65%=3	C4 _____
Component 5	# sum of scores 5b to 5j (0=0; 1-9=1; 10-18=2; 19-27=3)	C5 _____
Component 6	#6 Score	C6 _____
Component 7	#7 Score + #8 score (0=0; 1-2=1; 3-4=2; 5-6=3)	C7 _____

Add the seven component scores together _____ Global PSQI _____

A total score of "5" or greater is indicative of poor sleep quality.

If you scored "5" or more it is suggested that you discuss your sleep habits with a healthcare provider

Appendix C

Study 2 & 3 documentation including Biospecimen Approval, Recruitment Poster, Participate Information, and Informed Consent, Food Diary and Aerobic & Resistance Training Diary

INFORMATION SHEET

Circadian desynchronization, sleep architecture and the effect of exercise.**Research Team and Roles****Primary Investigator**

Mr Blake Collins
 PhD Student
 School of Exercise Science,
 Sport and Health
 Charles Sturt University
 0409598135
blcollins@csu.edu.au

Co-Investigator

Dr Melissa Skein
 Charles Sturt University
mskein@csu.edu.au

Dr Tegan Hartmann
 Charles Sturt University
thartmann@csu.edu.au

You have been invited to participate in a research study as part of a PhD project examining the effect of differing exercise intensities on health measures currently believed to be affected by working in rotational shift work. As outlined in the consent form, if you choose to partake in this study, you are free to withdraw at any time, with no consequences and any data collected up until that point will be discarded, not included in the study or published in any medium. Mr Collins will be available during the study at all times if you have any problems or questions about the study.

Purpose of the Study

Rotating shift work, defined by irregular or unusual working hours outside the conventional working day is currently associated with changes in key biological mechanisms. These biological disturbances may be precursors to ill health, specifically effecting sleeping patterns, cardiovascular health, and inflammatory status. Conversely, exercise is viewed as a successful therapeutic intervention, improving general health and sleep outcomes. Therefore the purpose of this study is to investigate the effect of both a one-off (acute) and chronic (3 days a week for 12 weeks) exercise bout of different intensity on sleep quality, cardiovascular and metabolic health.

In order to be considered for enrolment into this study, participants must:

- Male, currently employed in rotational style shift work (minimum 2 years including day and night shift splits)
- Aged between 25 and 55 years
- Never been diagnosed with a sleep disorder for example; sleep apnoea or insomnia
- Have no known immunological irregularities or inflammatory conditions

- Achieve a minimum of 5.5 hours of sleep most nights
- Not currently engaging in more than 60 minutes of exercise per week
- Free from any condition which may be exacerbated by exercise including cardiovascular disease
- Current non-smoker
- Be available for testing between July and December 2019

Study Procedure and Protocol

Following informed consent, you will undertake screening and baseline testing, attending four (4) visits to Charles Sturt University (CSU) for laboratory testing. If you choose to participate in the 12 week training intervention, one (1) additional testing session, at CSU will be conducted at the end of the training period.

Screening and baseline testing

You will be required to:

- Obtain a clearance from your GP for participation, you will be responsible for the cost of the GP.
- Complete a general health questionnaire, the Pittsburgh Sleep Quality Index (PSQI) and the Epworth Sleepiness Scale to screen for sleep behaviours.
- Wear an Actiwatch (device worn on the wrist that monitors sleep) and complete a sleep diary (including bed times, get-up times, total time in bed, total time asleep, subjective sleep quality, caffeine consumption and screen time before bed) for 14 nights prior to the start of the study.
- You will also be asked to complete a food/fluid and work diary during your 14-night Actigraphy period (identifying work start and finish times).

Please note that the Actiwatch is required to be worn at all times during this 14-day period, expect for when there is a chance it could get wet (i.e. shower, swimming) or could be damaged. Further, if the Actiwatch is damaged or lost while in your possession, there will be no liability to the participant.

Laboratory testing

You will be required to abstain from vigorous exercise, alcohol for 24 hour period and caffeine for 12 hours before testing. On arrival measures will be taken to assess cardiovascular health via a chest strap and inflammatory status via a blood sample. On the day, you will then be randomly assigned an exercise condition (High intensity interval training or moderate intensity continuous training) or non-exercising control before going home to sleep and returning the following morning (same time) for repeat laboratory testing of the same health measures. The third testing session will involve repeat standardised laboratory testing followed by alternating intervention (HIIT vs. MICE), standardised sleep, before a fourth testing

session compromising of repeat measures. Exercise intervention groups will then participate in a training intervention, 3 days a week for 12 weeks on a stationary exercise bike before all groups, including the control, are invited back to complete the final laboratory testing session. Training session will be conducted in groups, as such you may come into contact with other participants but no confidential information regarding testing measures will be shared with any other participant. The data collection procedures will be conducted by Mr Blake Collins who has the appropriate qualifications and has received University approval regarding data collection, storage and disposal procedures. During data collection, photos/videos may be recorded for presentation purposes to demonstrate data collection methods. In the case a photo/video is to be recorded, you will be notified verbally before it is taken and again via email before any use of the recording, with the right to refuse or withdraw consent at any time. In the case your photo/video is used there will be an additional option to be de-identified, with the blurring of face and any identifiable features which will be verbally and electronically explained before the use of any images/videos.

Ionising Radiation:

This research study involves exposure to a very small amount of radiation from the DEXA scan as we calculate body composition (body fat % vs. muscle %). The scan will occur on two separate occasions, once during the pre testing period and once during the post laboratory testing following the 12 week exercise intervention. The whole body scan radiation dose received during this study is equal to 0.2 millisieverts (mSv), which has no harmful effects of radiation and has a risk classification of minimal. To put that value into perspective, as part of every day living, everyone is exposed to naturally occurring background radiation, including sun exposure, receiving a dose of about 2 mSv each year. The DEXA scan will take place in a separate, private room within the laboratory in a clothed state (removal of shoes).

Time Commitment

We understand the study design requires a considerable time commitment from participants, which is outlined in the information sheet. Irrespective of the cohort group, across the duration of the study, participants will be required to complete 14 nights of actigraphy (sleep watch and diary), a familiarisation session and four additional laboratory visits make up the pre intervention testing protocol. One final testing session will be conducted at the conclusion of the 12 week training intervention to assess its success.

We also acknowledge that there are numerous benefits to volunteering in this project including provision of information about your health, wellbeing, exercise capacity, and sleep. In addition, you will be provided with education about your results, and the importance of the relationship between sleep, appetite and exercise. You will also be provided with the opportunity to access free training for twelve weeks under the supervision of a member of the research team in a community-based, supportive setting.

Activity	Duration	Notes
Testing Familiarisation	Approx. 2 hour	To become familiar with the laboratory equipment and testing protocols
Laboratory Testing (Visits 1 & 3)	Approx. 2 hours each	Complete blood sample, oral glucose tolerance test (OGTT) heart rate (HRV) monitoring and intervention
Actigraphy	14 days	Small watch like device that is worn for the 14 days including both testing sessions.
Laboratory Testing (Visits 2 & 4)	Approx. 30 mins each	Complete repeat testing procedures
Training Intervention	3 days a week for 12 weeks	Session will start at 30 minutes and progress incrementally towards 1 hour in weeks 11 + 12
Post Testing Session	Approx. 2 hours	Complete pre testing procedure including OGTT, DEXA, blood samples and HRV

Research Team

Mr Collins, Dr Skein and Dr Hartmann, are located at Charles Sturt University and will be present at data collection sessions.

Confidentiality

The privacy of all volunteers will be assured and all data acquired from individual participants will be kept strictly confidential on password-protected computers. Only the Chief Investigator and Research Supervisors will have access to your identity.

Receiving assessment information regarding general health and wellbeing may be potentially distressing. As such the research team will provide additional information including normative data, explanation of measures (how specific measure relate to health) and support in the event you find this information distressing or confronting. Individual results will provide additional context when analysed in addition to overall health status, therefore with your consent, results will also be passed onto your GP in the event further interpretation, diagnosis or medical intervention is required as it is outside the expertise of the research team. In the event you find any of the testing, associated procedures and/or results distressing additional information in regards to support services will be available including Lifeline (Phone: 131114), Headspace (phone: 63381100) and Bathurst Community Mental Health Services.

Charles Sturt University's Human Research Ethics Committee has approved this project. If you have any complaints or reservations about the ethical conduct of this project, you may contact the Committee via:

In writing: Ethics and Compliance Unit Locked Bag 588, Wagga Wagga, NSW 2678

Telephone: (02) 6338 4628 or Email: ethics@csu.edu.au.

Any issues you raise will be treated in confidence and investigated fully, and you will be informed of the outcome.

Rotating shift, circadian desynchronization and associated health outcomes.

Primary Investigator

Mr Blake Collins
School of Exercise Science,
Sport and Health
Charles Sturt University
0409598135
blcollins@csu.edu.au

Co-Investigator

Dr Melissa Skein
Charles Sturt University
mskein@csu.edu.au

Dr Tegan Hartmann
Charles Sturt University
thartmann@csu.edu.au

PURPOSE OF THE STUDY

The study aims to assess the acute and chronic effect of exercise modality and intensity on measures of health currently known to be affected by circadian disruption and short sleep duration associated with rotational shift work.

I, _____ have read the information contained in the *Participant Information Sheet* provided, the consent form and any questions I have asked have been answered to my satisfaction.

I agree to participate in this project, realising I am free to withdraw my participation at any time without being subject to any penalty or discriminatory treatment from the study.

I agree that the purpose of this research and potential risks or discomforts involved with the testing have been sufficiently explained to me, with the opportunity to ask questions.

I understand that any information or personal details gathered during this research about me is confidential and that neither my name nor any other identifying information will be used or published without my written permission. Additionally, you will be advised if any additional medical intervention is required in which case information can be passed onto your GP. I consent to photos and/or videos being collected throughout the research process and understand I have the ability to withdraw consent prior to them being published or used for presentation purposes.

General Practitioners Contact Name: _____ Number: _____

In the case of an emergency, please provide a name and contact number of a next of kin.

Name: _____ Phone: _____ Relationship: _____

Charles Sturt University's Human Research Ethics Committee has approved this study. I understand that if I have any complaints or concerns about this research I can contact:

Executive Officer
Human Research Ethics Committee, Office of Academic Governance
Charles Sturt University
Panorama Avenue, Bathurst NSW 2795
Ph: (02) 63384628 Fax: (02) 63384194

Signature of consent

Date

Signature of investigator

Date



DREAMING OF BETTER HEALTH?

Exercise intervention study

WHAT?

- Free health screening
- 4 laboratory testing sessions
- Sleep assessment
- 3 days/week for 12 weeks
- Free supervised training intervention

WHO? 25-55 y.o healthy males currently employed in shift work and exercise <60 mins/week

WHEN? July - December 2019

WHERE? Charles Sturt University

Interested?? Contact Blake Collins: bcollinsecsu.edu.au or 0409598135

Authority to Use Human Biological Specimens:

IBC Reference Number: IBC 19HB05
Name of Principal Investigator: Mr Blake Collins
Title of Project: Circadian desynchronization, sleep architecture and the acute and chronic effect of exercise
Type of Human Biological Specimen: Intravenous blood samples, collected via indwelling cannula
Facility to be used: Exercise Physiology Laboratory, building 1295, room 131
Duration of Project: 20 December 2019 until 20 February 2020
Date approved by Biosafety Committee: 20 December 2019

Lori Blechynden
Digitally signed by Lori Blechynden
 DN: cn=Lori Blechynden, o=Charles Sturt University, ou=Vetinary
 Enterprise, email=blechynden@csu.edu.au, c=AU
 Date: 2019.12.20 09:46:19 +1100

Signed: Presiding Officer Charles Sturt University Institutional Biosafety Committee

Date:

Submit all applications to: RadiationSafety@csu.edu.au

Electronic files with digital signatures are preferred. If this is not possible, please print, sign and scan.
Remember to attach any additional documents, such as copies of radiation licences, exemption forms or written approvals.

Remember to attach any required licences, exemption forms, written approvals or additional pages.

11	Radiation Safety Committee Use Only
Approval No: R19008	Signature:  <small>Dr Peter Simpson, Dr Radiation Safety Committee, CSU Deputy Chair radiation@csu.edu.au ORAU</small>
	Date: 8 Feb 2019
Radiation Management Licence Holder (or delegate)	

Day/Date				
Breakfast 350 ml Orange Juice 2 x slices of white toast 2 x eggs				
Lunch 300 g chicken Garden salad 2 x tbsp. ranch dressing				
Dinner 400 g mince 150 ml Spaghetti sauce 50 g thin pasta				
Snacks & Beverages 2 x coffees 2 x apples				

Circadian desynchronization, sleep architecture and the effect of exercise.

Aerobic Exercise Diary

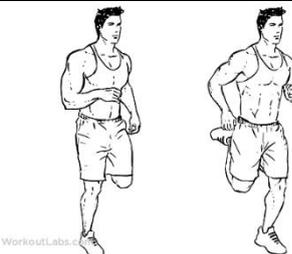
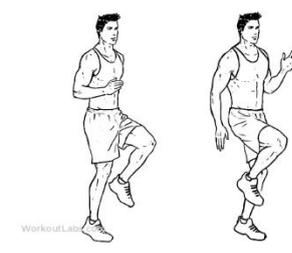
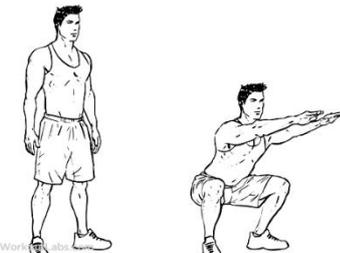
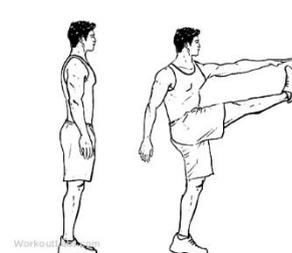
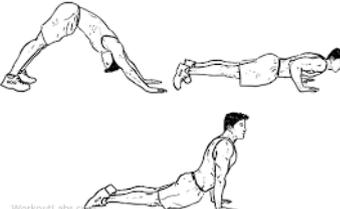
The program will be running for 12 weeks with the aim of completing 3 exercise sessions per week at the Charles Sturt University Gymnasium, operating hours are **Mon-Fri: 6am – 9pm** and **Sat-Sun: 9am-4pm**.

The Intervention includes three aerobic modality workouts totalling 40 minutes of work, which can be achieved using any combination (minimum of 10-12 minutes) of any of the modalities. Included in the diary are the aerobic workouts as well as templates to record the exercise, rest intervals, resistance (in Watts), rating of perceived exertion and any additional notes about the workout.

Start Date: _____

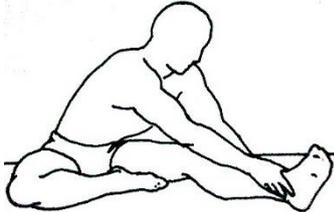
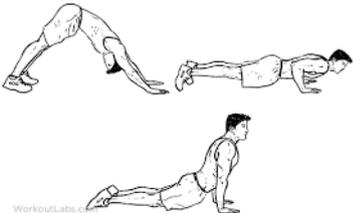
End Date: _____

Appendix C
Dynamic Warm up – 5 mins

Exercise	Repetition	Example
Butt Kicks	15 sec	
High Knees	15 secs	
Body Weight Squats	20	
Leg Swings	10 (each Side)	
Push up Complex	10	
Bird Dogs	10 (each side)	

Appendix C

Static Stretch – 5 mins

Stretch	Repetition	Example
Hamstring Stretch	10 sec (each side x 3)	
Quads Stretch	10 sec (each side x 3)	
Shoulder Stretch	10 sec (each side x 3)	
Push up Complex	10 sec (each side x 3)	
Bird Dogs	15 (each side)	

Aerobic - Continuous			
Warm Up		5 mins dynamic bodyweight exercises	
Exercise	Time (minutes)	Rest	Target HR (% of max)
Bike	10-12	60 seconds	60-70%
Rower	10-12	60 seconds	60-70%
Treadmill	10-12	60 seconds	60-70%
Warm Down		5 mins of static stretching of major muscles	

Date: _____

Exercise	Time	Rest	Workload	Target HR	HR	RPE	Notes
Bike	24 mins	1 minute	100 Watts	>130	135	6	
Rower	-	-	-	-	-	-	
Treadmill	12 mins	1 minute	9 Km	>130	138	7	

Workload will differ depending on the mode, for example Watts on the bike, fan resistance on the rower and speed on the treadmill

Date: _____

Exercise	Time	Rest	Workload	Target HR	HR	RPE	Notes

Date: _____

Exercise	Time	Rest	Workload	Target HR	HR	RPE	Notes

Date: _____

Exercise	Time	Rest	Workload	Target HR	HR	RPE	Notes

Date: _____

Exercise	Time	Rest	Workload	Target HR	HR	RPE	Notes

rating	description
0	NOTHING AT ALL
0.5	VERY, VERY LIGHT
1	VERY LIGHT
2	FAIRLY LIGHT
3	MODERATE
4	SOMEWHAT HARD
5	HARD
6	
7	VERY HARD
8	
9	
10	VERY VERY HARD (MAXIMAL)

Circadian desynchronization, sleep architecture and the effect of exercise.

Resistance Exercise Diary

The program will be running for 12 weeks with the aim of completing 3 exercise sessions per week at the Charles Sturt University Gymnasium, operating hours are **Mon-Fri: 6am – 9pm** and **Sat-Sun: 9am-4pm.**

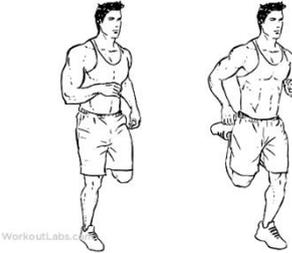
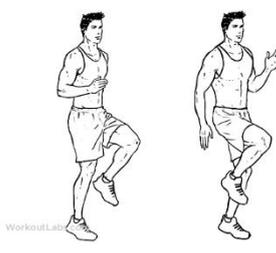
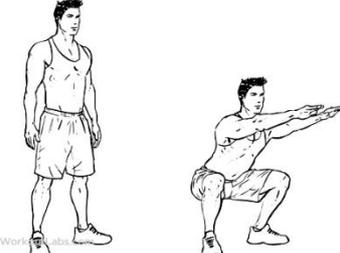
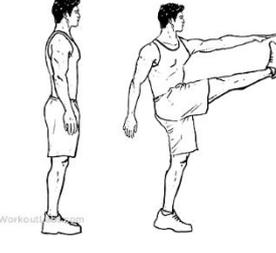
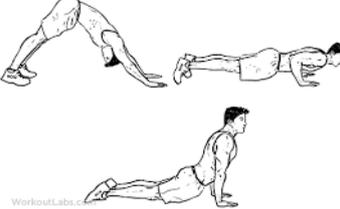
The Intervention includes two different resistance workouts that are to be alternated so that the same routine is not performed on consecutive days or workouts. Included in the diary are the two resistance routines as well as templates to record the exercise, repetitions, sets, rest intervals, resistance (weight in kg), rating of perceived exertion and any additional notes about the workout.

Start Date: _____

End Date: _____

Appendix C

Dynamic Warm up – 5 mins

Exercise	Repetition	Example
Butt Kicks	15 sec	
High Knees	15 secs	
Body Weight Squats	20	
Leg Swings	10 (each Side)	
Push up Complex	10	

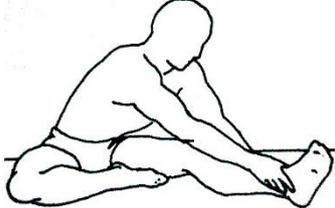
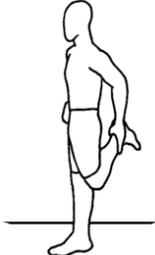
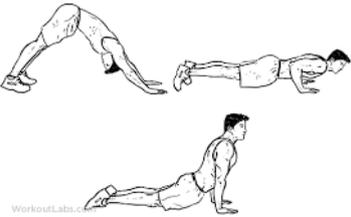
Bird Dogs

Appendix C
10 (each side)



Appendix C

Static Stretch – 5 mins

Stretch	Repetition	Example
Hamstring Stretch	10 sec (each side x 3)	
Quads Stretch	10 sec (each side x 3)	
Shoulder Stretch	10 sec (each side x 3)	
Push up Complex	10 sec (each side x 3)	
Bird Dogs	15 (each side)	

Resistance – Session 1			
Warm Up		5 mins dynamic bodyweight exercises	
Exercise	Repetitions	Sets	Rest
Chest Press	8-12	3	60 seconds
Lat Pull Down	8-12	3	60 seconds
Leg Press	8-12	3	60 seconds
Bicep Curl	8-12	3	60 seconds
Shoulder Press	8-12	3	60 seconds
Plank	30-60 seconds	3	30-60 seconds
Warm Down		5 mins of static stretching of major muscles	
Resistance – Session 2			
Warm Up		5 mins dynamic bodyweight exercises	
Exercise	Repetitions	Sets	Rest
Chest Press	8-12	3	60 seconds
Seated Row	8-12	3	60 seconds
Seated Leg Extension	8-12	3	60 seconds
Seated Hamstring Curl	8-12	3	60 seconds
Triceps Extension	8-12	3	60 seconds
Plank	30-60 seconds	3	30-60 seconds

Warm Down

5 mins of static stretching of major muscles

Session: 1 / 2

Date: _____

Exercise	Repetitions	Sets	Rest	Weight	HR	RPE	Notes
Chest Press	12, 12, 10	3	60 seconds	45	120	7	
Lat Pull Down	12, 12, 12	3	60 seconds	40	122	7	
Leg Press	12, 11, 9	3	60 seconds	65	125	8	
Bicep Curl	12, 12, 12	3	60 seconds	25	122	6	
Shoulder Press	11, 10, 8	3	60 seconds	30	130	6	
Plank	30 secs	3	60 seconds	BW	145	8	

Session: 1 / 2

Date: _____

Exercise	Repetitions	Sets	Rest	Weight	HR	RPE	Notes

Session: 1 / 2

Date: _____

Exercise	Repetitions	Sets	Rest	Weight	HR	RPE	Notes

rating	description
0	NOTHING AT ALL
0.5	VERY, VERY LIGHT
1	VERY LIGHT
2	FAIRLY LIGHT
3	MODERATE
4	SOMEWHAT HARD
5	HARD
6	
7	VERY HARD
8	
9	
10	VERY VERY HARD (MAXIMAL)