Building practical dietary strategies to improve the metabolic health of night shift workers

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Bachelor of Nutrition (Honours)

Bachelor of Nutrition and Dietetics

A Thesis submitted for the degree of Doctor of Philosophy at

Monash University in 2021

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Melbourne, Australia

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Thesis abstract

Observational studies show that shift workers face an increased risk of cardiovascular disease, type-2 diabetes and obesity. Research suggests that this is associated with the behavioural pattern that they adopt to align with their shift schedule, i.e. eating and working during the night, whilst fasting and sleeping during the day, opposing the regulation of our circadian clock system. The shift working population accounts for approximately 20% of employees in industrialised countries, yet, there are currently limited dietary guidance for this population, tailored to the protection of their metabolic health. Therefore, this Thesis aims to identify strategies to mitigate the metabolic impact of eating during the night, which was achieved through the combined use of quantitative and qualitative research methods.

Firstly, via a meta-analysis of diurnal studies, we confirmed the causal relationship between night time eating and reduced glucose tolerance. This finding indicates a physiological mechanism, which contributes towards the increased risk of metabolic diseases observed in shift workers. Furthermore, it suggests meal timing as a modifiable risk factor, providing rationale to the 'Shifting the Risk' study. This pilot randomised crossover study was the first meal timing intervention conducted in free-living night shift workers, and examined whether a 5-hour overnight fasting period is able to improve the metabolic health of night shift workers. Although no difference was observed in postprandial glucose and triglyceride responses after the intervention, a small loss of body weight was evident. This observation suggests that manipulation of meal timing may facilitate weight maintenance in night shift workers, and therefore needs to be verified in future studies. Critical to a pilot study, the feasibility of the 'Shifting the Risk' protocol was explored. Results showed that it was feasible for night shift workers to maintain a 5-hour overnight fasting period within their work environment. However, participants expressed that flexibility of meal break structure and the observation of more overt health improvements are required, to promote long-term sustainability of this dietary intervention. This finding stresses the influence of the workplace on night shift workers' ability to practise healthy eating, an observation that was further substantiated in the qualitative study. Moreover, results from this qualitative study emphasise that night shift workers' food choices and eating habits are driven by a complex interplay of individual, social and environmental factors, such as the experience of fatigue, presence of social networks and workplace environment respectively.

This Thesis substantiated the need to consider meal timing and food choices during the night, in the development of nutrition interventions and health promotion strategies for night shift workers. Further research is required to determine optimal meal timing and food choices for the night. Moreover, health promotion for this population require a multi-strategy approach, targeting individual and environmental factors concurrently. Modifications to the workplace environment and public policy reforms should be considered, to create a setting that supports night shift workers in adopting healthy eating practices. There is an urgent need to further develop this body of work, in order to protect the metabolic health of this essential workforce.

Publications during enrolment

Published manuscripts included in Thesis

Leung GKW, Huggins CE, Ware RS, Bonham MP. Time of day difference in postprandial glucose and insulin responses: systematic review and meta-analysis of acute postprandial studies. *Chronobiology International*. 2020;37(3):311-26. Doi: 10.1080/07420528.2019.1683856.
Included as Chapter 2; published manuscript available in Appendix A.

Leung GKW, Davis R, Huggins CE, Ware RS, Bonham MP. Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers. *Nutrition, Metabolism and Cardiovascular Diseases*. 2021;31(6):1890-1902. Doi: 10.1016/j.numecd.2021.03.008.
Included as Chapter 3a; published manuscript available in Appendix B.

Other associated works

Bonham MP, Kaias E, Huggins CE, Davis R, Leung GKW, Eikelis N, Shaw E, Murgia C. Effects of macronutrient manipulation on postprandial metabolic responses in overweight males with high fasting lipids during simulated shift work: a randomised crossover trial. *Clinical Nutrition*. 2020;39(2):369-77. Doi: 10.1016/j.clnu.2019.02.018.

Shaw E*, Leung GKW*, Jong J, Coates AM, Davis R, Blair M, Huggins CE, Dorrian J, Banks S, Kellow NJ, Bonham MP. The impact of time of day on energy expenditure: implications for long-term energy balance. *Nutrients*. 2019;11(10):2383-402. Doi: 10.3390/nu11102383.
**Joint first authors.*

Bonham MP, Kaias E, Zimberg I, Leung GKW, Davis R, Sletten TL, Windsor-Aubrey H, Huggins CE. Effect of night time eating on postprandial triglyceride metabolism in healthy adults: a systematic literature review. *Journal of Biological Rhythms*. 2019;34(2):119-30. Doi: 10.1177/0748730418824214.

Shaw E, Dorrian J, Coates AM, Leung GKW, Davis R, Rosbotham E, Warnock R, Huggins CE, Bonham MP. Temporal pattern of eating in night shift workers. *Chronobiology International*. 2019;36(12):1613-25. Doi: 10.1080/07420528.2019.1660358.

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Conference, symposia and webinar presentations during enrolment

Oral presentations

Invited Speaker; Australian Nursing and Midwifery Federation (Victoria), Working hours, shift and fatigue conference. *Food choices and meal-timing for shift workers*. Melbourne, Australia; Mar 2021.

Selected Speaker; Monash Health Translation Precinct Research Symposium – PhD Showcase Presentation. *Chrononutrition*. Melbourne, Australia; Oct 2018.

Leung GKW, Huggins CE, Ware RS, Bonham MP. *The effect of meal timing on postprandial glucose and insulin responses: a meta-analysis of acute experimental trials*. Asian Pacific Conference on Clinical Nutrition, Adelaide, Australia; Nov 2017.

Poster presentations

Leung GKW, Davis R, Huggins CE, Rosbotham E, Warnock R, Bonham MP. *Rearranging meal times during night shift work promotes weight change: a randomised crossover intervention in shift workers*. 13th European Nutrition Conference, FENS 2019, Dublin, Ireland; Oct 2019.

Bonham MP, Kaias E, Leung GKW, Davis R, Eikelis N, Murgia C, Huggins CE. *Effects of macronutrient manipulation on postprandial metabolic responses, in males at risk of cardiovascular disease during simulated shift work (RCT).* Nutrition Society of Australia Conference, Canberra, Australia; Dec 2018.

Webinar presentations

Nutrition Education Melbourne; *Meals and meal-timing for shift workers*; Dec 2019.

Nutrition Education Melbourne; *Nutrition considerations in shift work*; Sept 2019.

Nutrition Society of Australia; *Chrononutrition, relationship between circadian clock system and metabolic health*; Nov 2018.

Scholarships and awards

2017 – 2019: King and Amy O'Malley Trust Scholarship.

2018: Nutrition Society of Australia Nestle Emerging Researcher Award.

2018: People's Choice Award, Monash Health Translation Precinct Research Symposium – PhD Showcase Presentation.

Additional training

ANZBMS: Clinical Densitometry Training Course (for DXA Scanning); Melbourne, Australia; Mar 2019.

ACSPRI: An Introduction to Mixed Methods Research Design; Melbourne, Australia; May 2019.

Monash Institute for Health & Clinical Education: Qualitative research for health professions education; Melbourne, Australia; Sept 2018.

Melbourne GRADE Centre: Using the GRADE approach to summarise evidence for policy and practice; Melbourne, Australia; May 2018.

RMIT University: Venepuncture Course; Melbourne, Australia; Mar 2017.

Thesis including published works declaration

I hereby declare that this Thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this Thesis contains no material previously published or written by another person, except where due reference is made in the text of the Thesis.

This Thesis includes *two* original papers published in peer reviewed journals. The core theme of the Thesis is building practical strategies to improve the metabolic health of night shift workers. The ideas, development and writing up of all the papers in the Thesis were the principal responsibility of myself, the student, working within the *Department of Nutrition, Dietetics and Food* under the supervision of *A/Prof Maxine Bonham, Dr Catherine Huggins and Dr Suzanne Kleve.*

In the case of *Chapter 2 and Chapter 3a*, my contribution to the work involved the following:

Thesis Chapter	Publication Title	Status	Nature and % of student contribution	Co-author name(s) Nature and % of Co-author's contribution*	Co-author(s), Monash student Y/N*
2	*Time of day difference in postprandial glucose and insulin responses: systematic review and meta-analysis of acute postprandial studies*	*Published*	*Paper identification, data synthesis, manuscript preparation (65%)*	*Maxine P Bonham: conceptualisation, input into manuscript (15%)* *Catherine E Huggins: conceptualisation, input into manuscript (15%)* *Robert S Ware: data analysis, input into manuscript (5%)*	*No*
3a	*Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers*	*Published*	*Study design, data collection, data analysis, manuscript preparation (60%)*	*Maxine P Bonham: conceptualisation, study design, secure funding, input into manuscript (15%)* *Rochelle Davis: study design, data collection, input into manuscript (10%)* *Catherine E Huggins: conceptualisation, study design, input into manuscript (10%)* *Robert S Ware: data analysis, input into manuscript (5%)*	*Yes – Rochelle Davis*

I have renumbered sections of submitted and published papers in order to generate a consistent presentation within the Thesis.

Student name: Gloria KW Leung

Student signature: **Date: 10.02.2021**

I hereby certify that the above declaration correctly reflects the nature and extent of the student's and co-authors' contributions to this work. In instances where I am not the responsible author, I have consulted with the responsible author to agree on the respective contributions of the authors.

Main Supervisor name: Maxine Bonham

Main Supervisor signature: **Date: 17.02.2021**

Professional acknowledgements

To my three wonderful supervisors: *A/Prof Maxine Bonham, Dr Kate Huggins and Dr Sue Kleve*, I cannot express in words the gratitude and appreciation for you. Without your support, there is no way I would have made it this far. From a young age, my parents have taught me to respect and value my teachers, "一日為師 , 終生為父", which roughly translates to "*once a teacher, always respect them as you would respect your father*". No matter where my career takes me, I will always remember and value all that you have taught me.

To Maxine, thank you for introducing me to the world of research. Under your guidance, I went from a girl who didn't even know what a centrifuge was, to someone who can provide others with research support. Thank you for always trusting in my abilities and knowledge, and encouraging me to take up so many opportunities in addition to being a PhD student. You are never shy to introduce me to your colleagues; which means a lot to a young Chinese girl like me. I hope I have made you proud.

To Kate, thank you for being the voice of reason in this often stressful PhD journey. When we get carried away or overwhelmed with troubles, you are always there to tell us that "it's going to be okay," and then would calmly guide us in finding practical solutions. In addition to guiding me through my PhD, you have also offered words of wisdom, in ways to manage the workforce and life in general, which I'm forever grateful for.

To Sue, thank you for always being so positive and caring. Your endless words of encouragement motivated me to keep going, when I thought I had no more to offer. It goes without saying; thank you for introducing me to the world of qualitative research, and respecting the value of both quantitative and qualitative research methods.

To all the participants of my studies, thank you for the time, samples and stories that you have shared with us. Without your generosity, I wouldn't have a Thesis.

Thank you to the Department of Nutrition, Dietetics and Food, for embracing me as a member of the Department. Finally, I would like to express my appreciation to the Australian Government Research Training Program (RTP) Scholarship, as well as the King and Amy O'Malley Trust Scholarship, which provided financial support during my Candidature.

Personal acknowledgements

To mum, I cannot say enough times, how thankful I am for your love and care. Thank you for always being proud of me, telling me that I'm doing a fabulous job, even though half the time you are not sure what I get up to at Uni. I am so grateful for the love you put into making us a warm home; a shelter from all the chaos that we face in life. Many people say that rather than being smart, resilience is what you need to get through a PhD. Thank you for showing me the true meaning of persistence and resilience. Through all the things life has thrown at you, you never gave up, and so, there is no reason for me to either.

To Olive and Jono, thank you for being the best siblings I can ask for. As I got nerdier, you made sure that I was still in touch with the fun things in life. Thank you for being there for me when I was feeling stressed and defeated, and treating me to all the dinner dates, bubble tea runs and supermarket trips. You were always there to defend your big sister, when others say that I wasn't doing a good enough job, which means so much to me. And of course, thank you for being my personal chauffeur.

To dad and Aunty Brenda, thank you for all your encouragement and support from across the globe. Your jokes make everything seem a little less scary and a little more achievable. Thank you for always being so proud of me, and supporting my decisions. To our fur-baby Tavi, thank you for giving me kisses when I am upset and spending many late nights with me during this last leg of my PhD.

To Andy, here we are – we have conquered another milestone. Thank you for always having faith and confidence in me, more than I ever have for myself. This journey hasn't been easy, but every time I thought I couldn't make it, your faith and encouragement helps me take another step forward. The technical assistance that you have provided for all the illustrations in my Thesis is very much appreciated; they are gems in the sea of words.

To Rochelle, my study buddy who has now become my best friend. What would I have done without you? All the early mornings and late nights in the lab, all the stressful moments we had in the last four years, I'm so grateful to have spent them with you. Thank you for all your wisdom and emotional support. No matter how busy you are, you always come to the rescue when I am lost and defeated. It has been a pleasure getting to know you and to have worked with you.

To all the friends that I have made in the Department during my Candidature, thank you for always having laughs with me and telling me, "You can do it Gloria!" Especially Steph C, Christie and Alastair, you guys have always been there to provide reassurance and to listen to me complain when times were tough. To my dear friend Ivy, thank you for always being there to cheer me on, no matter the distance and time difference.

Lastly but very importantly, I would like to take this opportunity to express my gratitude towards my grandparents, who looked after me when I was little, in a language they are more familiar with.

親愛的爺爺嫲嫲，

君君博士畢業了！我想籍著這個機會，為你們對我從小的悉心照顧和栽培，向你們說聲感謝。

在我剛剛開始讀博士的時候，身邊很多長輩都質疑我這個選擇。大家都問我是不是因為找不到工作或想逃避出社會，才做出這個決定。因為這樣，我開始覺得我可能選擇了一條錯的路。在那年的某一天，我在整理我的舊卡片，翻開了爺爺在 2005 年寫給我的一封信。上面寫著 “君君上中學了，這是爺爺期盼許久的一天。希望君君以後可以讀上大學，碩士和博士，爺爺都會給予支持。” 謝謝爺爺，在我感到彷徨的時候，給予我鼓勵。原來在我還沒知道博士是什麼的時侯，你們已經對我有著這一個期望。謝謝你們從小的教導，令我知道長知識並不是為了炫耀，而是為了成為一個有教養，有學識和能夠回饋社會的人。在爺爺已經要坐輪椅的時候，他還在用 iPad 學英文，讓我深深明白到何謂活到老學到老。

現在君君長大了，我能夠在職場上找到自己的位置，不是因為我特別聰明，是因為我遵隨你們教我做人的道理。做事要努力、認真，做人要謙虛，學懂尊重別人，在別人有困難時要伸出緩手。

我永遠不會忘記，在我二年級剛剛從澳洲轉學去香港的時候，我連應該懂得的英文科都跟不上。每晚都是你們坐在我身旁，耐心幫助我完成作業。每個早上天還沒亮時，你們就起床，幫我準備校服和早餐，然後牽著我的手，送我上學。

沒有你們的悉心照料和教導，我便不會有今天的成就。謝謝你，爺爺嫲嫲。遺憾的是，我已經沒有機會陪伴在你們身邊，帶你們去喝茶，親口對你們說這番話, 來報答你們對我的付出。但是我希望你們在天堂會因為我的努力而感到驕傲和安慰。無論我將來做什麼樣的工作，我都不會忘記你們的教誨。

想念和愛你們的君君上。

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Abbreviations

% E	Percentage of energy
95% CI	95% confidence interval
AHA	American Heart Association
Activity EE	Physical activity energy expenditure
AUC	Area under the curve
BASE	Monash University Be Active Sleep Eat Facility
BMI	Body Mass Index
CVD	Cardiovascular disease
EOI	Expressions of interest
ESTA	Melbourne Emergency Services Telecommunications Authority
GI	Glycaemic index
HDL-C	HDL-cholesterol
HOMA-IR	Homeostatic Model Assessment for Insulin Resistance
HR	Hazards ratio
I^2	I-squared statistic
iAUC	Incremental area under the curve
IGTT	Intraperitoneal glucose tolerance test
LDL-C	LDL-cholesterol
MD	Mean difference
OGTT	Oral glucose tolerance test
OR	Odds ratio
RCT	Randomised controlled trial
RMR	Resting metabolic rate
SCN	Suprachiasmatic nucleus
SD	Standard deviation
SE	Standard error
SE Model	Social Ecological Model
SMD	Standardised mean difference
T2DM	Type-2 diabetes
TAG	Triglyceride
TEF	Thermic effect of food
WHO	World Health Organisation

Glossary

Circadian misalignment/ circadian disruption	Condition where the phases of master clock and behavioural cycle/ peripheral clocks are out-of-sync with each other.
Circadian rhythm/ circadian variation	Biological rhythm that cycles and repeats itself once every 24-hours. It is endogenously generated by the circadian clock system, and is maintained even in the absence of any external cues.
Diurnal rhythm/ diurnal variation	Any biological rhythm that cycles and repeats itself once every 24-hours. The expression of diurnal rhythms represents the composite effects of the circadian clock system, the external light/dark and behavioural cycles (i.e. feed/fast, sleep/wake, activity/rest)
Phase	The timing of a circadian rhythm.
Zeitgeber	External factors that can shift the phase of the master clock and peripheral clocks.

Chapter 1 . Thesis Introduction

"Begin to work as the sun rises, prepare to rest as the sun sets" is a Chinese phrase that has been used for centuries to describe our ancestors' way of living. It is also one of the key principles underpinning the maintenance of good health according to Traditional Chinese Medicine. This concept is echoed in modern science; humans are classified as diurnal animals, whereby activity and feeding are preferred during the day, whilst the night is reserved for sleep and fast. Such preference in timing of behaviour is set and governed by our circadian clock system (1, 2). However, industrialisation of modern society has led to changes in the temporal patterns of these behaviours, one of which is extending meal times later into the night (3). An analysis of the 2011-12 Australian National Nutrition and Physical Activity Survey data (n= 6053), showed that only 40% of Australian adults follow the 'conventional' three meals per day eating pattern. Approximately a quarter of the population was categorised as having a 'grazing' eating pattern; characterised by frequent eating occasions throughout the day, and commonly recording eating occasions after 2000h (4). A similar temporal eating pattern was observed in the U.S. population data (National Health and Nutrition Examination Survey 1992-2004, n= 9326) (5), with a small cross-sectional study of U.S. adults (n= 156) reporting that there were only five hours of the 24-hour day (0100h to 0600h) when the number of eating occasions were <1% of total events (6).

The behaviour of late night eating opposes the regulation of our circadian clock (i.e. circadian disruption) (7) and has been associated with metabolic disturbances such as hyperglycaemia, hyperlipidaemia and abdominal obesity (8, 9). As such, meal timing has been identified as an emerging modifiable risk factor for cardiovascular disease (CVD) and type-2 diabetes (T2DM) (10), in addition to other traditional lifestyle risk factors such as sedentary behaviour, inadequate fibre intake and excessive sodium intake (11). The impact of meal timing on metabolic disease risks warrants investigation, as CVD and T2DM are causing immense and escalating disease burden within Australia and worldwide. According to the Australian Institute of Health and Welfare, CVD is the second cause of premature deaths in Australian adults, contributing to 22% of total (12). While globally, CVD related deaths were reported to be 18.6 million in 2019 (13) and T2DM was the seventh leading cause of healthy life lost to disability (DALYs) in 2017 (14).

In particular, the shift working population face a disproportionate CVD and T2DM disease burden, with observational studies showing that their risk of developing CVD, T2DM and obesity are increased by 20 to 40%, compared to the general day-working population (15-21). Evidence suggests that this is related to the constant circadian disruption caused by shift work, in both the feed/fast and sleep/wake cycles (22). The shift work schedule also introduces numerous social and environmental stressors,

further exacerbating shift workers' ability to maintain healthy eating habits (23). Evidently, shift workers require specific dietary guidance, to combat the effects of circadian disruption and manage the impacts of shift work, in order to protect their metabolic health. Such guidance is currently lacking and is of public health significance, as up to 20% of working individuals in industrialised countries are engaged in shift work (24). In Australia, this essential workforce is comprised of 1.4 million individuals (25), employed across multiple industries including healthcare and social assistance, food service and transport (26).

This Chapter will provide relevant background literature, substantiating the need to develop strategies to mitigate the metabolic impact of night time eating experienced by night shift workers. It will firstly summarise emerging epidemiological and mechanistic studies, which indicate a relationship between late night eating and risk of metabolic diseases. A summary of the circadian clock system will be presented, which is required in order to understand the ways in which meal timing affects metabolism. This Chapter will then hone in on literature that describes the increased risk of metabolic diseases observed in night shift workers, a population that has little choice but to eat during the night. Relating to this, a description of night shift workers' typical dietary patterns and food choices during the night will be presented. It should be acknowledged that whilst circadian disruption in the sleep/wake cycle also affects night shift workers' metabolic health (22), this Thesis will focus on the disruption in the feed/fast cycle (i.e. meal timing) and its metabolic implications. Using animal and human experimental trials, improvements of metabolic health outcomes through manipulation of meal timing (i.e. avoidance of night time eating) will be demonstrated. Considering the disproportionate risk of metabolic diseases faced by night shift workers, the lack of evidence-based and tailored dietary guidance currently available for this population will be highlighted. This Chapter will conclude by explaining the need to understand social and environmental influences of night shift workers' food choices, in order to design practical dietary guidance for this essential workforce.

1.1 Eating during the night is associated with metabolic disturbances

1.1.1 Evidence from epidemiological studies

In the modern society, adults are extending the portion of day over which food is consumed (4, 6), with many consuming a large proportion of their daily energy intake during dinner (27). These night time eating habits have been associated with increased risk of metabolic diseases, as reported in epidemiological studies. In a prospective cohort study of non-obese Italian adults (n= 1245), Bo et al. showed that consuming majority of daily energy intake (≥ 48%) at dinner, was associated with a two-fold increase in odds of becoming obese at 6-year follow-up (28). This association remained valid after adjustment for age, gender, BMI at baseline, total energy intake and habit of breakfast skipping.

The effects extended beyond weight gain; whereby the odds of developing metabolic syndrome was also significantly increased (OR= 1.52) (28). This finding is supported by a cross-sectional study of Korean men (n= 5854); those who consumed ≥ 25% of their total daily energy intake at night (after 2100h) had 48% increased odds of metabolic syndrome, compared to non-night eaters (adjusted for age, BMI, physical activity level and total energy intake) (29). This association was also observed in a prospective cohort study of Japanese women (n= 3278), with those eating dinner within two hours of bedtime and snacking after dinner ≥ 3 times per week having 68% increased odds of developing metabolic syndrome at 4-year follow-up, compared to those who did not present with either night time eating habit (9).

The relationship between night time eating habits and individual CVD and T2DM risk indicators have also been reported. A recent cross-sectional study categorised 3362 Spanish adults with overweight or obesity, to early eaters (midpoint between breakfast and dinner time before 1454h) and late eaters (midpoint after 1454h) (30). In a model adjusted for age, meal frequency, soft drink consumption and sleep duration, late eaters had a higher waist circumference compared to early eaters (β-coefficient= 1.62cm). Furthermore, eating late was associated with having higher fasting triglyceride (TAG) (β-coefficient= 5.60 mg/dL) and insulin resistance levels (HOMA-IR β-coefficient= 0.03). In a Japanese cohort of 61,364 adults, eating dinner within 2 hours of bedtime ≥ 3 times per week was associated with 13% increased odds of hyperglycaemia (HbA1c ≥ 5.7% and/ or pharmacotherapy for diabetes), after adjustment for age, BMI and history of CVD (8).

Undoubtedly, the causal relationship between night time eating and increased risk of metabolic diseases cannot be confirmed through findings from observational studies, especially as many are of cross-sectional design. Nonetheless, the epidemiological studies described involved large samples across multiple countries, and consistently suggest metabolic implications of night time eating. This warrants investigation into physiological mechanisms that can potentially explain these observational findings.

1.1.2 Acute metabolic response of eating during the night

Findings from diurnal studies can provide physiological mechanisms that elucidate the relationship between night time eating and increased risk of metabolic disease. Diurnal studies are able to assess and reflect *diurnal rhythms*, i.e. fluctuation in physiological responses that occur during the 24-hour day. As such, this study design allows researchers to compare how nutrient intake is metabolised differently at various clock hours of the 24-hour day. Light exposure and behavioural cycles (i.e. several large nutrient loads and a sleep period during the 24-hour day) are also maintained in its experimental protocol (7). Therefore, this type of study can reflect whether night time eating, as a form of circadian

disruption, leads to acute metabolic disturbances. Diurnal studies were firstly utilised by Bowen et al in 1967; who reported a glucose load consumed at noon led to higher 2-hour postprandial glucose levels, compared to that observed in the morning (load consumed at 0800h) (31). Subsequently, numerous diurnal studies have examined the time of day difference in postprandial glucose and insulin responses, by administering acute meal challenges at different times of the 24-hour day.

Diurnal studies examining postprandial glucose response have in general shown an increased response during the night compared to the day. Our group has previously reported (32) that the postprandial glucose response after a meal consumed at midnight, was nine times higher compared to a meal consumed at 0800h (Figure 1-1a). A similar pattern was observed in the postprandial insulin response (Figure 1-1b), although findings from published studies are less consistent. Variation in findings may be partially attributed to protocol differences amongst the studies, such as the day and night clock times chosen as comparisons. For example, a diurnal study by Al-Naimi et al reported a higher glucose response at night (0100h) compared to day (1300h), which occurred concurrently with a reduced insulin response (33). In comparison, Santos et al found a higher postprandial glucose response in the evening (1800h) compared to noon, but no differences in postprandial insulin response were observed (34). Confirmation as to whether time of day differences exist in postprandial glucose and insulin response is warranted, as prolonged postprandial hyperglycaemia and insulin insensitivity are known independent risk factors for T2DM and CVD (35-38). Such investigation will help determine if circadian disruption, caused by night time eating, leads to direct and acute metabolic disturbances, indicating a potential physiological mechanism that explains the association between eating during the night and increased risk of metabolic diseases. Moreover, it will suggest meal timing as a modifiable risk factor, which could be manipulated to lower the risk of metabolic diseases in populations who habitually eat during the night, such as night shift workers. Therefore, Chapter 2 of this Thesis will synthesise findings from diurnal studies via a meta-analysis, to determine whether postprandial glucose and insulin responses are indeed higher during the night compared to the day.

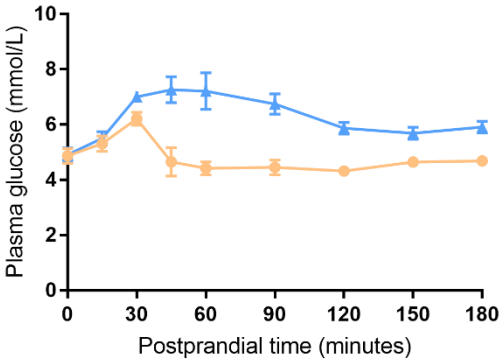

Figure 1-1a. Postprandial glucose iAUC after a meal consumed at midnight (▲) is 9 times higher compared to a meal consumed at 0800h (●) (median (IQR): 252.75 (84.80) vs. 27.90 (40.98) mmol/l.3h; p= 0.021).

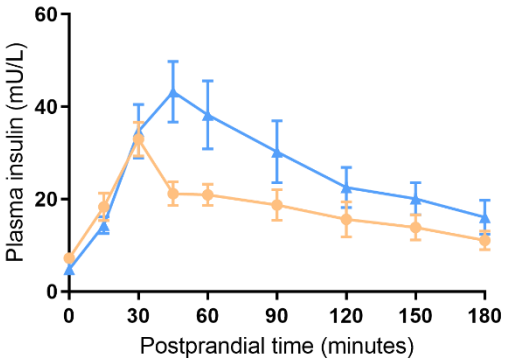

Figure 1-1b. Postprandial insulin iAUC after a meal consumed at midnight (▲) is doubled, compared to a meal consumed at 0800h (●) (median (IQR): 2945.1 (3665.2) vs. 1404.4 (885.2) mU/l.3h; p= 0.008).

Figures adapted from diurnal study by Leung et al, n= 9 (32). *Abbreviation: iAUC, incremental area under the curve.*

Literature reviews previously conducted by our research group have also suggested a time of day difference in postprandial TAG response and thermic effect of food (TEF) (39, 40). Firstly, in a systematic literature review, we identified five diurnal studies comparing postprandial TAG response between day (0700h to 1600h) and night (2000h to 0400h) (39). A meta-analysis was precluded, due to the small number of studies identified and inconsistent reporting of postprandial TAG outcome measures amongst studies (i.e. reporting either area under the curve, peak concentration or time-course kinetics). However, a narrative analysis showed that the majority of included studies reported a time of day difference in at least one of the TAG outcome measures examined. Mechanistic studies have shown that this observation may be attributed to the circadian variation of lipid absorption in the gastrointestinal tract, lipid synthesis in the liver and rate of uptake by white adipose tissues (41). As such, we concluded that increased postprandial TAG response at night may also contribute to the association between night time eating and increased risk of metabolic diseases. However, future diurnal studies with more standardised protocols and outcome reporting are required to confirm this finding.

Secondly, in a narrative review, we identified a small number of studies that suggest reduced TEF during the night compared to the day (40). TEF is the amount of energy expended after a meal, to digest and absorb the nutrient load, and represents 10% of total energy expenditure (42). In a study including 13 adults, TEF was reported to be 44% lower at 2000h compared to 0800h (43). These findings were supported by Bo et al's study comparing the same clock times (n= 20), which indicated a 380kJ difference between day and night (44). In a more recent study involving 16 healthy weight

men, Richter et al examined TEF after identical meals consumed in the morning (0900h) and at night (1900h), in a high caloric (4171 kJ) and a low caloric meal (1046 kJ) condition (45). Findings indicate that TEF at night was half of that observed in the morning, regardless of the energy content of the test meal. These studies suggest that our body is less efficient at burning energy from food intake during the night compared to the day. Although the difference seems to be trivial (380 kJ difference suggested by Bo et al (44)), it may contribute to weight gain in those who consistently eat during the night, such as night shift workers.

Findings from the above diurnal studies indicate eating during the night leads to acute metabolic disturbances, such as reduced glucose tolerance, insulin sensitivity and TEF, as well as increased postprandial TAG response. If sustained, these are likely contributing factors towards the increased risk of hyperglycaemia, hyperlipidaemia and obesity, observed in those who habitually eat during the night. These physiological responses to night time eating can be attributed to the regulation of the circadian clock system, which will be explored in the next section.

1.2 Circadian rhythms

The circadian clock system is aligned with the external light/dark cycle (i.e. solar day) to coordinate the timing of physiological, metabolic and behavioural processes, ensuring that they occur at the most appropriate time of the 24-hour day (1). This is achieved by organising body processes to follow *circadian rhythms*, i.e. a cycle of peaks and troughs repeated approximately every 24 hours (2). Any biological rhythms that follow such cyclic process within a 24-hour period is classified as a *diurnal rhythm*. Circadian rhythm is distinct in that it is endogenously generated by an organism's circadian clock system and is maintained even in the absence of any external cues. Whereas the expression of diurnal rhythms represents the composite effects of the circadian clock system, the external light/dark and behavioural cycles (i.e. feed/fast, sleep/wake, activity/rest) (2).

1.2.1 Architecture of the circadian clock system

The human circadian clock system has two main structural components: 1) the master clock, which is the suprachiasmatic nucleus (SCN) situated in the hypothalamus of our brain and 2) peripheral clocks, which are distributed across almost every peripheral tissue, including heart, muscles and the gastrointestinal tract (Figure 1-2). The *phase* (i.e. timing) of a circadian rhythm is set, and can be shifted, by external factors known as *zeitgebers*. The primary zeitgeber of the master clock is light from the external environment (predominantly sunlight exposure); received from the retina via direct photic input (46). Through this, the master clock is able to determine whether it is day time or night time in the external environment. The master clock then transmits this information to peripheral

clocks via neural and hormonal pathways, to ensure that the phase of all body clocks are in synchrony. The objective of this is to time behavioural and metabolic rhythms to the external light/dark cycle. In the condition where behaviour cycles are timed correctly to the signals from the master clock, and hence the light/dark cycle, circadian alignment is achieved (Figure 1-2). Having temporal separation of behaviours and their corresponding physiological pathways is a survival advantage (1). For example, nutrient digestion, absorption and storage occur at times of food intake; whilst nutrient stores are broken down to maintain homeostasis during periods of fasting. By timing food intake to the day and fasting to the night, these opposing physiological pathways will therefore occur at separate times, thereby optimising metabolic efficiency.

In addition to signals received from the master clock, behavioural factors including timing of food intake, sleep and physical activity also act as zeitgebers for the peripheral clocks. Emerging research has suggested that timing of food intake is a key zeitgeber and is able to shift the phase of peripheral clocks. The phases of the peripheral and master clocks can become misaligned, when their respective zeitgebers are out-of-sync (2), leading to disruptions in metabolism. In the general population, habitually eating late into the night is a form of such circadian misalignment. In the shift working population, circadian misalignment often occurs between multiple behavioural cycles and the master clock (Figure 1-2).

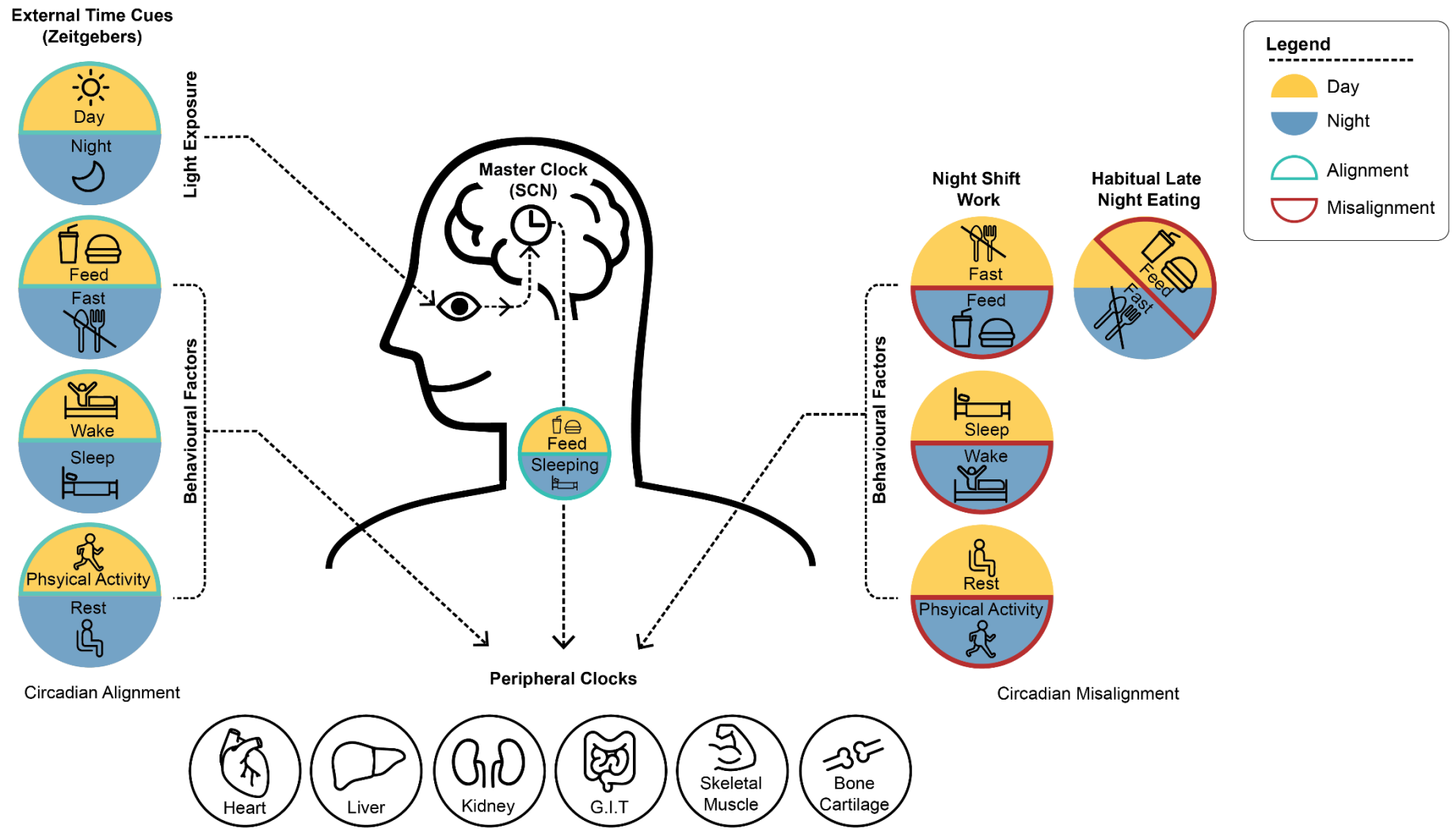

Figure 1-2. Circadian clock system of the human body.
The master clock receives light signal from the external environment, synchronising the peripheral clocks. As humans are diurnal animals, feeding and activity is preferred during the day, while night is reserved for sleep and fast. The diagram also illustrates scenarios where behavioural factors are aligned and misaligned with the signals of the master clock.

1.2.2 Circadian rhythms of metabolites and energy expenditure

Under conditions of circadian alignment, the master clock times food intake to occur during the day and a period of fasting to occur during the night. This is reflected in the circadian rhythms of plasma glucose and TAG, which naturally rise during the night, to maintain homeostasis in an anticipated period of fasting (1, 7). Circadian rhythms of metabolites can be measured through experimental protocols that eliminate external time cues, such as the constant routine, forced desynchrony and circadian alignment/misalignment studies. Briefly, in constant routine studies, external time cues are kept constant, whereby participants maintain constant wakefulness and are provided with evenly spaced iso-caloric snacks, under a dim-light environment for up to 40 hours (7). In a forced desynchrony study, participants undergo a series of new 'days' that are slightly shorter or longer than 24-hours. This causes a dissociation between behavioural rhythms and endogenous circadian rhythms. In a circadian alignment/ misalignment study, participants undergo each condition for two to four days (7). With both the forced desynchrony and the circadian alignment/misalignment studies, the effects of the circadian and behavioural rhythms are then separated using mathematical modelling (7).

Circadian rhythm of plasma glucose has been identified in a forced desynchrony study by Owens et al, who reported postprandial glucose response at 2000h to be 2.7 times higher than that at 0800h (47). Comparable findings were reported in circadian alignment/ misalignment and constant routine studies, which demonstrated an endogenous circadian rhythm in plasma glucose that peaks between the night hours of 2200h and 0600h (48-50). Similarly, circadian rhythmicity has been observed in plasma lipids (48), but the times of peaks and troughs are less consistent amongst published studies. A constant routine study Chua et al reported that majority of glycerolipids (TAGs and diglycerides) peak in the late night and early morning, while sphingolipids mostly peak in the afternoon and evening (51). Whilst a study by Dallman et al showed that the circadian rhythm of free fatty acids exhibits peaks between mid-morning and noon (52).

Lastly, a small number of studies have also indicated circadian rhythmicity in energy expenditure. Constant routine studies have reported circadian rhythm in resting metabolic rate (RMR), with a lull during the night (0000h to 0600h) and a peak in the morning (0900h to 1200h) (53, 54). Although the constant routine protocol is one of the recognised methodologies to measure circadian rhythms, the standard procedure for RMR measurement presents a unique situation. Participants are required to fast for at least six hours prior to RMR measurement (55), which contradicts with the constant routine protocol of hourly or bi-hourly snacks. Forced desynchrony study is therefore the preferred methodology. Using such protocol, Zitting et al has previously identified circadian rhythm in RMR, with

a lull between 0100h to 0500h (56), consistent with findings from constant routine studies. Collectively, these experimental trials indicate that the human body expends less energy during the night.

Taken together, these experimental trials indicate that plasma glucose and lipids are endogenously regulated by the circadian clock system. A natural rise in plasma glucose and TAG occurs during the night, while lipid species may peak at different times, most occur during the dark hours of the 24-hour day. Energy expenditure also dips during the night, indicating that the human body is burning less energy, independent of sleep and physical activity. It has been speculated that catabolic pathways are favoured during the night, such as the breakdown of glycogen into glucose, releasing glucose into the bloodstream to maintain homeostasis during this anticipated fast period (57). Therefore, when a nutrient load is consumed during this time, it adds to the natural rise of glucose and TAG in the bloodstream, leading to acute metabolic disturbances. Over time, it is likely to contribute to the development of metabolic diseases, as hyperglycaemia and hyperlipidaemia are independent risk factors for T2DM and CVD (36-38, 58). Furthermore, habitual food intake during the night, when energy expenditure is downregulated, is a probable causal factor to steady weight gain. These physiological responses may help explain the increased risk of metabolic diseases observed in night shift workers, a population who is likely to eat during the night.

1.3 Increased risk of metabolic diseases observed in shift workers

Shift workers is a hallmark population that experiences circadian misalignment; in that, they consistently work and eat during the night, whilst sleeping and fasting usually occur during the day (22). With multiple behavioural cycles being in constant and near complete misalignment with the signals of the master clock (Figure 1-2), shift workers are speculated to face a marked increase in metabolic disease risks, compared to individuals who habitually eat during the night and experience circadian disruption in only the feed/fast cycle (22). The associations between shift work and metabolic diseases have been recognised from as early as the 1980s. A 15-year cohort study published in 1986 in *The Lancet* showed that workers who have been engaged in rotating shifts for 11 to 15 years experienced a two-fold increased risk of ischaemic heart disease compared to day workers (18). This finding is supported by a 6-year cohort study, which reported that shift workers (permanent night and rotating) are at a 38% increased risk of developing coronary heart disease compared to day workers, adjusting for age, BMI, total cholesterol and systolic blood pressure (20). The association between shift work and CVD has since been systematically assessed in a meta-analysis including 34 cohort and case-control studies, with a total of 2,011,935 participants (21). Compared to day work, shift work was associated with an increased risk of myocardial infarction (risk ratio= 1.23) and coronary events (risk ratio= 1.24).

Shift work has also been identified as a risk factor for T2DM. In a 15-year cohort study of Danish female nurses (n= 19,873), both evening and night shifts were associated with increased risk of T2DM when compared to day shift, with hazard ratios of 1.29 and 1.58 for the two shift types respectively (59). The associations were independent of age, BMI, fruit and vegetable intake, hypertension and previous diagnosis of myocardial infarction. Similar findings were reported in a cross-sectional study of male rotating shift workers in Belgium (60). These associations were confirmed by a recent meta-analysis of 12 cohort studies, reporting a 14% increased risk of T2DM in shift workers compared to day workers (61). Subgroup analyses of studies with rotating shift workers (n= 10 studies) and evening or night shift workers (n= 7 studies) reported comparable findings.

Lastly, shift work has been associated with risk of overweight and obesity. In a cross-sectional study comparing permanent night workers (n= 800) and day workers (n= 406), shift work was associated with an increase in prevalence of overweight and abdominal obesity, with prevalence ratio of 1.27 and 1.45 respectively (62). Weight gain has been observed in a 14-year retrospective cohort study of rotating shift workers (n= 7254), with an odds ratio of 1.13 for a BMI increase of ≥10% in shift workers compared to day workers (63). In a recent meta-analysis, Sun et al substantiated the increased risk of overweight and obesity in shift workers, by pooling findings from both cross-sectional (n= 22; OR= 1.26) and cohort studies (n= 6; OR= 1.10) (19). Subgroup analyses revealed highest odds for abdominal obesity (n= 9 studies, OR= 1.35) and BMI ≥ 25 kg/m^2 (n= 14, OR= 1.32), amongst all different assessment criteria for overweight and obesity.

The above epidemiological evidence including large samples demonstrates that shift work, both permanent night and rotating schedule, is associated with increased risks of CVD, T2DM and obesity. These associations are independent of traditional risk factors such as age, gender and BMI. As such, the opposing sleep/wake pattern that night shift workers adopt is a major contributing factor to their increased disease risk (22). In addition, this population also typically eats during and throughout the night. This form of circadian misalignment is likely to play a significant role in the onset and progression of metabolic diseases.

1.4 Night shift workers' dietary patterns

Current evidence suggests that shift workers' increased risk of metabolic diseases is not associated with their energy intake. A meta-analysis conducted by our research group, including 10,367 day workers and 4726 shift workers from 12 cross-sectional studies, have shown that shift workers' overall energy intake does not differ to their day-working counterparts (64). However, night shift workers' timing of energy intake seems to be different to that of day workers. A Brazilian study of 66 garbage collectors reported that those who work night shift consumed 38% of their daily energy intake during

the night (2000h – 0459h), whilst those who work morning shift consumed 26% of their daily energy intake during the same period (65). Such redistribution of daily energy intake is also evident in rotating shift workers. A cross-section study of UK female nurses (n= 20) showed that on days where participants worked night shifts, 30% of daily energy intake occurred between 2100h and 0700h, whilst only 8% of daily energy intake occurred during this time period on non-night shift days (66). Similar results were reported in Canadian police officers (n= 31); whereby 30% of daily energy intake occurred between 2300h and 0600h on night shift days, compared to 1% on days with morning shifts (67).

Multiple cross-sectional studies have also shown that energy consumption during night shifts is typically characterised by frequent snacking of discretionary food items (68-70). In two separate studies of Australian fire fighters and Brazilian garbage collectors, it was reported that percentage of daily energy from sugar was higher during the 24-hour period that included a night shift, compared to day shift (68, 69). Within the Brazilian garbage collectors, a large proportion of this was attributed to the consumption of sugar-sweetened beverages (69). In comparison, Australian fire fighters reported consuming more sweet snacks, such as chocolate, ice cream and sweet pastries, on night shifts compared to morning shifts (68). These eating habits may also promote a higher daily fat intake. A cross-sectional study involving Australian shift workers (n= 118) from multiple industries reported that whilst all types of shift workers (permanent morning, permanent night, rotating) had a saturated fat intake higher than the national recommendations (< 10% daily energy) (71), it was highest amongst permanent night shift workers (72). This was consistent with findings from a Brazilian study involving 150 male bus drivers, which showed that a higher proportion of night bus drivers exceeded the recommended daily dietary oil intake, compared to day bus drivers (73).

Evidently, night shift workers' dietary patterns are different to that of day workers, in that a significant proportion of their energy intake is redistributed to the night, and is likely to include unhealthy food choices. Considering that shift workers' overall energy intake is similar to that of day workers (64), meal timing is a potential mediating factor between shift work and increased risk of metabolic diseases. As such, avoidance or reduction of energy intake during the night may elicit metabolic benefits in night shift workers.

1.5 Manipulating meal timing to improve metabolic health

Based on experimental studies summarised above, several metabolic benefits of overnight food avoidance can be inferred. Firstly, reducing eating occasions during the night can minimise frequent spikes in postprandial glucose and TAG levels, thereby reducing risk of T2DM and CVD. Secondly, reducing energy intake at a time when our body is less efficient at burning energy may also promote

weight maintenance. These hypotheses are supported by existing data from animal and human experimental trials, which have examined the effect of meal timing manipulation on metabolic risk factors.

Evidence from mice studies have shown that those who are fed during their 'biological day' (i.e. night time in mice) experience less weight gain compared to those fed during their biological night (74-76). At the end of a 4-week intervention, mice who were fed during their biological night gained 17 ± 2.8% more weight compared to the control group (fed ad libitum), while those fed during their biological day had similar weight gain as the control group (76). This is consistent with findings from a 6-week intervention by Arble et al, who in addition, reported similar energy intake and physical activity levels between the two groups of mice, emphasising that the difference in weight gain occurred independently of energy intake and output (74). Animal studies have suggested that the benefits of eating at the 'right time' extend beyond body weight outcomes. Mice who were fed during their biological night for four weeks had higher glucose responses at 15 minutes and 30 minutes postprandially in an intraperitoneal glucose tolerance test (IGTT), compared to those fed during their biological day (77). Similar findings were reported in study by Chaix et al, whereby mice with restricted intake to 9-hours of their biological day had lower postprandial glucose response to an IGTT, compared to mice who were fed ad-libitum during the 10-week intervention (78). Lower weight gain was also observed in the group with restricted feeding to their biological day, independent of overall energy intake.

Only a few human experimental studies have examined the effect of meal time manipulation on metabolic health, most of which were incorporated into energy restricted diets for weight loss. In a 12-week weight loss intervention, overweight and obese women (n= 74) were randomised into the 'breakfast' group consuming majority of energy in the morning (% daily energy for breakfast = 50%, lunch = 36%, dinner = 14%) or the 'dinner' group consuming majority of daily energy at night (% daily energy for breakfast and dinner reversed) (79). Although body weight was reduced in both groups of women, weight loss was 2.5 fold greater in the 'breakfast' group compared to the 'dinner' group (mean ± SE: –8.7 ± 1.4 vs. –3.6 ± 1.5 kg). Furthermore, mean fasting TAG concentration was reduced by 60.3 mg/dl in the 'breakfast' group, but increased by 26.0 m/dl in the 'dinner' group. Compared to the 'dinner' group, the 'breakfast' group also had a greater reduction in fasting glucose, insulin and HOMA-IR levels. Similar findings were reported in another 12-week weight loss study including overweight and obese women (n= 36), whereby those who consumed majority of daily energy (70%) at or before lunch had greater reduction in body weight and HOMA-IR, compared to the control group (55% daily energy at or before lunch) (80). Lastly, a large weight loss trial based on the Mediterranean diet, involving 2119 participants, showed that early eaters (midpoint between breakfast and dinner

time before 1454h) lost 80g more body weight per week than later eaters (midpoint after 1454h) (30). Although this may seem minor, it accounted for 1.5kg more weight loss at the end of the 5-month intervention.

In summary, emerging evidence from animal studies suggests that manipulating meal timing to prioritise energy intake to the biological day induces metabolic benefits. Although this has been observed in a few human experimental trials combined with energy restriction, improvements in metabolic health independent of energy restriction and weight loss have not been shown. The independent effect of meal timing manipulation on metabolic health is important to ascertain, as a simple recommendation to minimise food intake overnight offers an easily translatable public health message. However, none of the previous experimental studies was conducted in night shift workers. This population faces a heightened risk of metabolic diseases compared to their day-working counterparts, as a result of the circadian disruption that they experience (Section 1.3). To date, only one study has examined the potential effect of meal timing manipulation in a night shift setting; however, this was a short term simulated night shift study conducted in the laboratory (81). After four simulated night shifts, participants (n= 4) who ate at 0130h each night showed an increased glucose response to a meal challenge test, compared to baseline (i.e. before night shifts). While those (n= 7) who fasted between 1900h and 0700h each night, by redistributing energy intake to the day period, showed no changes in glucose tolerance to the meal challenge test. Currently unknown, is whether this acute response can be translated to long term benefits in free-living night shift workers. This is the primary aim of the 'Shifting the Risk' pilot randomised crossover trial (Chapter 3a); to examine whether a short period of fasting at night is able to improve CVD risk markers (postprandial TAG and glucose response), in at-risk night shift workers (i.e. with abdominal obesity). The intervention will be implemented for four weeks, whereby participants' energy intake will be redistributed to create an overnight fasting period between 0100h to 0600h. Contrary to the simulated night shift study aforementioned, a shorter fasting period has been chosen, to optimise feasibility of implementation by participants. If metabolic benefit is observed from this intervention, it can be translated to a meal-timing recommendation specific for the shift working population.

1.6 Tailored dietary guidance is needed for shift workers

There has been growing interests in the general public regarding shift workers' health, whilst research in this area is rapidly emerging. Concurrently, additional dietary recommendations targeted at shift workers have been disseminated via the internet, by various government bodies, independent health organisations and medical journals. However, the majority of recommendations are still focused on food choices to combat fatigue and gastrointestinal symptoms during work, and to improve sleep after

work, rather than prevention of metabolic diseases. Common recommendations include eating a light meal that is low in fat during night shift to avoid heartburn and indigestion, and eating a small meal before daytime sleep to avoid waking up from hunger (82-86). Limiting caffeine intake during night shift to improve sleep is a recommendation suggested by all organisations.

Recognising the metabolic disturbances of night time eating, recommendations published by a few medical journals have suggested avoidance of snacking during night shifts (87, 88). They suggest that during night shifts, workers should avoid high sugar and processed foods, and instead choose low energy, protein-rich snacks. In order to achieve these eating habits, shift workers are encouraged to prepare home-cooked meals to bring to work, as well as having a ready supply of healthy snacks at work (88). While the majority of recommendations described align with national healthy eating guidelines, they are largely based on expert opinion rather than evidence from research studies. Moreover, the factors that shape unhealthy eating habits such as excessive intake of caffeine and processed foods have not been considered. It is also unknown whether workers have the skills and ability to build healthy eating practices; and whether their workplace provides food preparation facilities and an eating environment that support healthy eating behaviours.

1.7 Lack of dietary interventions targeting night shift workers

To date, only a limited number of dietary interventions have specifically involved night shift workers. In a 2014 review of interventions that aim to prevent chronic diseases in shift workers, only one of the 38 interventions identified included a nutrition-related component (89). All other identified studies examined effects of controlled light exposure, changes in shift rotations or pharmacological therapies, with the majority aiming to improve sleep outcomes. A more recent systematic review published in 2018 focused on group-based workplace interventions that aim to improve physical activity, eating habits or weight loss in shift workers (90). Amongst the 22 studies identified, only two targeted eating habits specifically, with 12 others having a combined focus on nutrition and physical activity. The majority of nutrition interventions were based solely on the delivery of nutrition education on general healthy eating principles, with no interventions specifically targeting meal timing. Moreover, only three interventions involved modifications to the physical workplace environment, two of which were incentives-based. Closer examination of the included studies also revealed that some studies included occupations that typically involve shift work, but did not specifically recruit night shift workers (90). The population group of night shift workers was specifically investigated in a literature review recently published by Phoi et al (91). However, amongst the five dietary interventions identified, three were acute studies that tested the effects of meals with varying nutrient composition on cognitive function and postprandial metabolic responses, during single night shifts. Whilst the two remaining

interventions were again, nutrition education programs based on general healthy eating principles, which aimed to improve body weight status and dietary intake. This substantiates the uniqueness of the 'Shifting the Risk' pilot study, as it will be the first dietary intervention to examine the effect of meal timing manipulation on long-term health outcomes of night shift workers. Due to its novelty, it is unknown whether night shift workers would engage in and be able to implement such a meal timing intervention. These feasibility aspects of the study protocol will be explored in Chapter 3b, which will provide important areas of consideration for future dietary interventions that involve this population group.

1.8 Influencing factors of night shift workers' eating habits

Experimental evidence discussed in Section 1.1.2 and 1.5 have demonstrated the impact of meal timing on metabolic responses, in particular the habit of night time eating. In addition to meal timing, shift workers' food choices during their night shifts are also likely contributors to metabolic disease progression. As indicated in Section 1.4, frequent snacking, particularly of discretionary foods, is common during night shifts. Australian fire fighters have expressed that this eating habit is driven by the lack of food options after completing extended night calls (68). Whilst in nurses, "*The three C's*" (Coke, Chocolate, Coffee/ Chips) are commonly consumed during night shifts, as means to stay awake (92, 93), relieve stress (70) or as a reward for working night shift (84). Findings from these studies suggest that night shift workers' workload and work responsibilities influence their food choices whilst on night shift.

A qualitative study involving 109 Irish shift workers across multiple industries identified the workplace environment as one of the major barriers for workers to maintain healthy eating practices (23). This has also been reported in the few Australian studies published, which qualitatively explored factors that influence night shift workers' food choices and eating habits. However, each of these were focused on a discrete occupation, therefore the factors identified were specific to the workplace environment and structure of the occupation (68, 93-95). For example, the ambulance environment has been reported to limit paramedics' food choices to those that were convenient, non-perishable and required minimal preparation (94). Whilst nurses often reported interruptions in meal breaks to attend to their patients, which led to overeating when they had the opportunity to take a short break (93). It is currently unknown whether these workplace barriers are occupation specific or are generalisable to the Australian night shift worker population. It will be valuable to explore the factors that influence the food choices of night shift workers from a range of occupations, as it will provide insights into common barriers experienced by this population, which could inform broader health promotion strategies with a wider reach.

The majority of qualitative studies that explore influences of night shift workers' food choices and eating habits focus on their experiences whilst at work (94, 96). However, there has been some indications that night shift work may affect workers' eating habits in their daily lives. Irish healthcare shift workers have described opting for convenience foods following a night shift, as they were too tired to prepare a meal (23). Australian fire fighters have reported a lack of time to prepare food to take to their night shifts, as they had other family or work commitments during the day (68). To our knowledge, there has been no qualitative studies that specifically explored night shift workers' eating habits outside of work, as well as during work. It is important to determine whether night shift work has a lingering impact on workers' eating habits in their daily lives. If some of the unhealthy eating habits observed during night shift continue into their rostered days-off, this indicates that dietary recommendations and health promotion strategies for this population need to extend beyond the focus on habits at work.

Acknowledging the gaps in the literature identified above, Chapter 4 of this Thesis presents a qualitative study, exploring Australian night shift workers' experience with food and eating, both at work and outside of work. It will provide insight into common drivers of their food choices and eating habits, thereby informing health promotion strategies that can protect the metabolic health of this essential workforce.

1.9 Thesis overview

1.9.1 Thesis aim and objectives

The overall aim of this Thesis is:

To identify strategies to mitigate the metabolic impact of eating at night experienced by night shift workers.

A combination of quantitative and qualitative research methods will be used in this Thesis. Quantitative investigation will be used to assess the metabolic effects of meal timing, in particular night time eating. Qualitative exploration will be used to understand the complex shift work context, thereby informing ways in which nutrition interventions and health promotion strategies can be optimised to improve the metabolic health of this population.

Specifically, the objectives of this Thesis are:

- To understand the glycaemic response to food at night (Chapter 2).
- To examine the effect of a short period of overnight fasting on the metabolic risk factors of free-living night shift workers (Chapter 3a).
- To explore key determinants to successful implementation of a meal-timing intervention in free-living night shift workers (Chapter 3b).
- To determine factors that influence Australian night shift workers' food choices and eating habits (Chapter 4).

1.9.2 Thesis structure

This Thesis comprises five chapters. Chapter 2 is a systematic literature review and meta-analysis, synthesising findings from diurnal studies, to investigate the difference in postprandial glucose and insulin response between day and night in healthy adults. If eating at night is confirmed to induce acute metabolic disturbances, it provides a potential physiological mechanism that partially explains the association between late night eating and increased risk of metabolic diseases.

Chapter 3 presents a pilot randomised crossover trial referred to as 'Shifting the Risk', which is the first intervention study to explore meal timing in free-living night shift workers. This study will be reported in two separate chapters. Chapter 3a will examine whether the intervention, i.e. a nightly fast between 0100h to 0600h for a 4-week period, is able to improve CVD risk markers in at-risk night shift workers. Chapter 3b will explore whether the target population will engage in and be able to implement the intervention, which are important feasibility aspects of the study protocol. This

exploration will infer key areas of consideration for the design of feasible dietary interventions for free-living night shift workers.

Chapter 4 is a qualitative study, which explores Australian night shift workers' experience with food and eating, both at work and outside of work. This study involves night shift workers across different occupations, and identifies common influences of their food choices and eating habits. This knowledge can inform aspects that need to be considered in the design of practical nutrition interventions and effective health promotion strategies for this population.

Chapter 5 is the final chapter of this Thesis. It will summarise key research findings from each chapter, which will then be considered collectively, in a discussion of future research directions and health promotion strategies specifically targeted at night shift workers.

References cited in the Thesis Introduction, Preambles and Thesis Discussion chapters are included in the reference list at the end of the Thesis. References cited in the studies are listed within their corresponding chapters, and have not been duplicated in the reference list. This format was taken for consistency, as some of the studies have been published or submitted for publication.

Chapter 2 . Time of day difference in postprandial glucose and insulin responses: systematic review and meta-analysis of acute postprandial studies

Preamble

Modern lifestyles have prompted individuals to eat later into the night, with an epidemiological study showing that approximately a quarter of Australian adults have a 'grazing' eating pattern, characterised by frequent eating occasions throughout the day and after 2000h (4). This is consistent with data from the U.K., reporting that adults consume approximately 40% of their daily energy intake during dinner (27). Observational studies have shown that eating during the night is associated with increased risk of hyperglycaemia, hyperlipidaemia and obesity (9, 28, 29). Metabolic disease risks are further exaggerated in shift workers, who habitually eat during the night, with observational studies reporting that their risk of CVD, T2DM and obesity are 20 to 40% higher than that of their day-working counterparts.

It is therefore important to determine the physiological responses that mediate the relationship between food intake during the night and increased metabolic disease risks, to support the development of nutrition interventions that will improve the metabolic health of those who frequently eat during the night. A number of diurnal studies (referred to as 'acute postprandial studies' in this Chapter) have suggested the increase in postprandial glucose and insulin responses as potential contributing factors (32, 97, 98). However, conflicting results have been reported, likely to be due to variation in protocol. The meta-analysis reported in this Chapter is the first to quantitatively synthesise findings from diurnal studies, to examine the time of day difference in postprandial glucose and insulin response to identical meals consumed during the night (2000h to 0400h) compared to the day (0700h to 1600h). If night time eating is confirmed to induce an exaggerated postprandial glucose and insulin response, it will suggest a physiological response that modulates, in part, the association between night time eating and increased metabolic disease risks. This will also indicate meal timing as a modifiable risk factor for T2DM and CVD.

This Chapter has been published in *Chronobiology International* in 2020:

Leung GKW, Huggins CE, Ware RS, Bonham MP. Time of day difference in postprandial glucose and insulin responses: systematic review and meta-analysis of acute postprandial studies. *Chronobiology International*. 2020;37(3):311-326. Doi: 10.1080/07420528.2019.1683856.

Published manuscript available in Appendix A.

2.1 Introduction

In Westernised countries, there is an observed trend of humans consuming more energy during the latter hours of the 24-hour day. Surveys from the United Kingdom and Germany report that dinner accounts for ~40% of daily energy intake, while breakfast only accounts for half of that observed during dinner (1). This habitual night-time eating behaviour is in conflict with metabolic activities that follow a circadian rhythm. As a consequence, nutrient metabolism is inefficient during this time, which may result in increased risks of obesity and metabolic syndrome (2).

The circadian clock system, or 'body clock', receives light cues from the external environment (i.e. sunlight) and informs our body whether it is day time (favouring activity and feeding) or night time (favouring sleep and fasting) (3, 4). Experimental studies that remove the influence of daylight, bouts of sleep and nutrient loads are used to examine endogenous (natural) rhythms (5). These 'constant routine' studies show that endogenous rhythms promote a steady increase in blood glucose and insulin concentration through the 24-hour day and peak during the biological dark hours (6, 7).

Acute postprandial studies have examined metabolic responses to larger nutrient loads at different times of the day, in free-living individuals whose circadian rhythms are synchronised with the external light cues. This was firstly conducted by Bowen et al. in the 1960s, who reported a higher glucose response in the afternoon compared to the same meal consumed in the morning (8). In recent years, other postprandial studies have reported similar findings with higher postprandial glucose response during the night compared to the day, however results have been conflicting for insulin (9-12). Prolonged postprandial hyperglycaemia and reduced insulin sensitivity are both independent risk factors for T2DM and CVD (13-16). As a consequence, it is important to understand the impact of eating at night on metabolic responses, as this may be a modifiable risk factor.

This systematic literature review and meta-analysis aims to synthesise results from acute postprandial studies, and determine whether the postprandial plasma glucose and insulin concentration differ between daytime (0700h – 1600h) and night time (2000h – 0400h) in healthy adults. If night time eating is confirmed to be associated with dysregulated glucose metabolism, it will justify the importance to consider meal timing, when designing dietary interventions or recommendations to protect the metabolic health of those who habitually eat at night.

2.2 Methods

This review is reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) Statement (17).

2.2.1 Search strategy

A systematic search was conducted in February 2018, to identify articles investigating the impact of night time eating (food intake after 2000h) on postprandial glucose and insulin concentration in healthy participants. Electronic databases searched were CINAHL plus, Cochrane Library, EMBASE, Ovid MEDLINE, Informit, Psych Info and Scopus. The search was limited to English language articles and human studies only. An example of the full search strategy from Ovid MEDLINE is presented in Appendix C.

A keyword search was conducted by combining three sets of search terms with 'and' (Table 2-1). Truncations appropriate for each database were used. A subject term search was also conducted where available, using the terms listed in (Table 2-1).

All titles and abstracts retrieved from the literature search were screened for eligibility by two authors independently. Full-texts of accepted abstracts were reviewed independently by authors GKWL and MPB. Any discrepancies were discussed with author CEH before a final decision on inclusion was made.

Table 2-1. Systematic literature review search terms.

SET 1		SET 2		SET 3
Night Nighttime "night time" Nocturnal Diurnal Evening "night work" Shiftwork "shift worker" Circadian rhythm	**AND**	Food "Meal" Diet* Eat* "Nutrient" Energy Energy intake Feed* Snack* Nutrition "beverage" Drink* glucose	**AND**	Postprandial "post prandial" Postmeal "post meal" "tolerance test" "postprandial period"

2.2.2 Selection criteria

The PICOS (Participants, Intervention, Comparator, Outcomes, Study Design) criteria for study selection are presented in Table 2-2. Briefly, acute postprandial studies where the same participant consumed a test meal during the day (0700h to 1600h) and during the night (2000h to 0400h) were included. The limits of 1600h and 0400h were chosen, as these were the latest times that participants could be given a meal, before the three-hour postprandial period would become 'night' or 'day' respectively. 'Test meal' was defined as macronutrient administered orally in any form, i.e. as mixed-meal or single macronutrient load. Studies that administered nutrients intravenously were excluded. Test meals were identical and isocaloric, and preceded by a three-hour fasting period (minimum), which is typically long enough for glucose and/or insulin to return to basal levels. Healthy adult participants were included. Studies that reported participants to be 'healthy', but did not explicitly state exclusion of participants based on BMI or conditions listed as exclusion criteria in Table 2-2 were included.

The primary outcome measures were postprandial glucose and insulin area under the curve (AUC) or incremental area under the curve (iAUC). Studies were excluded if postprandial blood samples were collected hourly or bihourly; as AUC and iAUC could not be calculated using infrequent measurements. Studies that did not provide numerical data nor an overall conclusion regarding the effect of time of day on the primary outcome measures (after contacting the authors on two occasions) were excluded.

Table 2-2. PICOS criteria for inclusion and exclusion of studies.

Parameter	Inclusion criteria	Exclusion criteria
Participants	▪ Age: 18 to 50 years ▪ BMI: 18.5 to 30 kg/m^2 ▪ 'Healthy' participants	▪ Had conditions that affected glucose or lipid metabolism (impaired glucose tolerance, impaired fasting glucose, T2DM, CVD or metabolic syndrome). ▪ Used oral hypoglycaemic agents, insulin and/or lipid lowering medication.
Intervention	▪ A meal during the day (0700h – 1600h) – preceded by a 3-hour fast (minimum)	
Comparators	▪ A meal during the night (2000h – 0400h) – preceded by a 3-hour fast (minimum) ▪ Energy and macronutrient composition identical to day meal	
Outcomes	▪ Postprandial glucose AUC or iAUC ▪ Postprandial insulin AUC or iAUC	▪ Did not report numerical value and overall conclusion on primary outcome measures
Study design	▪ Acute postprandial studies ▪ Each participant completed both the day and night intervention	▪ Postprandial blood samples were collected hourly or bihourly

Abbreviations: AUC, area under the curve; BMI, Body Mass Index; CVD, cardiovascular disease; iAUC, incremental area under the curve; T2DM, type-2 diabetes.

2.2.3 Data extraction

A data extraction table was developed by author GKWL, based on a data extraction tool from the Dietitians Association of Australia (18). Data were extracted by two authors and included study design, location/setting, sample size, sample characteristics, intervention (pre-intervention fasting period, consumption time and macronutrient composition of test meal), blood collection protocol (time points and route) and data on the primary outcome measures (postprandial glucose and insulin AUC or iAUC). Attempts were made to contact corresponding authors if numerical data on the primary outcome measures were omitted from the article. Authors of three studies dating from 1973 to 1979 could not be contacted, as contact information were unavailable (19-21). Eight authors were contacted; three provided data, (22-24) four did not respond (25-28) and one responded but data were no longer available (29). The conclusions made by the authors of each article, regarding the effect of time of day on postprandial glucose and insulin responses were identified when available.

2.2.4 Quality assessment

Quality assessment of each study was completed by two authors independently, using the *American Dietetic Association (ADA) Quality Criteria Checklist: Primary Research* (30). This checklist includes four questions relating to the relevance of the study to the target population and 10 questions on validity of the study, which includes questions assessing bias.

Item two, which assesses participant selection bias, was awarded with a “yes” if health status of participants were sufficiently described. As all included studies had participants completing all intervention arms, it was assumed that study groups were comparable and item three was awarded with a “yes” for all studies. Item four was assessed as unclear, if it could not be determined whether all enrolled participants were accounted for; this formed a “no” when scoring the overall rating of the study quality. As primary outcome measures were determined from objective tests (i.e. plasma glucose and insulin concentrations), it was assumed that data collectors were blinded for outcome assessment and item five was awarded with a “yes” for all included studies.

Each study was designated with an overall rating of positive, neutral or negative. To be designated with a positive rating, the study must be free from selection bias; have comparable groups; describe the intervention in sufficient detail; and clearly define outcomes that were obtained from valid and reliable measurements. Studies awarded with positive ratings indicate that they have high validity. Studies that were designated with a negative rating were not included in the synthesis of results.

2.2.5 Meta-analysis

A meta-analysis was conducted for each of the primary outcome measures (postprandial glucose AUC/iAUC and insulin AUC/iAUC). Some studies included multiple time points, however only one day versus night comparison was selected for the meta-analysis. The first time point that occurred within the specified day-time bracket in the eligibility criteria (0700h – 1600h) was chosen, as this is typically the first meal after waking. The night time point chosen was 11 to 12 hours apart from the day time point. Primary outcome measures were reported using different units in included studies; hence, the standardised mean difference (SMD) and associated 95% confidence interval (CI) were reported.

Where a study included multiple intervention arms, each was included in the meta-analysis separately, given that each arm had different test meal compositions and was conducted on a separate day. The participants in the study by Gil-Lozano et al. (22) completed three protocols, each with different sleep conditions. Data chosen for meta-analysis were from sessions without sleep deprivation implemented. The first day time point included in the Holmback et al. studies were 0800h (23, 24). However, this time point was not chosen as it was preceded by different pre-test conditions compared to the night time comparison (i.e. longer fast and absence of standard pre-test meal). The next day time point (1200h), which had similar pre-test conditions as the night time point was chosen. The two studies by Morris et al (26, 27) exposed participants to a circadian alignment and a circadian misalignment condition and pooled data across the conditions for analysis. Raw data were not available from authors upon request; hence, this study was not included in the meta-analysis.

Heterogeneity was calculated using the I-squared (I^2) statistic. If I^2 was less than 50%, a fixed effects model was used, otherwise a random effects model was used. As participant level differences in glucose/insulin concentration between day and night were not provided by the included studies, when calculating within-participant differences, the correlation coefficient was estimated (31) using raw glucose data provided by Holmback et al (2002) (23) and raw insulin data provided by Holmback et al (2003) (24). The within-person correlation coefficient was estimated to be 0.4 and 0.3, for glucose and insulin respectively. These coefficients were used to calculate standard deviations of the difference between day and night for all included studies.

Publication bias was assessed by visual interpretation of funnel plots. Two types of sensitivity analyses were conducted. Firstly, the meta-analyses were re-run, excluding one study at a time, in order to determine whether the summary effect was overly influenced by a particular study. Secondly, several studies included a later time point that still fell within the day-time bracket specified in the eligibility criteria. Therefore we substituted the original day versus night comparisons with these (where available) as another form of sensitivity analysis. When testing the overall effect of time of day, a p-value of ≤ 0.05 was considered significant. Analyses were conducted using Stata statistical software (version 13.0, StataCorp, College Station, TX, USA).

2.2.6 Data synthesis

Studies that did not report numerical data on primary outcome measures could not be included in the meta-analyses. Instead, the conclusions made by each study's authors, on the effect of time of day on primary outcome measures, were summarised qualitatively. Participant characteristics and descriptions of the intervention are also presented for each study.

2.3 Results

2.3.1 Study selection

Electronic searches identified 7297 articles (Figure 2-1). Following removal of duplicates and screening of titles and abstracts, 172 articles remained, for which full text were retrieved and assessed for eligibility. Overall, 15 studies met the eligibility criteria and were included. Data from 10 studies were included in the meta-analysis. Thirteen comparisons were included in the glucose meta-analysis. Eleven comparisons were included in the insulin meta-analysis.

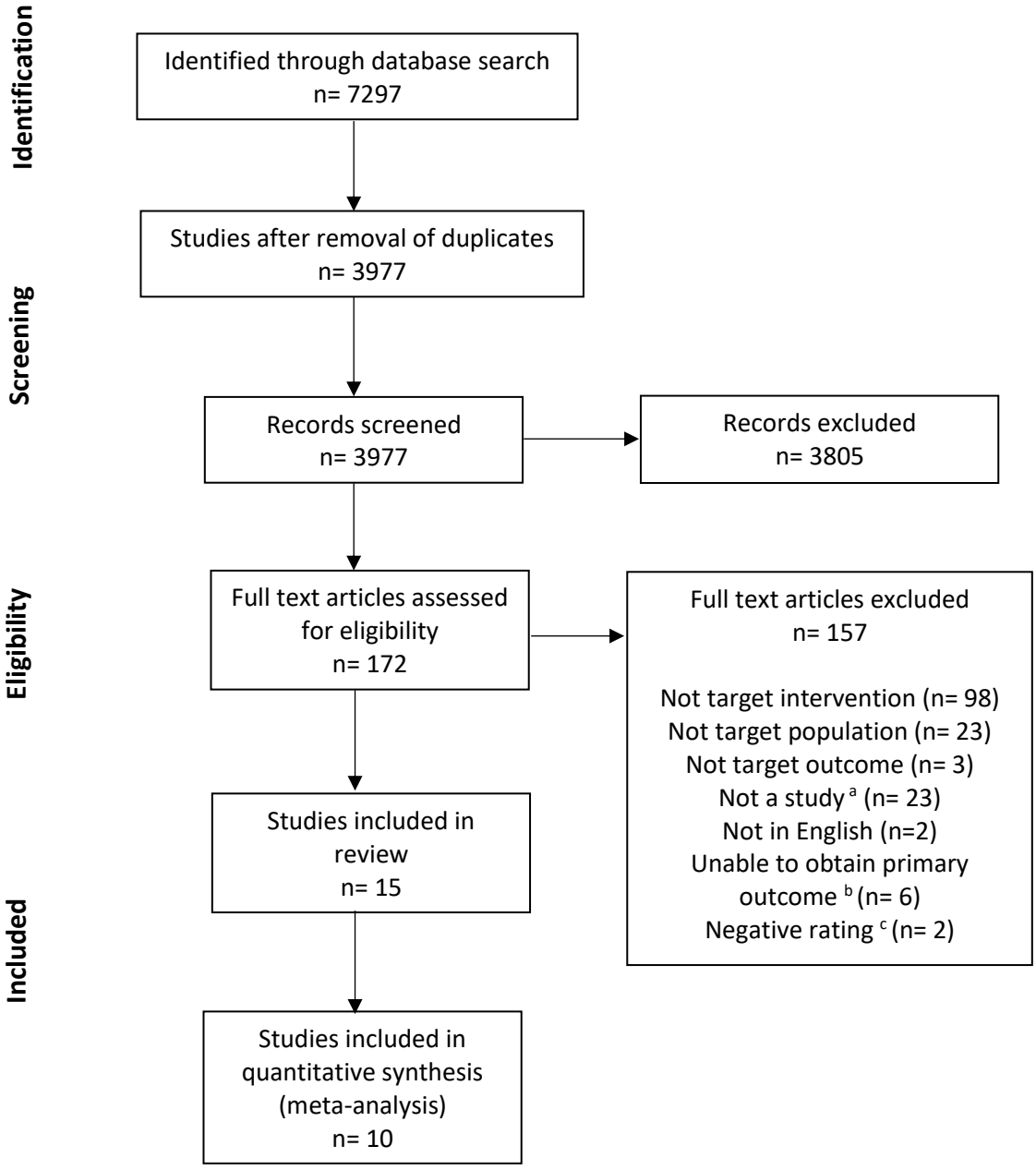

Figure 2-1. Selection process of included studies of systematic literature review.

[a] Includes narrative reviews, conference abstracts, news articles. [b] Studies that collected blood samples hourly or bi-hourly; did not provide numerical data or did not make overall conclusion on primary outcome measures. [c] Studies rated as "negative" in quality assessment, using the ADA Quality Criteria Checklist: Primary Research.

2.3.2 Characteristics of included studies

Characteristics of the 10 studies included in the meta-analysis (Table 2-3a (9, 12, 22-24, 32-36)) and five studies in the qualitative synthesis (Table 2-3b (19, 25-27, 29)) are presented. Each of the included studies had the same participant complete both day and night test sessions. The order to complete day or night session was randomised in four studies, and hence were classified as randomised crossover trials (12, 22, 32, 33). The order was not randomised in others, hence were classified as the following study design: two were pseudo-randomised controlled trials (26, 27) and nine were non-randomised experimental trials (9, 19, 23-25, 29, 34-36).

Included studies had 6 to 20 participants, aged from 19 – 43 years. Five studies included males only, (12, 19, 22-24) two included females only (29, 35) and eight included both genders (9, 25-27, 32-34, 36). Eleven studies included healthy weight participants (9, 12, 22-24, 26, 32-36), as assessed by mean BMI (or range when mean was not reported), and only one study reported a mean BMI in the overweight category (27). Participants' BMI were not reported in three studies (19, 25, 29), but were stated to be 'healthy', hence were assumed to be non-obese. Participants included in studies by Lund et al (25) and Morris et al (2016) (26) were shift workers. The two studies by Holmback et al (23, 24) appeared to have the same sample population, as they had identical participant characteristics, however one reported on glucose response (23) and the other reported on insulin response (24).

Included studies had varying protocols (Table 2-3a, Table 2-3b). Five studies administered multiple test meals at set intervals on a single occasion (e.g. four meals over a 24-hour period) (9, 23, 24, 29, 36). Similarly, three studies administered multiple test meals per occasion, however, the protocol was carried out multiple times (12, 26, 27). The remainder of the studies administered one test meal per occasion, over two or more occasions (19, 22, 25, 32-35).

Five of the studies included two arms. Both studies by Holmback et al (23, 24) had a high fat and a high carbohydrate intervention. Gibbs et al (33) had a low glycaemic index (GI) meal intervention and a high GI meal intervention. Van Cauter et al (36) had one arm which administered meals at 6 hour intervals and another at 12 hour intervals. Leung et al (34) had an oral glucose tolerance test (OGTT) and a low GI meal intervention. Each arm was included as a separate comparison in the meta-analysis. Aparicio et al (19) also administered its test meal as an OGTT, all other included studies administered mixed-meals.

Table 2-3a. Characteristics of studies included in meta-analyses.

Reference	Study Design	Participant characteristics			Intervention				
		Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (Day-time, Night-time) [1]	Fast period	Pre-test meal (MJ)	Test meal (MJ)
Al-Naimi et al. (2004) (12)	Randomised crossover trial	M (8)	R: 20 - 33	R: 20 - 25	2 occasions, each with 3 meals (1: 1300, 1600, 1900h; 2: 0100, 0400, 0700h)	a. 1300, 0100h b. 1600, 0400h	3 hrs 3 hrs	1.0 3.2	3.2 1.0
Biston et al. (1996) (9)	Non-randomised experimental trial	M (6) F (3)	24.5 ± 2.5 ($\bar{x}$ ± SEM)	22.7 ± 2.1 ($\bar{x}$ ± SEM)	1 occasion, 4 identical meals over 24 hrs: 0830 (pre-test meal), 1400 (test 1), 2000 (test 2), 0830h next day (test 3)	a. 0830, 2000h b. 1400, 2000h	- 12.5 hrs for 0830h arm - 5.5 hrs for 1400h arm - 6 hrs for 2000h arm	2.9	2.9
Bo et al. (2017) (32)	Randomised crossover trial	M (10) F (10)	27.6 ± 3.4 ($\bar{x}$ ± SD)	23.4 ± 3.2 ($\bar{x}$ ± SD)	2 occasions, each with 1 meal (0800, 2000h)	0800, 2000h	8 hrs	4.5	4.9
Gibbs et al. (2014) (33)	Randomised crossover trial	M (1) F (9) n=9 included in original insulin analysis	25.5 ± 8.8 ($\bar{x}$ ± SD)	21.9 ± 1.7 ($\bar{x}$ ± SD)	1. low GI 2. high GI Each protocol with: 2 occasions, each occasion with 1 meal (0800, 2000h)	0800, 2000h	10 hrs for 0800h arm 8 hrs for 2000h arm	2.6	1.3 (both low GI and high GI)
Gil-Lozano et al. (2016) (22)	Randomised crossover trial	M (6)	21.1 ± 0.9 ($\bar{x}$ ± SEM)	23.9 ± 1.0 ($\bar{x}$ ± SEM)	2 occasions, each with 1 meal (1100h, 2300h)	1100, 2300h	5 hrs	1.3	3.6

Reference	Study Design	Participant characteristics			Intervention				
		Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (Day-time, Night-time) [1]	Fast period	Pre-test meal (MJ)	Test meal (MJ)
Holmback et al. (2002) (23)	Non-randomised experimental trial	M (7)	32 (26-43) ($\bar{x}$ (R))	23.8 (19.9-26.6) ($\bar{x}$ (R))	1. high carbohydrate (65% energy) meal [2] 2. high fat (45% energy) meal [2] Each protocol with: 1 occasion, 6 identical meals over 24hrs (0800, 1200, 1600, 2000, 0000, 0400h)	a. 1200, 0000h b. 1600, 0400h	4 hrs	~ 1.9 [3]	~ 1.9 [3]
Holmback et al. (2003) (24)	Non-randomised experimental trial	M (7)	32 (26-43) ($\bar{x}$ (R))	23.8 (19.9-26.6) ($\bar{x}$ (R))	1. high carbohydrate (65% energy) meal [2] 2. high fat (45% energy) meal [2] Each protocol with: 1 occasion, 6 identical meals over 24hrs (0800, 1200, 1600, 2000, 0000, 0400h)	a. 1200, 0000h b. 1600, 0400h	4 hrs	~ 1.9 [3]	~ 1.9 [3]
Leung et al. (2019) (34)	Non-randomised experimental trial	M (2) F (8)	23 (4) (med(IQR))	22.6 (3.0) (med(IQR))	1. Oral glucose tolerance test 2 occasions, each with 1 meal (0800, 2000h)	0800, 2000h	10 hrs	2.7	1.3
		M (2) F (7)	26 (15) (med(IQR))	23.6 (3.9) (med(IQR))	2. Low GI meal 3 occasions, each with 1 meal (0800, 2000, 0000h)	0800, 2000h	10 hrs	2.7	3.3

Reference	Study Design	Participant characteristics			Intervention				
		Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (Day-time, Night-time) [1]	Fast period	Pre-test meal (MJ)	Test meal (MJ)
Owens et al. (1996) (35)	Non-randomised experimental trial	F (9)	R: 19 - 20	20.2 ± 3.0 ($\bar{x}$ ± SD)	4 occasions, each with 1 meal (0200, 0800, 1400, 2000h)	a. 0800, 2000h b. 1400, 0200h	10.5 hrs	n/a	2.4
Van Cauter et al. (1992) (36)	Non-randomised experimental trial	M (4) F (4)	R: 22 - 35	21.5 ± 2.2 ($\bar{x}$ ± SD)	1. 1 occasion, 5 identical meals over 34hrs: 2000h, 0200h, 0800h (test 1), 1400h, 2000h (test 2) 2. 1 occasion, 3 identical meals over 34hrs: 2000h, 0800h (test 1), 2000h (test 2)	0800, 2000h	1. 6 hrs 2. 12 hrs	2.1 2.1 [4]	2.1 2.1 [4]

[1] Comparison (a) used in main meta-analysis. Comparison (a) was replaced with (b) in sensitivity analysis.
[2] Participants followed the assigned diet for 7 days, data for day and night comparison collected on the last day.
[3] Energy content of test meal was matched to each participant's energy requirement. Approximate energy content of each test meal was estimated by dividing mean daily energy intake of participants (11.3MJ/24hrs.) by 6 (meals).
[4] Did not specify energy content, assumed to be the same as protocol 1.
Abbreviations: F, female; GI, glycaemic index; hrs, hours; M, male; med, median; R, range; SEM, standard error of means; SD, standard deviation; $\bar{x}$, mean; yrs., years.

Table 2-3b. Characteristics of studies included in qualitative synthesis.

Reference	Study Design	Participant characteristics			Intervention				
		Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (Day time, Night time)	Fast period	Pre-test meal (MJ)	Test meal (MJ)
Aparicio et al. (1974) (19)	Non-randomised experimental trial	M (6)	31 ± 7 ($\bar{x}$ ± SEM)	n/s	4 occasions, each with 1 meal (0600, 1200, 1800, 0000h)	1200, 0000h	10 hrs	Consumed, but energy content not reported	1.7
King et al. (1994) (29)	Non-randomised experimental trial	F (10)	R: 19 – 42	n/s	1 occasion, 7 identical meals over 42 hrs: Day 1 – 1200, 1800, 2400h, Day 2 – 0600 (start of blood sampling), 1200, 1800, 2400h, Day 3 – 0600h (end of blood sampling)	n/s	6 hrs	2.0	2.0
Lund et al. (2001) (25)	Non-randomised experimental trial	M (10) F (2) Shift workers	28 ± 1.9 ($\bar{x}$ ± SEM)	n/s	3 occasions, each with 1 meal (1^{st} (of 7) day shift – 1330h , 2^{nd} (of 7) night shift – 0130h, 2^{nd} day after return to day shift –1330h)	1330, 0130h	5 hrs	2.1	3.3
Morris et al. (2015) (27)	Pseudo-randomised controlled trial	M (8) F (6) n=10 included in analysis	28 ± 9 ($\bar{x}$ ± SD)	25.4 ± 2.6 ($\bar{x}$ ± SD)	Circadian alignment protocol – 2 occasions, each with 4 meals over 27 hrs: 2000, 0800 (test), 1130, 2000h (test) Circadian misalignment protocol – 2 occasions, each with 4 meals over 27 hrs: 0800h, 2000h (test), 2330h, 0800h (test)	0800, 2000h	Alignment: 12 hrs for 0800h arm; 8.5 hrs for 2000h arm Misalignment: 12hrs for 2000h arm; 8.5 hrs for 0800h arm	Consumed, but energy content not reported	33% ER

Reference	Study Design	Participant characteristics			Intervention				
		Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (Day time, Night time)	Fast period	Pre-test meal (MJ)	Test meal (MJ)
Morris et al. (2016) (26)	Pseudo-randomised controlled trial	M (3) F (6) Shift workers; n=7 included in analysis	34 ± 8 ($\bar{x}$ ± SD)	24.2 ± 3.4 ($\bar{x}$ ± SD)	Circadian alignment protocol – 1 occasion, 4 meals over 27 hrs: 2000h, 0800h (test), 1130h, 2000h (test) Circadian misalignment protocol – 1 occasion, 4 meals over 27 hrs: 0800h, 2000h (test), 2330h, 0800h (test)	0800, 2000h	Alignment: 12 hrs for 0800h arm; 8.5 hrs for 2000h arm Misalignment: 12hrs for 2000h arm; 8.5 hrs for 0800h arm	33% ER	33% ER

Abbreviations: ER, energy requirement; F, female; GI, glycaemic index; hrs, hours; M, male; n/s, not stated; R, range; $\bar{x}$, mean; SEM, standard error of means; SD, standard deviation; yrs., years.

2.3.3 Difference in postprandial glucose response between day and night

2.3.3.1 Meta-analysis

Thirteen comparisons, from nine studies, were included in the meta-analysis for postprandial glucose response. Two sets of comparisons were included from studies by Gibbs et al (33), Holmback et al (2002) (23), Leung et al (34) and Van Cauter et al (36), as each included two intervention arms. The day time points included in the meta-analysis were within the morning hours (0800h to noon), except one study, which had 1300h (12). The meta-analysis showed that postprandial glucose response was lower during the day compared to that observed in the night, after an identical meal (SMD = -1.66; 95% CI, -1.97 to -1.36; $p < 0.001$) (Figure 2-2). Heterogeneity was low ($I^2 = 33.0\%$, $p = 0.12$). Data used for meta-analysis are presented in Appendix E.

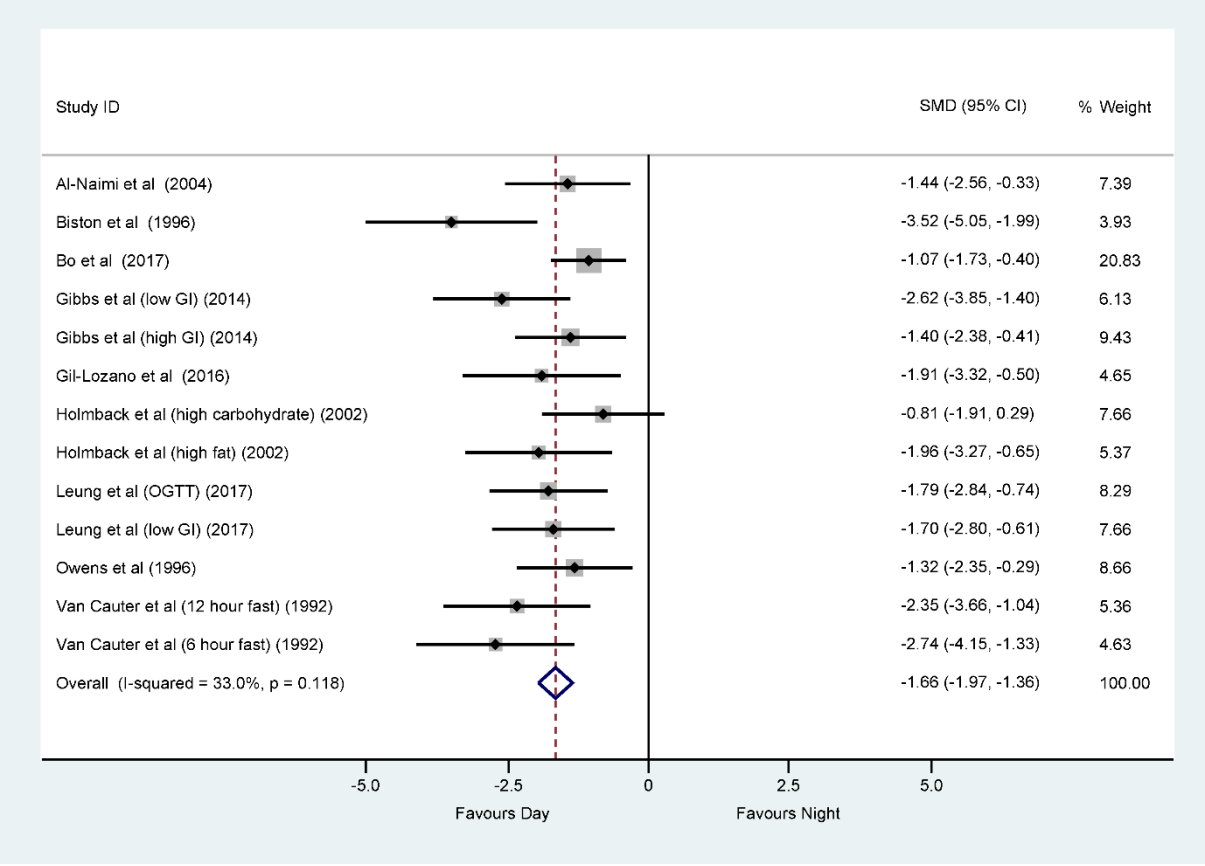

Figure 2-2. Glucose response meta-analysis: comparison of postprandial glucose response between day and night. Fixed effects model was used.

2.3.3.2 Qualitative synthesis

Five studies did not report numerical AUC/iAUC and overall study conclusions were summarised qualitatively (Table 2-4) (19, 25-27, 29). All reported a lower response during the day compared with at night; this was confirmed by statistical tests in four studies (25-27, 29), while one study did not perform statistical tests (19).

Table 2-4. Qualitative synthesis of studies not included in meta-analysis.

Reference	Conclusion on postprandial glucose response	Conclusion on postprandial insulin response
Aparicio et al. (1974) (19)	Postprandial glucose response is affected by time of day: lower levels in the morning (0600h) and higher levels at 1800h and midnight (p= n/p).	Postprandial insulin levels were higher in the morning (0600h) compared to 1800h and midnight (p= n/p).
King et al. (1994) (29)	In the period of 0000h to 0600h, postprandial glucose rise was greater compared to the other periods of intervention (p< 0.001).	Postprandial insulin response was affected by time of day (p< 0.01), which reflected the slower rate of decline after meal consumption at 0000h, compared to the other periods.
Lund et al. (2001) (25)	Postprandial blood glucose (p< 0.01) response is affected by time of day: higher values during night shift compared with 1st day shift.	Postprandial blood insulin (p< 0.01) response is affected by time of day: higher values during night shift compared with 1st day shift.
Morris et al. (2015) (27)	There is a circadian effect on postprandial glucose response, independent of behavioural effects. Two-hour postprandial glucose AUC was 12% higher at night compared to morning (p< 0.0001).	Early-phase postprandial insulin AUC was 27% lower at night compared to morning (p< 0.0001). Late phase postprandial insulin AUC was not affected by circadian phase (p= 0.26).
Morris et al. (2016) (26)	There is a circadian effect on postprandial glucose response, independent of behavioural effects. Postprandial glucose was 6.5% higher at night compared to morning (p= 0.0041).	There is a circadian effect on postprandial insulin response, independent of behavioural effects. Both early-phase and late-phase postprandial insulin was 18% lower at night compared to morning (p= 0.011, p<0.0001 respectively).

Abbreviations: AUC, area under the curve; n/p, not provided.

2.3.4 Difference in postprandial insulin response between day and night

2.3.4.1 Meta-analysis

Eleven comparisons, from eight studies, were included in the meta-analysis for postprandial insulin response. Two sets of comparisons were included from studies by Gibbs et al (33), Holmback et al (2003) (24) and Van Cauter et al (36), as each included two intervention arms. The day time points included in the meta-analysis were within the morning hours (0800h to noon), except one study, which had 1300h (12). The meta-analysis showed that postprandial insulin response was lower during the day compared to that observed in the night, after an identical meal (SMD = -0.35; 95% CI, -0.63 to -0.06; p = 0.016) (Figure 2-3). Low heterogeneity was detected (I^2 = 31.0%, p = 0.15). Data used for meta-analysis are presented in Appendix F.

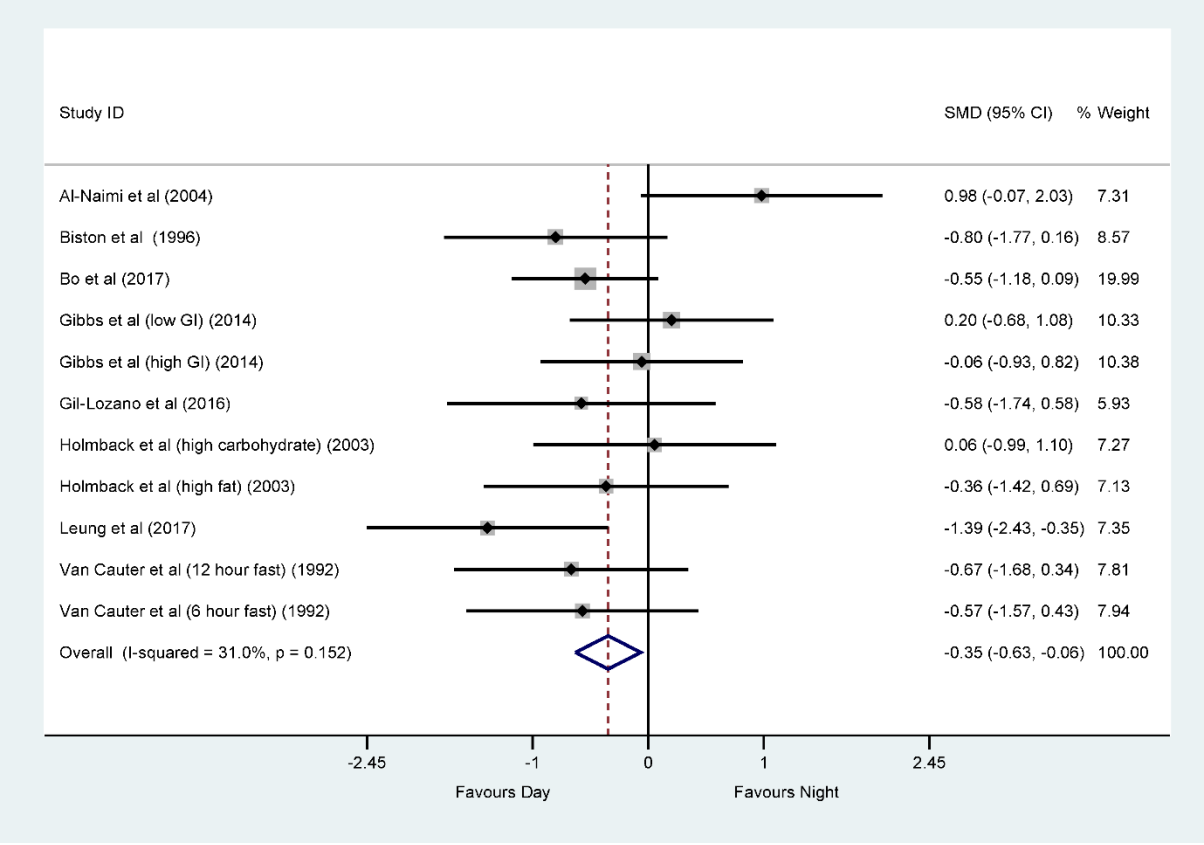

Figure 2-3. Insulin response meta-analysis: comparison of postprandial insulin response between day and night. Fixed effects model was used.

2.3.4.2 Qualitative synthesis

Five studies did not report AUC/iAUC and overall study conclusions were summarised qualitatively (Table 2-4), results varied amongst studies. Three studies reported lower response at night compared to the day (19, 26, 27). This was not confirmed by statistical tests in the study by Aparicio et al (19). Both studies by Morris et al (26, 27) analysed early-phase and late-phase insulin separately. Both reported lower early-phase insulin response at night (defined as 0 – 30 minutes postprandial (27) or 10 – 30 minutes postprandially (26)). Morris et al (2016) (26) found that late-phase insulin response (40 – 90 minutes postprandially) was also lower at night, but included permanent shift workers as participants. In contrast, Morris et al (2015) (27) reported that late-phase insulin response (30 – 120 minutes postprandially) was not affected by time of day, but included day workers as participants.

Lund et al (25) reported significantly greater postprandial insulin response at night compared to the day, and included shift workers based in Antarctica as participants. King et al (29) concluded that postprandial insulin response was significantly affected by time of day; however, no magnitude of response was reported.

2.3.5 Risk of bias within studies

Based on the ADA quality assessment checklist (30), seven of the included studies were rated positive (9, 22-24, 32-34), eight were rated neutral (12, 19, 25-27, 29, 35, 36) and two were rated negative (21, 37). The main difference between the positive and neutral studies was that those rated positive were free from participant selection bias. Studies rated neutral did not specify the exclusion of participants based on metabolic conditions that are likely to affect primary outcome measures of the current review, such as T2DM and CVD. The reporting of weight status was also omitted in some studies rated neutral. Studies rated negative were excluded in the synthesis of results. See Appendix D for checklist for each individual study.

2.3.6 Publication bias and sensitivity analysis

Assessment of funnel plots (Appendix G) showed no evidence of publication bias in glucose and insulin meta-analyses. Take-one-out sensitivity analyses did not lead to any notable changes in the meta-analysis results for both primary outcome measures (Appendix H).

Four studies examined glucose response at additional time points during the day, all of which were in the afternoon (1400h or 1600h). One study included two arms, therefore provided five afternoon versus night comparisons that were substituted into the original metal-analysis as a form of sensitivity analysis. This did not change the results of the original meta-analysis; postprandial glucose response

remained lower during the day compared to the night (SMD = -1.03; 95% CI, -1.32 to -0.74; $p < 0.001$) (Appendix I). However, heterogeneity was higher ($I^2 = 79.2\%$, $p < 0.001$).

Similarly, three studies examined insulin response at additional time points during the day. This provided four afternoon (1400h or 1600h) versus night comparisons that were substituted into the original meta-analysis as a form of sensitivity analysis. This changed the results of the original meta-analysis; postprandial insulin response were no longer different between day and night (SMD = -0.23; 95% CI, -0.51 to 0.05; $p = 0.11$) (Appendix J). However, low heterogeneity was detected ($I^2 = 28.5\%$, $p = 0.17$).

2.4 Discussion

2.4.1 Summary of findings

This systematic review and meta-analysis of 15 studies, which included paired day/night data from the same participant, found that both postprandial glucose and insulin responses are significantly higher at night compared to the day. This was important to establish as modern lifestyles have led individuals to extend their eating period during the 24-hour day and eat later into the night (38, 39). The results of this review further substantiates the recommendations by the American Heart Association (AHA) Statement on meal timing and CVD prevention, that meal timing can form the basis of dietary strategies to protect metabolic health (2).

A propensity for raised plasma glucose following meal consumption later in the 24-hour day and evening, may negatively impact the metabolic health of individuals who habitually consume large proportions of their energy intake at these times. Observational studies have reported that eating late at night is associated with increased risk of hyperglycaemia, metabolic syndrome and obesity (40-42). The shift working population is a classic example of those who habitually eat at night. Meta-analyses have shown that shift workers experience up to 40% increased risks of T2DM and CVD compared to those who have never been exposed to shift work (43-45). Higher odds of weight gain have also been reported (46, 47), but without differences in daily energy intake compared to day workers (48). This indicates that timing of energy intake may be a key contributor to increased metabolic disturbances observed in this population.

The higher postprandial glucose response at night (compared to the day) reported in the current meta-analysis may be caused by a reduction in insulin sensitivity (49-51). A reduced responsiveness of the liver and peripheral tissues to insulin stimulation at night would reduce glucose uptake by these tissues. Concomitantly, at night the body is under circadian rhythm regulation that dampens anabolic processes (as shown in constant routine studies) (6) and increases endogenous glucose production through the breakdown of stores (e.g. glycogen) (51). The current meta-analysis also showed higher postprandial insulin concentrations at night compared to the day, which is likely to be a counteractive response to the postprandial glucose elevation observed. Whilst this is probably a normal response for the healthy participants included in these acute postprandial studies, frequent hyperinsulinemia is observed in the development of T2DM (52).

The day time points included in the main meta-analysis were all during the morning hours (0800h to noon), except for one comparison (12). In a sensitivity analysis, morning time points were substituted with afternoon time points provided by the included studies. Whilst this did not alter the results of

the glucose meta-analysis, it resulted in a loss of significance for insulin. The postprandial plasma insulin concentration is a composite effect of numerous physiological mechanisms, including β-cell responsiveness and insulin sensitivity. These have influences on insulin secretion and insulin action/clearance respectively, and together will alter the amount of insulin present in the circulation (53, 54). Aforementioned studies have reported a reduction in insulin sensitivity and β-cell responsiveness at night (49, 51). There is great inter-individual variability in these mechanisms (55) and hence are likely to decline at different times and rates during the 24-hour day. This may have contributed to the loss of statistical significance in the sensitivity analysis. Therefore, time of the day differences in β-cell responsiveness, insulin secretion, sensitivity and clearance should be further examined specifically, to provide a better idea of the changes in insulin function throughout the 24-hour day.

2.4.2 Strengths and limitations of review

Strengths of this study include the comprehensive literature search of seven databases, and the inclusion of only studies with paired data from each participant. Excluding hours in the afternoon/evening when defining 'day' and 'night' in the eligibility criteria reduced heterogeneity and allowed us to conduct a meta-analysis that made clear time of day comparisons. There is considerable variation in individuals' circadian rhythms (56, 57), therefore it is difficult to pinpoint the specific hour of the night at which glucose tolerance and insulin sensitivity begin to be less favourable. Nonetheless, this meta-analysis conducted confirms that there is a time of day difference in postprandial glucose and insulin response. These findings support the need for further work in this area, as this is a potential mechanism for increased risk of metabolic disorders.

Although the eligibility criteria of the current review included participants who were overweight (BMI up to 30 kg/m^2), participants of all included studies were actually within the healthy weight range, which reduces the effects of confounding factors such as adiposity (58), however this may also reduce the generalisability of these findings. We were unable to explore gender differences in the meta-analyses, as included studies had a combination of single- and mix-gender samples. However, as all included studies made within-participant comparisons, gender differences should have minimal influence on primary outcomes examined.

2.4.3 Future research directions

Whilst the AHA Statement suggests the maintenance of consistent overnight fast periods, this can be challenging for specific populations who work and eat at atypical times, such as shift workers. The feasibility and effectiveness of overnight fasts should be specifically examined in such populations. Moreover, investigation into the dietary strategies to attenuate postprandial hyperglycaemia observed during the night is recommended. Manipulation of macronutrient composition (to avoid carbohydrates) may be effective, as meals with higher proportions of protein and/or fat have been shown to reduce postprandial glucose and insulin response, at least in the day (24, 59).

The actions of other glucoregulatory hormones such as glucagon and GLP-1 (60) should be examined, as some studies have shown that they also exhibit circadian rhythms. Glucagon, a pancreatic hormone, stimulates endogenous glucose production by the liver, thereby increasing blood glucose concentration. Results from a hypoglycaemic clamp study showed that fasting and peak glucagon concentration is higher at night (2300h) compared to day (0900h) (61). GLP-1, an intestinal hormone, promotes glucose-dependent insulin secretion and suppresses glucagon secretion after food intake (60, 62). Gil-Lozano et al (22) showed that postprandial GLP-1 concentration was higher at night (2300h) compared to the day (1100h). In addition to the postprandial glucose elevation that occur, this may also contribute to the increased postprandial insulin response observed at night.

2.5 Conclusion

The current review highlights the importance of meal timing, to all those who eat at night habitually, such as night shift workers. Our meta-analyses showed that postprandial glucose and insulin responses are higher during the night compared to the day. This may be a physiological mechanism causing the increased risks of T2DM and CVD observed in shift workers, and also metabolic syndrome and hyperglycaemia observed in late-night eaters. Future intervention studies should investigate the benefits of food or carbohydrate avoidance at night, especially in at risk populations such as night shift workers.

Acknowledgements: We would like to express thanks to University of Ulster (Northern Ireland) placement student Hazel Windsor-Aubrey and Monash University research assistant Claire Bristow who assisted with the study selection process of this review.

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Chapter 3 . 'Shifting the Risk' study

Chapter 3 a. Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers.

Preamble

Findings from Chapter 2 and literature reviews published by our research group have indicated that eating during the night is associated with a reduction in glucose tolerance, postprandial hyperlipidaemia and a reduction in TEF (39, 40). Night shift workers are likely to have frequent exposures to such acute metabolic disturbances, as they regularly eat during and throughout the night whilst on night shifts (96, 99, 100). This behaviour opposes the regulation of the circadian clock system (7, 22), and therefore, is a probable contributing factor towards shift workers' increased risk of CVD, T2DM and obesity (19, 21, 61). Moreover, a published meta-analysis by our research group has also reported no difference in overall energy intake between night shift workers and their day-working counterparts (64), emphasising meal timing as a potential modifiable risk factor for metabolic diseases.

Despite this, there is currently no tailored meal timing recommendations for shift workers targeting the prevention of metabolic diseases. The novel pilot randomised crossover trial reported in the current chapter is the first step towards building these recommendations. It examined, for the first time, whether a short 5-hour overnight fasting period can improve the metabolic health of night shift workers. The study will be reported over two chapters: Chapter 3a will report on CVD risk factors (postprandial TAG and glucose response and body weight changes), while Chapter 3b will explore the protocol's feasibility.

The findings from this study are relevant for 20% of the workforce in industrialised countries, who are engaged in shift work (24). The American Heart Association highlighted the importance of maintaining a consistent overnight fast period, in their 2017 position statement on meal timing and CVD risk (10). Evidence for the development of suitable meal-timing strategies for the shift working population is therefore urgently required.

This Chapter has been published in *Nutrition, Metabolism and Cardiovascular Diseases* in June 2021:

Leung GKW, Davis R, Huggins CE, Ware RS, Bonham MP. Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers. *Nutrition, Metabolism and Cardiovascular Diseases*. 2021;31(6):1890-1902. Doi: 10.1016/j.numecd.2021.03.008.

Published manuscript available in Appendix B.

3.1 Introduction

Shift workers form a significant proportion of the workforce in industrialised countries (1). Approximately 16% of the Australian workforce are shift workers (2), who work across multiple industries and are integral to the operations of the 'round-the-clock' modern society. Epidemiological studies have reported that shift workers are at an increased risk of metabolic diseases compared to their day-working counterparts. Meta-analyses of observational studies have shown that the risk of shift workers developing T2DM is 10% higher (3), while the risk of developing CVD is 10 to 20% greater (4, 5). In a cohort of 1806 shift workers employed in manufacturing, the association between shift work and risk of CVD remained after adjustment for traditional risk factors such as BMI, physical activity level and age (6). Multiple observational studies have reported an increased risk of obesity in shift workers (7, 8) that could not be explained by increases in energy intake. These findings are supported by a meta-analysis of 12 cross-sectional studies, which showed shift workers' daily energy intake was similar to that of their day-working counterparts (9).

Whilst shift workers' overall energy intake may be similar to that of day workers (9), the times at which they eat differ. Shift workers often eat during and throughout the night, typically fitting their meal times around their shift schedules (10-12). However, as humans are diurnal animals, feeding is favoured during the day, whilst the night is reserved for rest and fast. This temporal separation of feed/fast activity is set by the circadian clock system (13). This system regulates the timing of numerous physiological and metabolic processes, synchronising them with each other and to the anticipated feed/fast cycle. This mechanism evolved as a way to optimise metabolic efficiency (14). Shift workers' habitual night time eating behaviour is therefore out of synchronisation with the timing set by the circadian clock, and its impact on metabolism is reflected in findings from diurnal studies. In these studies, postprandial lipaemic and glycaemic responses are measured at regular intervals, after participants consume identical meals at different times of the 24-hour day. They are performed under controlled laboratory settings, with consistent energy intake and fast periods prior to the testing session, and restricted food intake and physical activity during the session. They have reported compelling evidence, showing increased postprandial glucose and insulin responses during the night compared to the day. These findings are supported by a recent meta-analysis of ten diurnal studies (15) and suggest a potential physiological mechanism contributing to the increased CVD and T2DM risks observed in shift workers. Similar diurnal variations have been observed in postprandial lipid metabolism. In a recent systematic review, all five included studies reported at least one parameter of postprandial TAG response (e.g. total concentration or time course kinetics) that were different due to time of day (16). However, findings are less convincing than for postprandial glycaemic response, as variations in study protocols precluded a meta-analysis. Recent evidence also suggests time of day

differences in energy expenditure, with lowered TEF and possibly RMR during the night (summarised in Shaw et al (17)). These findings provide plausible explanations for the comparatively rapid weight gain observed in shift working populations, in the absence of increases in energy intake. Together, these reviews suggest poor glucose tolerance, reduced insulin sensitivity, compromised lipid handling and energy expenditure during the night; all of which are independent risk factors for the development of CVD, T2DM and obesity (18-21). Given that shift workers are typically awake working and eating during the night (22), their time of energy intake is a probable contributor to their increased risks of metabolic diseases.

Considering the exaggerated postprandial metabolic responses that occur at night, minimising energy intake during these hours by maintaining a small period of fasting, should induce metabolic benefits in night shift workers. In this pilot randomised crossover trial, we implemented a short overnight fast (0100h – 0600h) in the participants' temporal eating pattern for four weeks, by redistributing energy intake to other hours of the 24-hour day. We aimed to examine this meal timing intervention's effect on CVD risk markers of at-risk night shift workers (i.e. with abdominal obesity). This Chapter reports on biochemical (postprandial TAG, glucose and insulin response) and anthropometric (body weight) risk factors of CVD, before and after the intervention crossover. Adherence to the intervention is examined through dietary assessment. This is a novel study, translating effects of meal timing observed in acute laboratory studies, to a dietary intervention implemented in free-living night shift workers for a comparatively extended period. As such, sample size determination was precluded in this pilot; considerations and requirements for a future definitive randomised controlled trial (RCT) will be discussed.

3.2 Methods

3.2.1 Trial design

This pilot randomised crossover trial, referred to as the 'Shifting the Risk' Study, was conducted in Melbourne, Australia, between July 2017 and October 2018. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12617000791336) and the study protocol has been published (23). This study was approved by the Monash University Human Research Ethics Committee (Project ID: 2017-8619) and was conducted in accordance with the Declaration of Helsinki for human studies.

3.2.2 Participants

We aimed to have 20 participants complete the study; an attrition rate of 30% was assumed, therefore we aimed to recruit and randomise 28 participants (23). A formal sample size calculation prior to study commencement was not conducted, due to the pilot nature of the trial. Eligible participants were permanent or rotating night shift workers involved in night shift work for a minimum of 12 months consecutively prior to study commencement and were expecting to work at least three night shifts per fortnight during the study's duration. They were aged between 18 and 60 years and had abdominal obesity, as measured by researchers during the screening session prior to enrolment, and defined as waist circumference >94 cm (non-Asian men), >90 cm (Asian men) or >80 cm (all women) (24).

Night shift workers were ineligible for the study if they did not work or routinely eat between 0100h and 0600h whilst on night shift, had an existing diagnosis of CVD or T2DM, on medication for diabetes, hyperlipidaemia or medications known to alter metabolism (e.g. thyroxin, insulin sensitisers, glucocorticoids or anti-depressants). Further exclusion criteria are outlined in the published study protocol (23).

3.2.3 Experimental protocol

The study was 11 weeks in duration; the experimental protocol is summarised in Figure 3-1. In the run-in period, participants were asked to complete a 4-day food diary that included two night shifts, to capture their usual dietary intake on shift. Participants were then randomised to begin with either the intervention or the control period (both four weeks minimum). These two test periods were separated by a wash-out period of two weeks minimum. Three acute meal challenge sessions were conducted at the Monash University Be Active Sleep Eat (BASE) Facility (Melbourne, Australia) for outcome assessment: at baseline, end of first period (challenge session 2) and end of second period (challenge session 3).

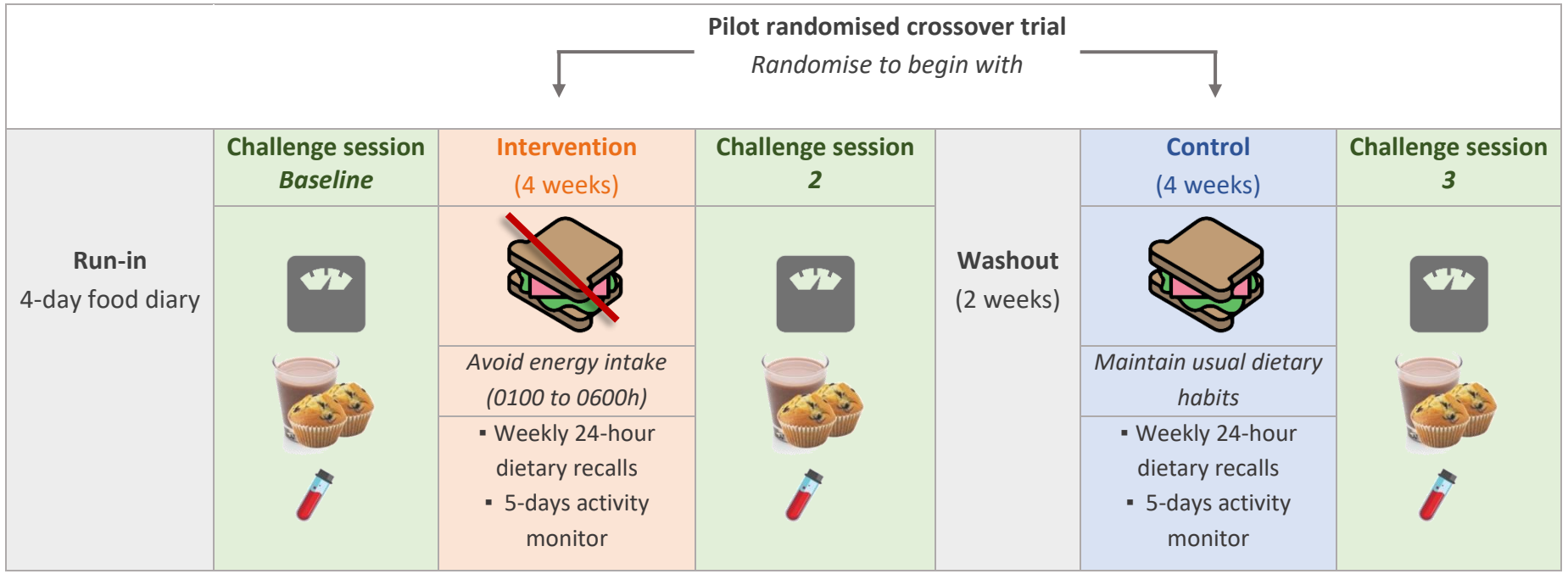

Figure 3-1. Experimental protocol of 'Shifting the Risk'.

All challenge sessions were seven hours in duration, running from approximately 0730h to 1430h.

3.2.3.1 Test periods

During the intervention period, participants were asked to avoid energy intake for a fixed 5-hour window during the night (0100h to 0600h). They were instructed to do so for the entire 4-week period, including the non-night shift days. Participants were asked to change their meal times only, and not the types of foods and beverages consumed, with an aim to maintain usual total daily energy intake. The duration of the intervention period was based on published studies that have shown changes in metabolic outcomes after a 4-week dietary intervention (25, 26). These studies implemented changes to dietary composition only, in the absence of changes in energy intake, as such reflect the intervention proposed in the current study (i.e. a change to diet via meal timing with no changes to energy intake).

Prior to starting the intervention period, each participant met with the study dietitian (GKWL), who advised on strategies to redistribute meal times. Participants received SMS text messages on a night shift (at least once weekly), reminding them not to consume any food or beverages (besides water) during the designated fast window. During the control period, participants were asked to maintain (or return to) their usual dietary habits for four weeks.

The study was designed to keep participants weight stable during the study's duration. As such, participants were asked to avoid changing their food choices and physical activity levels. During both test periods, participants were asked to wear a SenseWear activity monitor (Armband Model MF-SW, Bodymedia, Pennsylvania, USA) for the 5-days prior to attending the challenge session. This monitor provides an estimate of physical activity level. Adherence to the dietary intervention was monitored by 24-hour dietary recalls, which were conducted over the phone with a researcher. These were completed once weekly, after a night shift, during both test periods. Participants were asked to report their dietary intake from 0600h the day prior to 0600h of the current day.

3.2.4 Outcome measures

Primary outcome measures are postprandial TAG and glucose response, assessed by calculating iAUC. The secondary outcome measure is postprandial insulin iAUC. Hourly concentrations from 0 to 6 hours postprandially were used to calculate TAG iAUC, while concentrations from 0 to 3 hours postprandially (eight time points) were used to calculate glucose and insulin iAUC. Other blood lipid and glucose outcomes examined include fasting total cholesterol, LDL-cholesterol (LDL-C) and HDL-cholesterol (HDL-C) levels; lipid ratios; peak and time to peak postprandial TAG and glucose; postprandial TAG concentration at four hours; postprandial glucose concentration at two hours and HOMA-IR. Body weight and adherence to the dietary intervention were also recorded.

3.2.5 Outcome assessment

3.2.5.1 Acute meal challenge session (for biochemical and anthropometric measures)

Three challenge sessions were conducted: at baseline, end of first period (challenge session 2) and end of second period (challenge session 3). Outcome data, including postprandial TAG, glucose and insulin levels and body weight were collected during each session. Each session was scheduled on the second or third day after a night shift. Participants were asked to refrain from strenuous exercise and alcohol on the day prior to each session. They were given a standardised meal to consume between 1800h and 2000h the night prior (2810kJ, 56% energy (E) from carbohydrate, 24% E fat, 17% E protein), then fast overnight, until arriving at the BASE facility for the session.

Each session ran for seven hours, from approximately 0730h to 1430h. Upon arrival at the BASE facility, anthropometric measures were taken in the fasted state. Waist circumference was measured using standardised procedures; body weight and composition were measured using the SECA Bioelectrical Impedance Analyser (515/514, SECA Group, Hamburg, Germany). Blood pressure was taken in duplicate on participant's right arm, using an automated blood pressure monitor (ProBP 3400 Digital Blood Pressure Monitor, Welch Allyn, New York, USA), according to standardised procedures. A cannula was then fitted in the antecubital fossa or back of hand by a trained nurse or phlebotomist and a fasting blood sample was taken. Participants were provided with a test meal high in fat, to be consumed within 15 minutes. It consisted of two muffins and a milkshake, providing a total of 3779kJ (56% E fat, 36% E carbohydrates, 8% E protein). A high fat load (58g: 22% saturated, 30% monounsaturated; 48% polyunsaturated) was included to maximise the postprandial lipaemic response (27). Blood samples were taken at regular intervals for six hours post-meal (15, 30, 45, 60, 90, 120, 180, 240, 300, 360 minutes). During the challenge session conducted at baseline, participants completed the Morningness and Eveningness questionnaire (28), to determine their chronotype. Participants were not permitted to consume any other food or drinks (except water) during the postprandial period and remained sedentary.

3.2.5.2 Adherence

Adherence to the dietary intervention was determined from weekly 24-hour dietary recalls. It is reported as the percentage of dietary recalls collected in the intervention period, which documented nil energy intake between 0100h to 0600h. Estimates of usual daily physical activity energy expenditure (activity EE) during each test period were derived from the SenseWear activity monitor data.

3.2.5.3 Biochemical analysis

Serum lipid and plasma glucose concentrations were measured using the Indiko Clinical and Specialty Chemistry System (Thermo Fisher Scientific, Vantaa, Finland). Plasma insulin concentration was measured using the Human Insulin Specific RIA kit (HI-14K, Merck Millipore, Massachusetts, USA) according to manufacturer's instructions and read on a Gamma Counter. HOMA-IR was calculated using the following formula: [fasting plasma insulin (μU/ml) x fasting plasma glucose (mmol/l)] ÷ 22.5.

3.2.6 Randomisation

The randomisation sequence was generated by a researcher who was not involved in study recruitment or baseline data collection (C.E.H), through a computer-generated random number sequence using a permuted block design. Researchers responsible for eligibility assessment and data collection (G.K.W.L and R.D) were provided with the treatment allocation in sealed envelopes after the enrolment of each participant.

3.2.7 Statistical analysis

Descriptive statistics are summarised as mean ± standard deviation (SD) for continuous data and as frequency (percentage) for categorical data. Outcome variables were visually tested for normality using residual plots. The association between test period (intervention/ control) and biochemical outcome measures (challenge sessions 2 and 3) were assessed using mixed effects linear regression models, with test period (intervention/ control) included as a fixed effect and participant included as a random effect. In each model, the individual participant's baseline challenge session outcome value was included as a fixed-effect covariable, to adjust for any baseline differences between participants. For example, in the model comparing the difference in fasting glucose concentration between intervention and control, the fasting glucose concentration measured at the baseline challenge session was included as a covariate. Effect estimates are presented as mean difference (MD) and 95% confidence intervals (95% CI). A MD >0 indicates the outcome was greater post intervention compared to post control, and vice versa. A preliminary examination showed there was no detectable study period effect, that is, results did not depend on the order of the test sequence, and consequently, period was not included as a covariable in the analytic models. Sensitivity analyses investigated the effect of log-transforming outcome data. However, models were robust therefore untransformed data and analyses are presented. Analysis was conducted using Stata statistical software (version 14.1, StataCorp, College Station, TX, USA).

3.2.8 Sample size estimation for future definitive RCT

Using results obtained from this pilot, we have estimated sample sizes required for fully-powered definitive RCTs, based on postprandial TAG and glucose iAUC (primary outcome measures), peak postprandial TAG concentration, glucose concentration at two hours postprandially and body weight. For each outcome, a sample size was estimated based on the observed SD of the difference in values between the two test periods from the same participant (29).

3.3 Results

3.3.1 Participant enrolment

There were 405 individuals who expressed interest in participating and completed the screening questionnaire. Thirty-one individuals were confirmed to be eligible from the screening session and 28 were randomised. Nineteen (68%) participants completed the study, giving an attrition rate of 32%. Amongst the nine participants who withdrew, four had commenced the study. One participant withdrew because they could not maintain the dietary intervention and another because they could not complete the outcome assessment procedures. All nine participants withdrew prior to completion of their first test period, and consequently did not contribute available data for analysis. Their age and anthropometric measures were taken during the screening session; and were not significantly different to those who completed the study (assessed by independent t-test, Appendix K). Figure 3-2 details study participant flow.

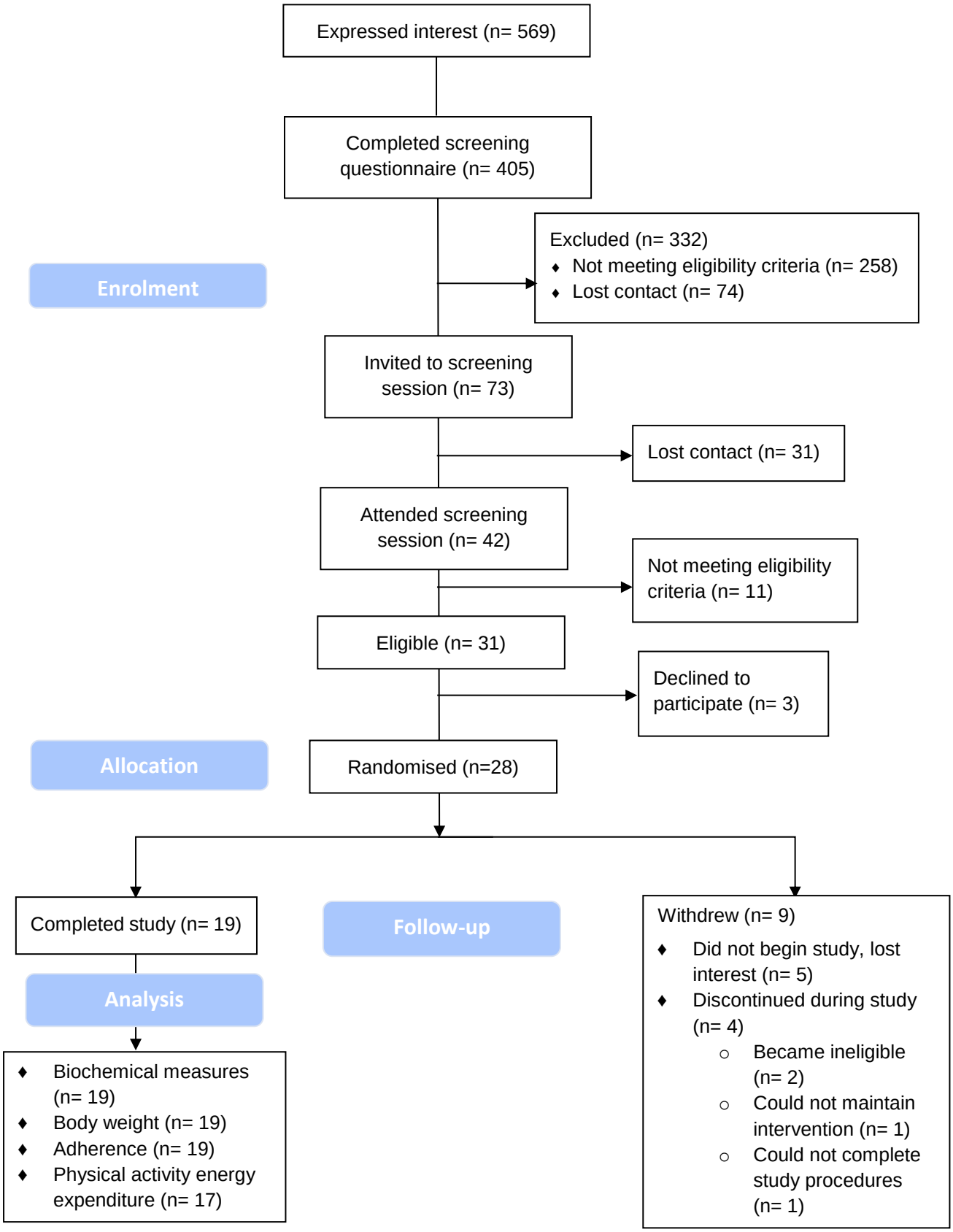

Figure 3-2. Study participant flow of 'Shifting the Risk'.

3.3.2 Baseline characteristics

Baseline and demographic characteristics of participants were taken at the baseline challenge session and are presented in Table 3-1. Most participants were female (n= 13) and worked permanent night shifts (n= 12). The mean (± SD) age was 41 ± 10 years; females had a mean waist circumference of 96.9 ± 13.4 cm, males' was 98.2 ± 4.4 cm and the sample's mean BMI was 30.7 ± 5.7 kg/m^2. The mean blood pressure, fasting TAG and glucose were within the healthy ranges (24, 30). The mean (± SD) postprandial TAG and glucose iAUC were 145.8 ± 89.4 mmol/l.6h and 89.5 ± 64.1 mmol/l.3h respectively, measured at the baseline challenge session.

Table 3-1. Demographic, employment and clinical characteristics of study participants in 'Shifting the Risk', measured at baseline (n= 19).

Demographics	**Frequency (percentage)** [1]		
Age (years), mean ± SD	41 ± 10		
Gender			
Female	13 (68)		
Male	6 (32)		
Occupational fields			
Healthcare and emergency services support	11 (58)		
Hospitality and food service	3 (16)		
Machinery operations and transport services	4 (21)		
Protective services	1 (5)		
Form of shift work			
Permanent night	12 (63)		
Rotating [2]	6 (32)		
Permanent morning [3]	1 (5)		
Years in shift work, median (range)	14 (1.2 – 28)		
Chronotype [4]			
Moderate morning	3 (16)		
Intermediate	14 (74)		
Moderate evening	2 (11)		
Non-smoker	17 (89)		
Body composition [5]	**Mean ± SD**		
	Total (n= 19)	*Female (n= 13)*	*Male (n= 6)*
Body weight (kg)	86.2 ± 17.2	84.7 ± 19.6	89.4 ± 11.1
BMI (kg/m^2)	30.7 ± 5.7	31.7 ± 6.6	28.6 ± 2.4
Waist circumference (cm)	97.3 ± 11.2	96.9 ± 13.4	98.2 ± 4.4
Fat mass (kg)	32.8 ± 12.8	36.6 ± 13.7	24.7 ± 5.0
Fat mass (%)	37.5 ± 9.0	42.1 ± 6.5	27.5 ± 3.3
Fat free mass (kg)	53.3 ± 10.8	48.1 ± 7.6	64.7 ± 7.4
Metabolic measures [5]	**Mean ± SD**		
Blood pressure (mmHg)	123 ± 13 / 79 ± 7		
Fasting biochemical measures [5]	**Mean ± SD**		
TAG (mmol/l)	1.1 ± 0.6		
Total cholesterol (mmol/l)	4.7 ± 0.8		
HDL-cholesterol (mmol/l)	1.4 ± 0.4		
LDL-cholesterol (mmol/l)	2.9 ± 0.8		
Glucose (mmol/l)	5.9 ± 0.5		
Insulin (μU/ml)	15.7 ± 5.9		
HOMA-IR	4.1 ± 1.8		
Postprandial biochemical measures [5]	**Mean ± SD**		
TAG iAUC (mmol/l.6h)	145.8 ± 89.4		
Glucose iAUC (mmol/l.3h)	89.5 ± 64.1		
Insulin iAUC (μU/ml.3h)	7592.8 ± 4521.3		

[1] Unless otherwise stated. [2] Rotating shift schedule included a combination of morning, afternoon and night shifts. [3] Shift hours were from 0400 to 1200, participant met eligibility criteria. [4] Assessed by Morningness and Eveningness Questionnaire [26]. [5] Measures taken at baseline acute meal challenge session. *Abbreviations: iAUC, incremental area under the curve; HOMA-IR homeostatic model assessment of insulin resistance; SD, standard deviation; TAG, triglyceride.*

3.3.3 Comparison of outcome measures between intervention and control

3.3.3.1 Test periods

Within those who completed the study, ten participants began with the intervention period. The intervention period had a median (range) duration of 29 (24 – 49) days; and included 12 (7 – 24) night shifts. The control period had a median duration of 33 (26 – 48) days; and included 16 (7 – 29) night shifts. The washout period had a median duration of 15 (0 – 109) days. Participant whose washout period was 109 days completed the intervention period first.

Outcome measures taken at challenge sessions 2 and 3 were used, to compare the differences between intervention and control. Participants attended the challenge sessions between 2 to 6 days after their last night shift post intervention (84% met the requested 2 to 3 day timeframe) and between 2 to 11 days after their last night shift post control (74% met the requested 2 to 3 day timeframe).

3.3.3.2 Fasting TAG, glucose and insulin

Fasting TAG and glucose levels remained within healthy ranges after both test periods (24, 30). Comparing measures taken at the end of intervention to control, there were no differences in mean fasting TAG, glucose and insulin levels (Table 3-2).

Table 3-2. Association between test period (intervention/ control) and clinical outcomes for 'Shifting the Risk' participants (n= 19).

Outcome	Intervention (mean ± SD) [1]	Control (mean ± SD) [1]	Mean difference	95% CI	p-value
Fasting measures					
TAG (mmol/l)	1.3 ± 1.1	1.1 ± 0.5	0.2	-0.1 – 0.4	0.14
Total cholesterol (mmol/l)	4.8 ± 1.0	4.6 ± 0.7	0.1	-0.1 – 0.3	0.17
HDL-C (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	0.0	-0.1 – 0.0	0.45
LDL-C (mmol/l)	2.9 ± 0.8	2.8 ± 0.8	0.1	0.0 – 0.2	0.09
Glucose (mmol/l)	5.8 ± 0.6	5.8 ± 0.5	0.1	-0.1 – 0.3	0.39
Insulin (μU/ml)	16.6 ± 9.8	15.0 ± 7.2	1.5	-1.2 – 4.2	0.27
HOMA-IR	4.4 ± 3.3	3.9 ± 1.9	0.6	-0.3 – 1.4	0.17
Total cholesterol : HDL-C	3.6 ± 1.3	3.5 ± 1.2	0.1	-0.1 – 0.3	0.28
LDL-C : HDL-C	2.2 ± 0.8	2.2 ± 1.0	0.1	-0.1 – 0.2	0.43
Postprandial measures					
TAG response					
TAG iAUC (mmol/l.6h)	174.5 ± 129.0	149.7 ± 109.7	24.8	-12.7 – 62.3	0.20
Peak TAG (mmol/l)	2.1 ± 1.6	1.9 ± 0.9	0.2	-0.1 – 0.6	0.20
Time to peak TAG (mins)	196 ± 77	202 ± 75	-6	-50 – 38	0.78
TAG concentration at 4h (mmol/l)	1.9 ± 1.6	1.6 ± 0.9	0.3	0.0 – 0.7	0.06
Glucose response					
Glucose iAUC (mmol/l.3h)	82.9 ± 71.1	93.9 ± 85.5	-11.0	-45.1 – 23.2	0.53
Peak glucose (mmol/l)	7.5 ± 1.3	7.4 ± 1.2	0.1	-0.2 – 0.5	0.41
Time to peak glucose (minutes)	48 ± 30	44 ± 23	4	-9 – 17	0.54
Glucose concentration at 2h (mmol/l)	5.6 ± 1.2	5.4 ± 1.4	0.2	-0.3 – 0.7	0.40
Insulin response					
Insulin iAUC (μU/ml.3h)	7470.2 ± 5125.2	7378.2 ± 4516.8	92.1	-807.1 – 991.2	0.84
Body weight (kg)	86.2 ± 17.4	87.1 ± 17.7	-0.9	-1.3 – -0.4	<0.001
Daily activity energy expenditure (kJ)	4983 ± 2329 [2]	5254 ± 2324 [2]	-188	-658 – 282	0.43

Associations between test and outcomes assessed using mixed effects linear regression models. [1] Measures taken at acute meal challenge session 2 and 3. [2] Measures taken during test period. *Abbreviations: CI, confidence interval; h, hour; HDL-C, HDL-cholesterol; HOMA-IR homeostatic model assessment of insulin resistance; iAUC, incremental area under the curve; LDL-C, LDL-cholesterol; mins, minutes; SD, standard deviation; TAG, triglycerides.*

3.3.3.3 Postprandial TAG response

During the challenge session post intervention, a steady increase in postprandial TAG concentration was observed, which reached a mean (±SD) peak of 2.1 ± 1.6 mmol/l between three and four hours postprandially (Figure 3-3a). Visual examination of the time-course curve shows that TAG concentration did not return to fasting levels at the end of the session (mean: 1.6 ± 1.4 mmol/l at six hours postprandially). A similar postprandial response was observed at the challenge session post control, with no significant difference in postprandial TAG iAUC between the test periods (MD: 24.8 mmol/l.6h; 95% CI: -12.7 – 62.3; p= 0.20; Table 3-2).

3.3.3.4 Postprandial glucose and insulin response

During the challenge session post intervention, postprandial glucose concentration reached a mean (±SD) peak of 7.5 ± 1.3 mmol/l at approximately 45 minutes postprandially (Figure 3-3b). A steep decline was observed thereafter; and returned to fasting levels by approximately 90 minutes postprandially. Peak glucose concentration and time-to-peak were similar at challenge session post control (Table 3-2), however, visual examination shows that it took slightly longer to return to fasting levels (between 90 and 120 minutes postprandially). Overall, time-course curves observed during challenge sessions post intervention and control were similar, with no significant difference in postprandial glucose iAUC between the test periods (MD: -11.0 mmol/l.3h; 95% CI: -45.1 – 23.2; p= 0.53; Table 3-2).

At the challenge session post intervention, insulin concentration rose concurrently with glucose, reaching a mean (±SD) peak of 100.7 ± 48.5 μU/ml at approximately 45 minutes postprandially (Figure 3-3c). Visual inspection of time-course curve showed that insulin concentration at three hours postprandially did not return to fasting levels (mean: 38.1 ± 42.8 μU/ml), likely due to sustained postprandial TAG response. A similar postprandial response was observed at the challenge session post control, with no significant difference in postprandial insulin iAUC between the test periods (MD: 92.1 μU/ml.3h; 95% CI: -807.1 – 991.2; p= 0.84; Table 3-2).

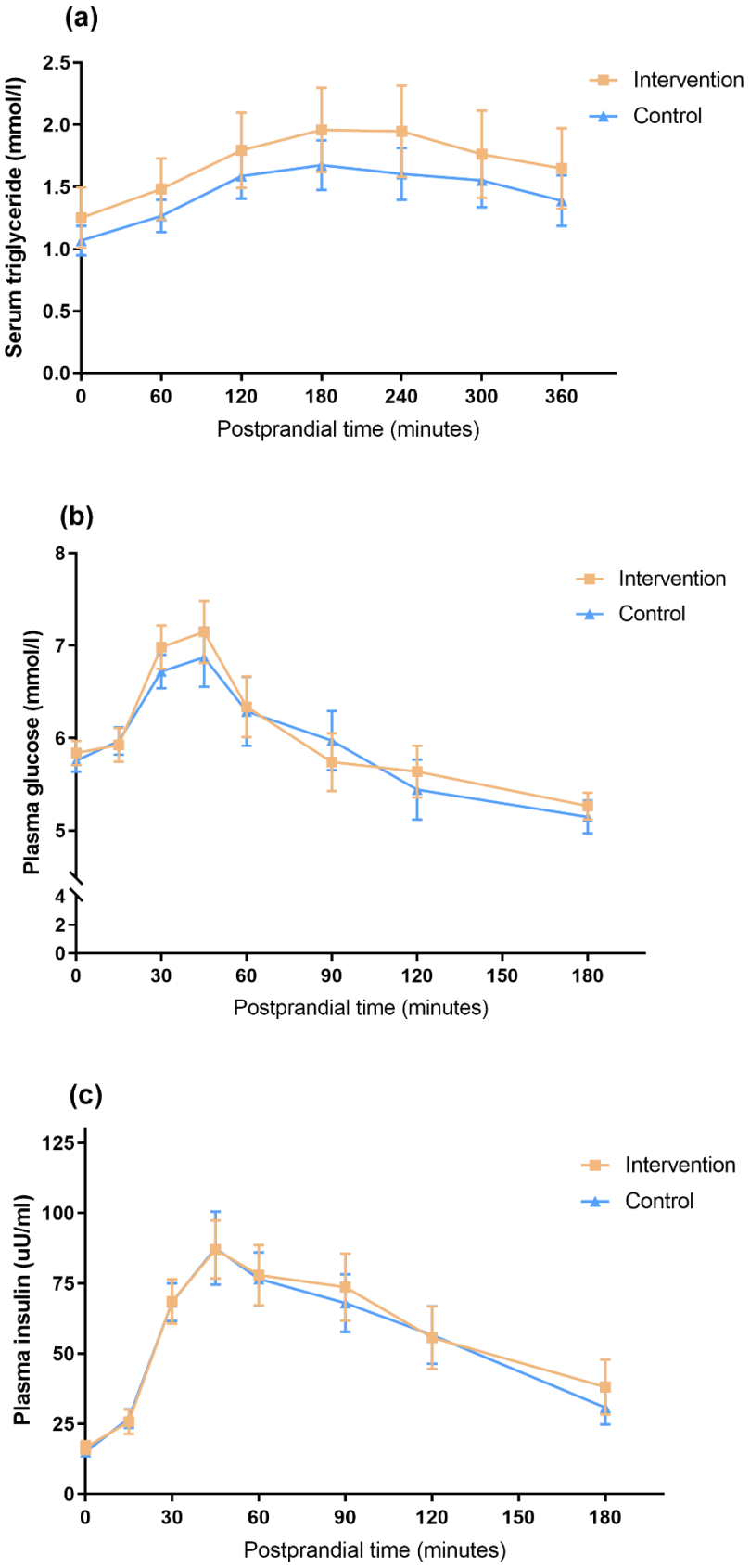

Figure 3-3. Postprandial time-course of triglyceride (a), glucose (b) and insulin (c) during acute meal challenge sessions conducted after intervention and control period.
Values expressed as mean ± standard error of means; n= 19.

3.3.3.5 Body weight and physical activity energy expenditure

Mean (±SD) body weight at the end of intervention was slightly lower compared to the end of control (MD: -0.9 kg; 95% CI: -1.3 – -0.4; p< 0.001; Table 3-2).

Activity EE during each test period was estimated from the SenseWear activity monitor (n= 17 participants). Participants wore the monitor for 108.4 ± 22.5 hours (approximately 4.5 days) in the intervention period and 92.1 ± 27.5 hours (approximately 3.8 days) in the control period. There were no difference in daily activity EE between the two test periods (MD: -188 kJ; 95% CI: -658 – 282; p= 0.43; Table 3-2).

3.3.3.6 Adherence

During the control period, 68 dietary recalls were collected (89% of expected total). During the intervention period, 73 dietary recalls were collected (96% of expected total). These were used to assess adherence to the period of fasting (0100h – 0600h). The overall adherence rate was 95%; with three non-adherence episodes recorded in three separate dietary recalls. Intervention adherence rate will be further explored in Chapter 3b.

3.3.3.7 Sample size estimation for future definitive RCT

Using the results obtained, sample sizes required for fully-powered definitive RCTs have been estimated (Table 3-3).

Table 3-3. Sample size estimations using data obtained, for future definitive randomised controlled trials.

Outcome	Observed SD of difference for values from same participant [1]	Observed MD for values from the same participant [2]	MD between intervention and control periods used for sample size estimation	Sample size estimated
Postprandial TAG iAUC (mmol/l.6h)	87	24.8	25	97
Postprandial glucose iAUC (mmol/l.3h)	79	-11.0	25	81
Peak postprandial TAG concentration (mmol/l)	0.8	0.2	0.5	23
Glucose concentration at 2 hours postprandially (mmol/l)	1.1	0.2	0.5	40
Body weight (kg)	1.3	-0.9	1	16

Estimations assume α= 0.05, β= 0.2 (or power= 80%). [1] Standard deviation based on data obtained in this pilot study. [2] MD based on data obtained in this pilot study, presented as treatment minus control.
Abbreviations: iAUC, incremental area under the curve; MD, mean difference; SD, standard deviation; TAG, triglyceride.

3.4 Discussion

'Shifting the Risk' is the first study to examine the effects of a meal timing intervention on CVD risk markers in night shift workers. We showed that it is feasible for night shift workers to maintain a small 5-hour overnight fast. Adherence to the 1am to 6am fast was high during the 4-week intervention period, with only three episodes of non-adherence reported and only one participant withdrawing due to inability to maintain the fasting period. Critical to a pilot, the practicality of outcome assessment methods was examined, all of which were tolerated and successfully completed by participants. Our estimation of a 30% attrition rate was confirmed; with 19 of 28 randomised participants completing the study. Whilst this pilot was not powered to detect significant changes in the primary outcome measures (postprandial blood TAG and glucose iAUC), findings indicate a small change in body weight at the end of the intervention period, warranting further investigation on the effects of meal timing on weight management in this population. Using the data generated in this pilot, we are able to provide sample size estimations based on blood TAG, glucose and body weight, which serves as a useful starting point for future meal timing interventions conducted in this population.

Examination of participants' baseline temporal eating pattern collectively, shows that while on night shifts, eating occasions occurred at all hours of the 24-hour day with no distinct fast period (31). This closely resembles the temporal eating pattern of night shifts observed during the control period, (Chapter 3b). In contrast, participants were able to adhere to the designated period of fast (0100h to 0600h) during the 4-week intervention period. This reduced the number of eating occasions during the night, creating a temporal eating pattern that is more aligned with the regulation of our circadian clock system (14). Metabolic disruptions associated with eating into the night have been reported in epidemiological studies. Using large population data sets that adjusted for traditional risk factors such as age and physical activity level, 'eating at night' has been associated with increased odds of being obese, in both Swedish (OR: 1.62) and Japanese cohorts (OR: 1.28) (32, 33). In the Japanese cohort of 61,364 healthy participants, 'eating at night' was also associated with increased odds of hyperglycaemia (OR: 1.13) (33). By applying statistical modelling on cross-sectional data, Chen et al showed that displacing 100 kcal of intake in the morning or noon to the night (2030h – 0459h), was associated with increased LDL-C levels in a healthy Taiwanese cohort (34). Furthermore, night shifts are typically characterised by frequent snacking of mainly discretionary food items and increased intake of sugar sweetened beverages (10, 12, 35), a finding we observed at baseline (31). The introduction of the overnight fast inadvertently reduced the intake of these discretionary items, at a time when lipid and glucose metabolism are least efficient (15, 16).

Exploratory analysis showed that body weight was slightly lower at the end of the intervention period, compared to the end of control, in the absence of changes in physical activity EE. This finding suggests that a short overnight fast may promote changes in energy expenditure. A number of diurnal studies have indicated lowered TEF after eating during the night, compared to during the day (17). It could therefore be speculated that participants in our study may have experienced a small reduction in body weight after the intervention period, by avoiding energy intake during the night, a time at which our body is less efficient at "burning" energy. Although the difference was small, it could be clinically relevant, if it is maintained in the long term by shift workers. For better understanding of this speculation, it would be valuable to incorporate measures of energy expenditure in future long-term meal timing interventions. In the context of the 'Shifting the Risk' protocol, the incorporation of TEF assessment during the acute meal challenge session may be a feasible addition, assessing one component of energy expenditure. This should be supported by a comprehensive assessment of usual dietary intake during the test periods, to distinguish the effect of energy intake on body weight changes, from the effect of energy expenditure. Considering the night shift worker population, a 7-day food diary may be required, to capture the potential differences in daily energy intake between shift types (11, 36, 37), as to provide an accurate proxy of usual daily energy intake. Lastly, future weight management studies in shift workers should consider harnessing the potential time of day difference in TEF, by incorporating a nightly fast window into their energy restriction regime. The combination of "how much" and "when to" eat is more likely to produce metabolic benefits that are of clinical significance.

Critical to a pilot study, is to assess the appropriateness of selected outcome measures and outcome assessment methods. Postprandial TAG and glucose responses were selected as primary outcome measures because they are stronger predictors of CVD in healthy individuals, compared to fasting TAG and glucose levels (38-40). A prospective cohort study of 26,509 healthy American women found that those in the highest postprandial TAG tertile at baseline (1.93 mmol/l) have twice the risk of developing CVD (HR: 1.98, median follow up: 11.4 years) compared to those in the reference postprandial TAG category of 1.18 mmol/l (38). This association was not observed with fasting TAG levels after adjustment with BMI and HDL-C levels. Moreover, a meta-analysis of 13 prospective cohort studies with non-diabetic participants reported that, postprandial glucose response has a positive linear relationship with CVD risk, from the ranges of 3.1 to 11.1 mmol/l. In contrast, the predictive effect of fasting glucose was capped at a threshold of 5.6 mmol/l (40). Postprandial TAG and glucose responses were therefore chosen as primary outcome measures in the current study, as participants were relatively healthy, with only one known risk factor of CVD (abdominal obesity), whilst fasting TAG and glucose levels were within healthy ranges. However, we acknowledge that due to the small

sample size, the minute difference in postprandial TAG and glucose responses between intervention and control might have been missed, leading to type II error. As such, future studies should consider including participants with higher risks of CVD, such as those with diagnosed metabolic syndrome (24). This population may be more likely to respond to the intervention, thereby maximising the magnitude of difference observed between intervention and control.

In this pilot, acute meal challenge sessions were conducted to assess postprandial outcomes. They were tolerated by participants and the test meal used successfully stimulated a metabolic response. Whilst we were able to capture the full postprandial glucose response (returned to fasting levels by two hours), this was not observed with postprandial TAG response (remained elevated at six hours). Therefore, the effect of the intervention on postprandial TAG clearance could not be properly assessed. Some studies have shown that postprandial TAG response can extend up to eight hours (41, 42). Extending challenge sessions beyond six hours is challenging, as it will increase participant burden. Peak postprandial TAG concentration, which occurred at three to four hours postprandially, may therefore be a more practical outcome to assess compared to postprandial TAG iAUC. This approach is supported by a meta-analysis of 113 studies, which showed that the plasma TAG concentration measured at 4-hours after an oral fat load was highest, compared to 2, 6 and 8-hours postprandially. The authors of this meta-analysis concluded that plasma TAG concentration measured at 4-hours postprandially is therefore most representative of the peak postprandial TAG concentration (43). Moreover, the aforementioned large prospective cohort study reported that postprandial TAG concentration measured at two to four hours postprandially had the strongest association with CVD risk (HR: 4.48), compared to other postprandial time periods (38).

As part of the protocol, participants were asked to attend all three challenge sessions two to three days after their last night shift. This was based on findings from Lund et al, who showed that elevated postprandial TAG response induced by a block of night shifts remained two days after the last night shift (44). Choosing this timeframe also reduced the independent effect that sleep deprivation has on glucose tolerance (45), which participants are likely to experience on their first day-off. However, approximately a quarter of participants were unable to attend the challenge sessions two to three days after their last night shift as per protocol; effects of the intervention may have diminished due to delayed outcome assessment. Acute diurnal studies have consistently shown that postprandial lipid and glucose metabolism are less efficient during the night compared to the day (15, 16). Considering this, perhaps outcome assessments should be conducted closer to the end of consecutive night shifts, so that the immediate effects of the intervention can be captured. In this case, peak postprandial TAG concentration may be better suited as an outcome measure compared to postprandial TAG iAUC, as it will be unlikely for participants to attend extended assessment sessions immediately after a night

shift. As such, we have provided sample size estimations using peak postprandial TAG concentration and postprandial glucose concentration at two hours, another postprandial risk marker of CVD commonly referred to in the literature (38, 40, 46-48).

The study design of 'Shifting the Risk' has numerous strengths. Prior to the intervention period, participants were provided with tailored advice on how to redistribute their meal times around the designated fast window, based on 4-day food diaries collected during the run-in period. The outcome assessment method was tightly controlled, with a standardised meal and fast prior to the challenge session, and a standardised test meal provided to all participants. During the intervention period, SMS messages reminding participants to fast were sent prior to each night shift, or at least on the first of a series of night shifts. Dietary recalls were collected once weekly as a method to assess adherence to the intervention, although the possibility of non-adherence on days when dietary recalls were not conducted should be acknowledged. The high SD and wide 95% CI is a limitation of our data, possibly due to high inter-participant variation. This may be attributed to participants' varied age, body weight and employment status, such as years in shift work, number of night shifts worked and shift schedule (i.e.: permanent night/ rotating). In order to account for this heterogeneity, larger sample sizes will be required, to allow for covariate adjustments in statistical analyses or additional subgroup analyses. Lastly, whilst we acknowledge circadian disruption from misaligned feed/fast, sleep/wake and activity/rest cycles have a composite effect on night shift workers' metabolic health (49, 50), we were unable to assess whether the current intervention could shift the participants' circadian rhythms and minimise circadian disruption. In order to assess an individual's circadian rhythm, extensive protocols are required, such as 48-hour urine collection protocol to measure melatonin rhythm (phase marker of master clock) (51) and constant routine studies to measure TAG and glucose rhythms (phase marker of peripheral clocks) (50). These protocols are time consuming and are likely to be burdensome for free-living night shift workers, hence were not included in this pilot.

3.5 Conclusion

In this pilot study, we showed that for shift workers who usually eat throughout the night whilst on night shift, it is possible to maintain a 5-hour overnight fast. This created a defined fast period during the 24-hour day, which was previously absent (31). Our pilot data suggest that reducing the number of eating occasions during the night may promote a small shift in body weight, which may be related to lowered TEF observed during the night. This warrants further investigation into the role of meal timing, in mitigating the metabolic consequences of night shift work. Sample size estimations have been provided using data generated, allowing future definitive RCTs to test whether an overnight fast is able to improve postprandial TAG and glucose metabolism in night shift workers. For future meal timing interventions examining CVD risk, postprandial TAG and glucose response remain as appropriate outcome measures to target. The assessment of energy expenditure would also be favourable, allowing for better explanations in weight changes that may occur. Any dietary interventions targeting the shift work population should consider "when" to eat in conjunction with "what" and "how much" to eat. Due to shift workers' atypical temporal eating pattern, meal timing is likely to play a significant role in the prevention of metabolic diseases in this population.

Acknowledgements: We would like to sincerely thank the National Heart Foundation (Australia) for funding this research. In addition, we would like to thank Dr Nina Eikelis (Swinburne University of Technology, Australia) for the biochemical analysis of insulin samples. Lastly, we would like to express appreciation to University of Ulster (Northern Ireland) placement student Jenny Robinson, for her assistance with day-to-day running of the study.

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Chapter 3 b. An exploration on the feasibility of the 'Shifting the Risk' protocol

Preamble

'Shifting the Risk' is the first intervention study to explore meal timing in free-living night shift workers. Due to its novelty, little was known about the feasibility of several aspects of the study protocol, and therefore will be explored in this Chapter. Undertaking a feasibility assessment on the pilot study's protocol allows researchers to elucidate any potential issues that should to be rectified before progressing to efficacy testing, thereby optimising chances of completion in any future efficacy trials (101). This is imperative, as failure to complete is one of the key contributors to financial loss in medical research (102).

Guided by Bowen et al's framework on feasibility studies, three feasibility focus areas are examined in this Chapter (103). Firstly, we assess whether the recruitment strategy and eligibility criteria are able to reach and recruit our target group of night shift workers. These aspects of a study protocol are recognised as key processes that determine success of trials (104). Secondly, the acceptability of the fasting intervention (i.e. fasting from 0100h to 0600h) is examined, as at the outset, it was unclear whether participants would be able to implement this intervention in their usual work environment. These findings inform whether this intervention is practical in the real world setting and whether it has the potential to be translated into a dietary recommendation (103). Lastly, we explore participants' reasons for participation and their intentions to continue the fasting intervention after study completion. These factors indicate the extent to which the intervention will be adopted in the community (103). Together with the generation of sample size calculations and the assessment of appropriateness of data collection methods in Chapter 3a, findings from this Chapter will inform the protocol of any future efficacy trials. Furthermore, it will provide areas of consideration for similar trials that involve changes in meal timing and/or night shift workers.

3b.1 Introduction

'Shifting the Risk' is the first study to examine the effect of meal timing manipulation on CVD risk factors of free-living night shift workers. Summarised in review by Neil-Sztramko et al, there is only a small number of health-related interventions targeting shift workers in the published literature (1). Amongst these, the majority aimed to improve sleep outcomes through interventions such as controlled light exposure and changes in shift rotation schedules; very few were nutrition-related (1). The few dietary interventions identified aimed to increase general healthy eating habits (e.g. increase fruit and vegetable intake, reduce fat intake), through provision of nutrition education and counselling (2, 3). Furthermore, the majority of dietary interventions involved all types of shift workers, including those who only worked day shifts (2), limiting the generalisability of findings to the night shift working population. A more recent systematic review by Phoi et al examined dietary interventions that exclusively involved night shift workers, and identified only five studies eligible for review (4). Three were acute interventions, which examined the effect of meals with varying macronutrient composition on cognitive or postprandial biochemical outcomes. These were conducted on single night shifts; providing minimal indications on long-term health implications. The remaining two studies identified were long-term interventions, which aimed to improve weight status and dietary intake of night shift workers. However, they were again, focused on education of general healthy eating principles, with no recommendations on meal timing (4).

The uniqueness of the 'Shifting the Risk' protocol and its potential to improve the health of an under-researched at risk population was recognised by the National Heart Foundation (Australia), whom provided funding for this study. To date, only one other study has examined the metabolic effects of an overnight fasting period in shift work. However, this study was conducted under simulated night shift settings within a laboratory, and only included four simulated night shifts (5). Whilst a difference in glucose tolerance was observed between the fasting and non-fasting conditions, the 12-hour fast (1900h to 0700h) involved in the fasting condition is not practical for free-living night shift workers. Night shift workers in the community, especially those who are involved in high-stress occupations such as paramedics, fire fighters and nurses, expressed that it is unsafe and detrimental to their health to not consume any food during their 8-hour shift (6-8). They indicated that fasting for an extended period would affect their judgement and work performance, and therefore, would typically make an effort to create eating opportunities during their shift. These factors were considered in the design of the 'Shifting the Risk' protocol; the fasting period (0100h to 0600h) is shorter compared to the simulated night shift study, and reflected a typical interval between main meals (e.g. between lunch and dinner). Moreover, the fasting protocol was implemented for a longer

period (i.e. four weeks compared to four simulated night shifts), to assess the long term effects of a nightly fasting period.

Due to the novelty of 'Shifting the Risk', little was known about the feasibility of the study protocol, as such, it was conducted as a pilot study. In the last decade, the value of pilot studies has increasingly been recognised. In 2009, a paper published in *The Lancet* reported that approximately 85% of investment into medical research is regarded as financial loss (9). With one of the contributing factors being unsuccessful completion of trials, attributed to poorly designed study protocols. Pilot studies are able to reduce this financial loss, by identifying potential problems in the study protocol that should be rectified before progression to efficacy testing, thereby optimising chances in completion of the efficacy trial (10).

Feasibility outcomes that are included in conventional reporting of randomised controlled trials were discussed in Chapter 3a, including participant retention rate and sample size estimation using pilot data obtained. Feasibility of the 'Shifting the Risk' trial is further explored in this Chapter. In a published framework, Bowen et al outlined eight aspects of a study protocol, for which feasibility can be examined (referred to as 'feasibility focus areas') (11). The authors explained that it is not necessary to assess all focus areas in a single feasibility study; researchers should instead select the focus areas with minimal existing evidence supporting their practicality, and are critical to the successful implementation of the particular study. Based on this recommendation, three feasibility focus areas were selected to be assessed in this Chapter. Firstly, the ability of the recruitment strategies and eligibility criteria to reach and engage our target population will be examined. Defined as 'process' outcomes (12), these are critical processes of a trial because they directly influence the sample size attained. Failure to reach the target sample size can lead to early termination, delay or unsuccessful completion of research trials (13). Secondly, the 'acceptability' of the fasting intervention will be assessed (11), via intervention adherence rate and an exploration of participants' experience of the intervention. The latter will be explored qualitatively, using semi-structured interview data collected from participants at trial completion. Exploring participants' perceived acceptability of the intervention provides an understanding on whether the intervention is practical to implement in the real shift work setting. Lastly, participants' reasons for partaking in the study and their intentions to continue the fasting intervention post study completion will be explored, via semi-structured interview data. Defined as 'demand' outcomes (11), these factors provide an indication on the level of intervention uptake by night shift workers in the community and the areas of health research that they perceive as important. This informs the ways in which health research can be promoted and pitched to the shift working population, in order to drive interest and recruitment.

There are minimal guidance in the literature on successful implementation of meal timing or shift work related interventions. In a recent literature review, Dashti et al suggested a myriad of factors that are able to facilitate the design of successful meal timing-based interventions, such as age, genetic predisposition, chronotype and sleep (14). Although comprehensive, the recommendations were based on a combination of observational and experimental studies, and were not specific to the shift working population. This emphasises the value of the current feasibility exploration, which provides insights into the challenges of conducting real world interventions in night shift workers. This is necessary when considering progression to a fully-powered efficacy trial, but is also valuable information for researchers conducting trials in this population.

3b.2 Methods

3b.2.1 Trial design

'Shifting the Risk' is a pilot randomised crossover trial conducted in Melbourne (Australia), between 2017 and 2018. The study is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12617000791336). Ethics approval was provided by the Monash University Human Research Ethics Committee (Project ID: 2017-8619). The study protocol of 'Shifting the Risk' is detailed in Chapter 3a.

3b.2.2 Participants

In brief, eligible participants were permanent or rotating night shift workers, aged between 18 and 60 years with abdominal obesity. Participant eligibility was assessed in two stages. Firstly, interested individuals were invited to complete an online screening questionnaire, to determine whether they worked night shift and assess eligibility based on age, shift work schedule and health status. Those considered eligible from the questionnaire were then invited to attend a screening session at our research facility, where waist circumference measurements were taken to confirm eligibility.

3b.2.3 Recruitment

Initially proposed in the funding application, an 8-month period was allocated for recruitment and data collection, based on recruitment rates into other research trials at Monash University. The recruitment target was 28 participants, with 20 to complete the study, accounting for a 30% attrition rate. The intervention was promoted as a study which aimed to investigate the relationship between meal timing and cardiovascular health of night shift workers. At enrolment, we reiterated to participants that the study is not a weight loss intervention. A financial reimbursement of $300 was provided to participants in three instalments, which was stated on all advertisements.

Numerous recruitment strategies were employed, including direct contacts with workplaces and utilisation of social media platforms. A member of the research team had existing contacts with management personnel of the Melbourne Emergency Services Telecommunications Authority (ESTA). Therefore, direct engagement was established with ESTA, who employ approximately 600 emergency call takers working rotating shifts. Advertisements were posted in their monthly newsletters and flyers were placed around the workplace. Cold contact was made with other shift work employing workplaces via email, including security services, aged care, printing services, steel manufacturers and hotels. Paid advertisements were placed on the online marketplace 'Gumtree' (Adevinta, Oslo, Norway) and social media platform 'Facebook' (Facebook Inc., California, United States). A target audience of 'Melbourne shift workers' was selected for Facebook advertisements. The advertisements

included a photo and a brief description, and were placed under two accounts including 'Monash Medicine, Nursing and Health Sciences' and 'Monash Nutrition'. Two Facebook advertisement formats were utilised; firstly, the 'traffic generation' format, which generated link clicks to the study's online screening questionnaire. Secondly, the 'lead generation' format, which prompted interested individuals to provide their contact details, for a member of the research team to follow-up. Advertisement was also placed on a Melbourne metropolitan radio station. Printed flyers were distributed to suburbs surrounding our research facility by a letterbox drop service. During the recruitment period, our research group prepared two blog posts, which disseminated research findings from Chapter 2. These were published on the Monash Nutrition Blog Post and the online magazine 'Monash Lens', and both made mention of 'Shifting the Risk' to enhance recruitment potential.

3b.2.4 Outcome measures and data analysis

3b.2.4.1 Study 'process' outcomes

Exploration of recruitment strategies

Several indicators were used to evaluate the performance of each recruitment strategy outlined in Section 3b.2.3. For clarity, the way in each indicator was derived is illustrated in Figure 3b-1, using the total number of eligible participants as an example.

Firstly, the total number of expressions of interest (EOI) was calculated. EOI includes the number of completed online screening questionnaires, as well as the number of individuals who enquired about the study (via email, SMS or telephone) but did not complete the questionnaire (n= 569). From the online screening questionnaires, we firstly excluded individuals who did not work night shifts, in turn identifying a total of 333 night shift workers. The proportion of night shift workers within the number of EOI was calculated as a performance indicator (333 ÷ 569 x 100 = 59%). Participant eligibility was further assessed against the remaining eligibility criteria (outlined in Table 3b-3). A total of 31 participants met all eligibility criteria. The proportion of eligible participants within the number of EOI was calculated as another performance indicator (31 ÷ 569 x 100 = 5%).

The advertisement cost associated with each recruitment strategy is listed in Table 3b-2. To calculate the 'total recruitment cost per randomised participant', we divided the total spending on recruitment by the total number of randomised participants.

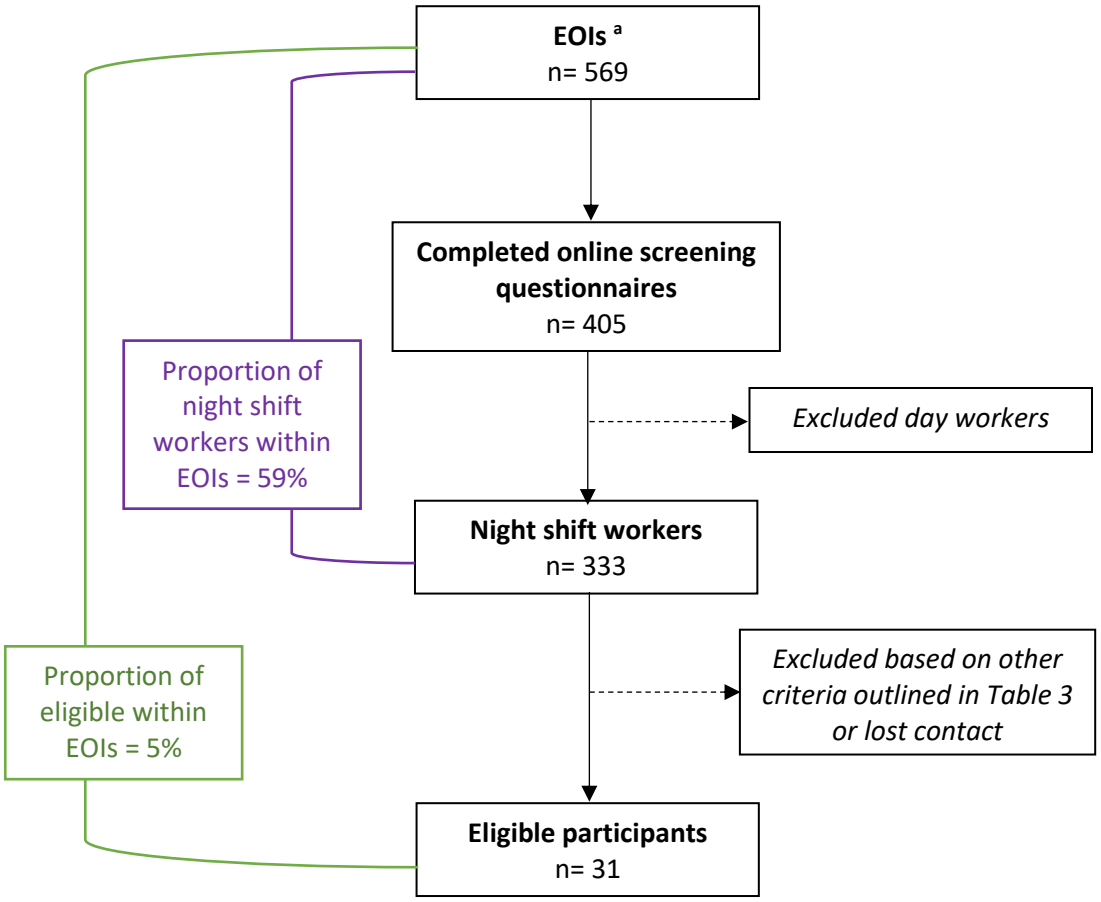

Figure 3b-1. Recruitment flow including all expressions of interests received for 'Shifting the Risk'; to demonstrate the way in which recruitment strategy performance indicators (Section 3b.2.4.1) was derived.

[a] Expressions of interest (EOIs) include completed online screening questionnaires, as well as enquiries received from SMS, email and telephone that did not proceed to completion of questionnaire.

Impact of eligibility criteria on recruitment rate

From the online screening questionnaire, a participant could be excluded on 18 potential eligibility criteria (Table 3b-3). In the screening session, eligibility is further assessed against three criteria. To assess the impact of eligibility criteria on recruitment rate, we divided the number of individuals excluded from each criterion by the total number of individuals excluded via the online screening questionnaire or the screening session. For example, 45 individuals were excluded because they worked less than 3 night shifts per fortnight. This was divided by the total number of individuals excluded from the online screening questionnaire, to denote the impact of the exclusion criterion (45 ÷ 258 x 100 = 17%).

3b.2.4.2 Participant 'acceptability' outcomes

Intervention adherence rate

Adherence to the fasting intervention was assessed via 24-hour dietary recalls conducted over the phone with a trained nutritionist. These were completed once weekly, after a night shift, during both intervention and control periods. Participants were asked to report their dietary intake from 0600h the day prior to 0600h of the current day. The proportion of dietary recalls collected in the intervention period, which documented nil energy intake between 0100h and 0600h, was calculated to denote the intervention adherence rate.

Participants' experience of the fasting intervention

Upon study completion, participants were invited to complete a semi-structured interview to share their experiences. In order to understand the breadth of their experience, four key areas were explored: (i) reasons for food intake during night shift, (ii) strategies implemented by participants to maintain the fasting period, (iii) participants' motivation to partake in the study and continue the fasting intervention after study completion and (iv) influences of their workplace environment on their ability to maintain the fasting period (interview guide in Table 3b-1). Study researcher (C.E.H), who had no prior contact with participants, conducted all interviews via the phone or in-person (in a meeting room at our research facility). Participants were reminded to speak freely and honestly regarding their experience, as this would provide an understanding of the study's feasibility. Interviews were audio-recorded and subsequently transcribed.

Interview data analysis was undertaken via an inductive coding process, to explore participants' experiences of partaking in this meal timing intervention. Data were coded independently by two researchers (J.J and S.P) to minimise bias, as author G.K.W.L conducted the intervention procedures. Author G.K.W.L facilitated discussions with the coders, to cross-check and define meaning of codes

generated, in order to develop the final codebook. The NVivo software (QSR International, Version 12.0, Melbourne; Australia) was used to manage and support data analysis.

Specifically pertaining to the outcome of 'acceptability' examined in this Chapter, two aspects were explored: (i) strategies implemented by participants that helped them maintain the fasting period and (ii) modifiable aspects of the study protocol that could potentially assist with maintenance of the fasting period. Author G.K.W.L grouped codes from the interview transcripts into categories; these were then combined into subthemes corresponding to the two aspects outlined above. Subthemes with illustrative quotes are presented in the Results.

3b.2.4.3 Participant 'demand' outcomes

The feasibility focus area of 'demand' was examined via semi-structured interview data, collected using the procedure outlined in Section 3b.2.4.2. Two aspects were explored: (i) participants' motivations and reasons to partake in the study and (ii) participants' motivations and intentions to continue the fasting intervention after study completion.

Table 3b-1. Interview guide for semi-structured interview completed with participants at study completion.

Key area (i): Reasons for food intake during night shift
1. What are some of the foods you ate on night shifts and why?
Key area (ii): Participants' experience of implementing the fasting intervention
2. Tell me what it's like working shift work.
3. Tell me about what night shift was like on the days you avoided food intake.
4. What are some of the things you did to help you change your eating times?
Key area (iii): Influences of their workplace break environment on their ability to maintain the fasting period
5. Describe your workplace environment and what options you have for places to go on your break. – What foods/ smells/ cooking occurs in these spaces? – How does it make you feel seeing/ smelling other people's food during your shift?
Key area (iv): Participants' motivation to partake in the study and continue the fasting intervention after study completion
6. What are some of the reasons why you got involved in this study?
7. How would you feel about restricting your eating times long term? – What should we consider to make this easier to follow in the future?

3b.3 Results

3b.3.1 Study 'process' outcomes

3b.3.1.1 Exploration of recruitment strategies

Recruitment began in July 2017 and data collection was completed in October 2018. Slow recruitment rate led to extension of the recruitment and data collection period, from the anticipated eight months to a total of 16 months. The online screening questionnaire was completed by 405 individuals, of whom 31 were confirmed to be eligible after the face-to-face screening session. Recruitment target was met, with 28 individuals randomised and 19 completing the study. Please refer to Chapter 3a for detailed participant flow.

Of the EOIs received, 59% was identified as night shift workers (Table 3b-2). Amongst the variety of recruitment strategies utilised, Facebook advertisement using the 'traffic generation' format (i.e. prompting interested individuals to visit the online screening questionnaire) reached the most number of night shift workers. Direct engagement with shift work organisation ESTA and the blog post on meal timing promoted by a social media Facebook boost were also effective, with 83% and 89% of EOIs identifying as night shift workers respectively. The blog post coupled with Facebook promotion was the quickest recruitment strategy, generating 85 responses in one week.

Of the 569 EOIs received, 31 participants were confirmed to be eligible (5%, Table 3b-2). Facebook advertisements ('traffic generation' format) identified the most number of eligible participants (n= 17). This was placed eight times during the recruitment period, with each round ranging from a week to a month. The total recruitment cost per randomised participant (n= 28) was $273.77.

Of the 23 EOIs received from employees of ESTA, 22% were confirmed to be eligible. This rate was the highest amongst all recruitment strategies, as recruitment through ESTA allowed for direct engagement with a group of night shift workers. Direct contact with shift work organisations also had the lowest recruitment cost, while radio advertisement was the most costly.

Table 3b-2. Number of expressions of interest received from each recruitment strategy utilised in 'Shifting the Risk', with corresponding proportion of night shift workers and eligible participants identified.

Recruitment Strategy	EOIs received (n) [a]	Online screening questionnaires completed (n)	Night shift workers identified (n)	Proportion of night shift workers within EOIs (%)	Confirmed eligible participants (n)	Proportion of eligible participants within EOIs (%)	Recruitment cost ($)
Direct industry engagement (ESTA)	23	22	19	83	5	22	0.00
Industry engagement through cold contacts	1	1	1	100	0	0	0.00
Gumtree advertisement [b]	14	9	4	29	1	7	39.00
Facebook paid advertisement (traffic generation) [c]	230	219	166	72	17	7	1856.49
Facebook paid advertisement (lead generation)	171	31	30	18	0	0	353.98
Blog post on Monash Lens	2	2	2	100	1	50	0.00
Blog post on Monash Nutrition Blog, promoted by Facebook	85	82	76	89	1	1	22.61
Letterbox drops of flyers	5	5	3	60	1	20	1543.47
Radio advertisement on Melbourne metropolitan radio station	38	34	32	84	5	13	3850.00
Total	**569**	**405**	**333**	**59**	**31**	**5**	**7665.55**

[a] EOIs (expressions of interests) include completed online screening questionnaires, as well as enquiries received from SMS, email and telephone that did not proceed to completion of questionnaire. [b] Two rounds were conducted. [c] Eight rounds were conducted. *Abbreviations: EOIs, expressions of interest; ESTA, Melbourne Emergency Services Telecommunications Authority.*

3b.3.1.2 Impact of eligibility criteria on recruitment rate

Of the 405 individuals who completed the online screening questionnaire, 258 were screened out (64%), based on one of eighteen exclusion criteria (Table 3b-3). A quarter of those excluded were not night shift workers (n= 72). A significant proportion of individuals were ineligible because of their shift work schedule or they had been engaged in night shift work for less than one year (total 35%, n= 91). Nineteen individuals (8%) were excluded, as they were already habitually fasting between the hours of 0100 to 0600h. A further 11 individuals were excluded after the screening session, primarily due to their waist circumference being below the cut-off (n= 8).

Table 3b-3. Number and proportion of total individuals screened out from the online screening questionnaire (n= 258) and screening session (n= 11).

Exclusion criteria	Excluded (n)	Proportion of total excluded (%)
Online screening questionnaire	**258**	
Not a night shift worker	72	28
Age >60 years	6	2
health-related		
BMI <20 kg/m^2	13	5
Diagnosed with medical condition (e.g. type-2 diabetes, previous ischaemic attack)	6	2
On pharmacotherapy that affects metabolism (e.g. for diabetes or hyperlipidaemia)	7	3
Lost >10% of body weight in previous 6 months	6	2
Gained >10% of body weight in previous 6 months	7	3
Pregnant, planning pregnancy or breastfeeding	2	1
Actively trying to lose weight	1	0
Work schedule and history		
Working <3 night shifts per fortnight	45	17
Working alternating blocks of night shifts and days shifts (i.e. does not have night shifts every week)	12	5
History of night shift work <1 year	34	13
Already routinely fast between 0100h and 0600h		
No habitual food intake between 0100h and 0600h	5	2
Sleep opportunities during night shift	2	1
Night shift hours do not encompass any hours within 0100h to 0600h	14	5
Study protocol related		
Unable to consume food provided in study (e.g. allergies)	4	2
Unable to travel to research facility	12	5
Unwilling to complete study protocol	10	4
Screening session	**11**	
Actively trying to lose weight	2	18
waist circumference below cut off	8	73
Diagnosed with medical condition (e.g. type-2 diabetes, previous ischaemic attack)	1	9

3b.3.2 Participant 'acceptability' outcomes

3b.3.2.1 Intervention adherence rate

During the control period (usual dietary intake), 68 dietary recalls were collected (89% of expected total). During the intervention period, 73 dietary recalls were collected (96% of expected total). These were used to assess adherence to the period of fasting (0100h – 0600h). The intervention adherence rate was 95%. Amongst dietary recalls collected during the control period, 112 eating occasions (≥ 1kJ) were recorded between 0100h to 0559h; compared to only nine during the intervention period (Figure 3b-2). Six of these nine occasions occurred at 0100h (i.e. right at the start of the fasting period), the remaining three were non-adherence episodes recorded in three separate dietary recalls. The reasons given for these were consumption of caffeinated beverages to maintain wakefulness and fresh produce tasting for work purposes. During the intervention period, high frequencies of eating occasions were recorded in the periods of 0000h to 0059h and 0600h to 0659h (Figure 3b-2). Participants may have included eating occasions during these time periods, to prepare for or to break the designated period of fasting.

Examination of the temporal eating pattern during the control period (Figure 3b-2) shows that eating occasions occurred least frequently between 1000h and 1559h, likely coinciding with sleep episodes after night shifts. No distinct fast periods were observed, with eight eating occasions between 1100h and 1159h being the lowest frequency recorded during the 24-hour period (accounting for all dietary recalls). During the intervention period, frequencies of eating occasions remained low between 1000h and 1559h, except for a spike at noon.

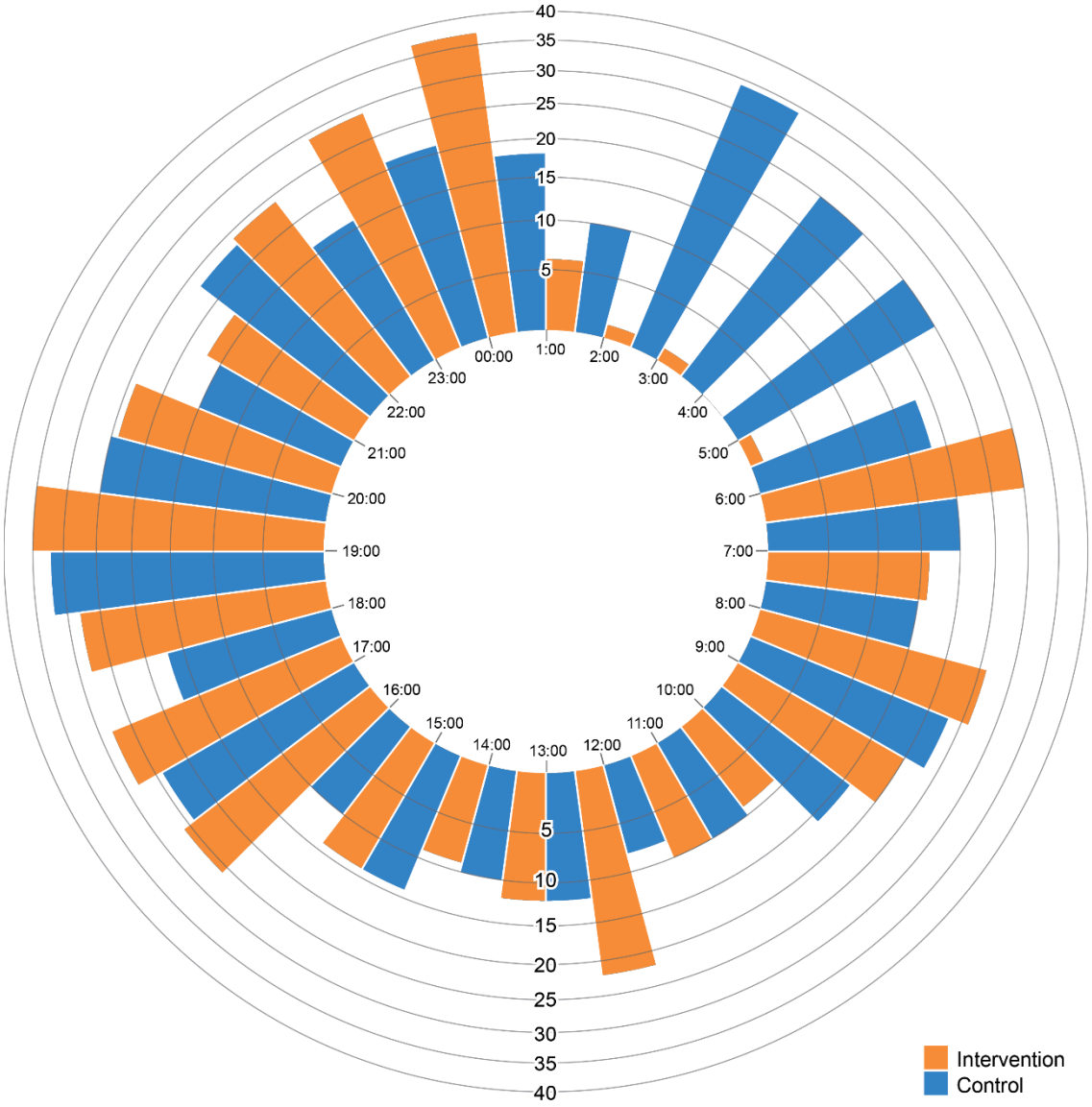

Figure 3b-2. Radar plots of eating occasions (≥ 1kJ) by time-of-day (24-hour time) during intervention and control periods; n= 19 participants.
Data collected via weekly 24-hour dietary recalls, conducted after a night shift (71 recalls in intervention, 68 recalls in control). Circular axis of the plot represents the clock time of the 24-hour day. Spoke axes indicate cumulative frequency of eating occasions during each hour (i.e. sum of eating occasions across all recalls at that time). For example, within all dietary recalls collected during the intervention period, 20 eating occasions were recorded at 0900h.

3b.3.2.2 Participants' experience of the fasting intervention

Of the 19 participants who completed the study, 17 participants agreed to be interviewed (11 women and 6 men), with age ranging from 25 to 55 years. Most participants were employed as healthcare professionals, labourers or hospitality workers, with years of experience in night shift work ranging from 14 months to 28 years.

Majority of participants expressed that fasting from 0100h to 0600h was "*quite easy*" and "*doable*". Some participants required time to adjust to the intervention and reported that maintenance of the fasting period gradually became easier. There were common strategies implemented by participants that helped them maintain the fasting period. However, a few common challenges were described, which could be addressed through modifications of the fasting intervention protocol.

Subtheme 1: Strategies implemented by participants

Participants typically attempted to have a meal or snack close to 0100h, in order to prepare for the fasting period, a finding supported by Figure 3b-2. As such, the highest number of eating occasions were recorded between 0000h and 01000h during the intervention period. Participants reported that they usually created this eating opportunity by delaying their main evening meal, or advancing the meal or snack they previously had during 0100h to 0600h. Adherence was enhanced by food choices that were more satiating or larger than the usual snack choice during 0100h to 0600h. While others preferred to have a snack at this time, but have a larger serving during their main evening meal. Planning ahead and establishing set meal times helped participants implement this new temporal eating pattern.

"It's just about being mindful really, that ok, I'm going to strategically time my eating of my dinner, um, so that I'm not having coffees or anything after midnight. You know, by 11:30[pm], that's it." (P01)

A common strategy used by participants to prevent or curb feelings of hunger was to drink large amounts of water. Majority of participants expressed that feelings of hunger were bearable or in some cases absent, as the fasting period was only five to six hours in duration, given that they could arrange meal breaks close to 0100h and 0600h.

Some participants expressed that it was the individual's responsibility to have the *"willpower"* to maintain the fasting period. This *"willpower"* was derived from their commitment to the research study, that there were *"rules"* to abide by and it will come to an end after four weeks.

Subtheme 2: Inability to tailor meal breaks to fasting period

The structure and duration of meal breaks varied greatly amongst participants and were often dictated by their workplace management and/or workload during the shift. As a result, many expressed difficulties in scheduling meal breaks close to 0100h in order to prepare for the fasting period and/or just after 0600h to break the fasting period. For some participants, this led to an extended period of fasting and increased feelings of hunger.

"Your intervention was between 1[am] and 6[am], so and depending on what breaks I had, I might not have been eating until 7[am], or just after 7 o'clock so I could be fasting for nearly 14 hours. Which… some mornings is difficult." (P07)

For participants who experienced such extended periods of fasting, the combination of hunger and fatigue affected their mood and ability to focus at work.

Subtheme 3: Importance of caffeine

Majority of participants expressed the desire to have tea and/or coffee during the fasting period. They indicated that avoiding caffeinated beverages was more challenging than abstaining from food.

"I drink a lot of black tea, and that was one… probably the biggest thing, more than the chocolate, that I missed." (P014)

Typically, caffeinated beverages were used as means to stay awake during night shifts and also during the commute home from work, which was essential to ensure safety while driving. For some, hot caffeinated beverages provided relief from the stresses of night shift work.

3b.3.3 Participant 'demand' outcomes

Participants indicated that health-related concerns prompted their participation in this research study. Accordingly, majority expressed that the biggest motivator to continuing this fasting intervention after study completion, would be seeing improvements in tangible health outcomes.

Subtheme 1: Health concerns prompted participation

Participants with abdominal obesity were recruited for this study, and most indicated that concerns of weight gain, CVD and T2DM prompted them to participate in this research study. Concerns regarding weight gain were particularly evident, with many participants reporting to have experienced weight gain since starting shift work and that prevalence of overweight and obesity is high in their industry.

"We're all like, with our shift work, we're all prone to being a little chubby." (P012)

Some participants also indicated that education around the adverse health effects of eating at night may encourage shift workers to implement and maintain dietary interventions. Numerous participants explained, *"You [humans] don't normally eat after midnight"*, so the fasting intervention in this study was sensible and made sense.

Subtheme 2: Perceived benefits of participation

For some, the intention to *"help out"* was the main reason for their participation. Financial reimbursement was not a key motivator to partake in this study, as they would earn an equivalent amount from a few hours overtime at work, which was commonly available.

Some participants indicated that they intended to continue the fasting intervention after study completion because it made them *"feel better"*, referring to improvements in gastrointestinal symptoms during night shifts and sleep quality after night shifts. However, most reported that they were unlikely to continue, as they did not observe any overt health benefits. When asked about the benefits that they would like to see, most indicated weight loss, even though they were aware that this was not the objective of the current study. It should be noted that even though results from Chapter 3a indicated a small amount of weight loss after the intervention, participants did not report noticing this change and this finding was not disclosed to them during the interview. Some participants also voiced that rather than generalised research findings, a personal and *"direct benefit"* would need to be observed, to encourage them to continue the fasting intervention.

3b.4 Discussion

This Chapter examined the feasibility of the 'Shifting the Risk' protocol and identified important areas of consideration for the implementation of any potential future efficacy trial. Our recruitment target of 28 participants was achieved; however, the recruitment period was extended due to slow recruitment rate. Amongst all recruitment strategies utilised, paid advertisements via social media platform Facebook and direct engagement with shift work organisation (ESTA) identified the most number of eligible participants. From interested individuals who completed the online screening questionnaire, a significant proportion of individuals were ineligible because they worked less than three night shifts per fortnight or had less than one year of night shift work experience. The acceptability of the fasting intervention was assessed via dietary recalls and interview data, which confirmed that participants were able to maintain the fasting intervention during the 4-week intervention period. To enhance adherence, majority of participants restructured their temporal eating pattern to include a meal or a snack immediately prior to the fasting period (i.e. between 0000h and 0100h). Although high adherence rate was recorded during the study, long term sustainability of this fasting intervention remains unclear. Participants expressed that this would require support from their workplace management, through allowing flexibility in meal break scheduling. Furthermore, participants highlighted health concerns as the major driver for participation in health research, and expressed that observation of tangible health benefits (e.g. weight loss) would promote long term maintenance of dietary changes.

In order to comprehensively evaluate the study protocol's feasibility, the results will be considered using the *'Can it work? Does it work? Will it work?'* feasibility framework proposed by Bowen et al (11). *'Can it work?'* explores whether there are indications that the study protocol and/or dietary intervention might work. The ability of the recruitment strategies and eligibility criteria to reach the target sample size will be summarised, as an indicator of *'can the study protocol work'*. Participants' acceptability of the fasting intervention will be discussed, as an indicator of *'can the fasting intervention work'*. The second feasibility question of *'Does it work?'* refers to the efficacy of the intervention, i.e. whether maintaining a fasting period from 0100h to 0600h for four weeks is able to improve night shift workers' metabolic health, which is the focus of Chapter 3a. Lastly, the question of *'Will it work?'* examines whether the dietary strategy proposed will be effective in real world contexts and settings. To answer this, the second part of this discussion will explore the potential for this fasting intervention to be implemented and maintained within the workplace setting.

3b.4.1 Can it work?

3b.4.1.1 Can the recruitment strategy reach our target population?

The recruitment protocol was effective, as the target sample size of 28 participants was reached. However, due to slow recruitment, the recruitment period was extended from the anticipated eight months to a total of 16 months. Amongst all implemented recruitment strategies, direct industry engagement with ESTA and social media Facebook paid advertisements (traffic generation format) identified the highest number of eligible participants, because they provided a targeted audience. Consistent with previous literature (15), direct industry engagement relied upon existing relationships between members of the research team and workplace management personnel, while cold calling companies had little success. When considering recruitment from only a single workplace, researchers should consider its effects on external generalisability and internal validity of findings. While homogeneity regarding shift work structure and workplace environment will be optimised, external generalisability of findings to other occupations and workplaces may be limited. In comparison, paid advertisements via social media Facebook allowed us to identify shift workers from a variety of occupations. This was achieved as Facebook campaigns have a wide reach, but concurrently allow specifications of target audience using broad characteristics (e.g. 'nurse' or 'night shift'). The effectiveness of social media strategies in recruiting participants for health research is becoming increasingly recognised (16, 17), with Facebook paid advertisements being the most effective in targeting individuals with specific characteristics (16). In addition to its potential reach, these two recruitment strategies also differed in the recruitment costs associated. Whilst industry engagement with ESTA had no direct costs, paid Facebook advertisements were the second most costly recruitment strategy we utilised. Overall, the recruitment cost per randomised participant was $273.77, a factor that should be considered in the budget of any potential future efficacy trial.

Night shift workers' interest in the current dietary intervention is indicated by the high number of EOIs received during the recruitment period. Interview data suggest that health concerns may be the reason for this interest; particularly shift work related weight gain, which was either experienced personally or observed from colleagues. The observation of health concerns as the driver for study participation can be explained by the Health Belief Model (18). According to this Model, 'perceived severity' and 'perceived susceptibility' of a disease are factors that prompt behaviour change. Accordingly, participants may have been motivated to partake in the study (i.e. adopt behaviour change), because they were aware of the detriments of weight gain (i.e. 'perceived severity') and have recognised that they are at high risk of weight gain due to their work (i.e. 'perceived susceptibility'). Therefore, future dietary interventions targeting night shift workers should consider focusing on

tangible health outcomes (e.g. weight loss) as outcome measures, to increase engagement. In addition, participants expressed that education around the adverse health effects of night time eating may promote participation in research studies such as 'Shifting the Risk'. This was observed when we published a blog post, indicating health implications of night shift work (results from Chapter 2), which led to a sharp increase in EOIs in the several days following.

3b.4.1.2 Can the eligibility criteria engage the target population?

The eligibility criteria of 'Shifting the Risk' were designed to optimise homogeneity within the sample, which is essential in order to properly assess efficacy of the fasting intervention. However, a number of the exclusion criteria had an impact on recruitment rate. A considerable proportion of identified night shift workers were excluded because their shifts did not encompass hours between 0100h and 0600h, or they already avoided food intake during this period. When the protocol of 'Shifting the Risk' was developed, literature suggested that during the night, food intake between the hours of 0100h and 0600h was the most detrimental to health (19, 20). This was based on constant routine studies, which indicated a natural rise in plasma glucose levels during this time period, while food intake, sleep and activity were kept constant (19, 20). However, findings from more recent diurnal studies, such as that conducted in my Honours Thesis, have shown that food intake as early as 2000h in the night leads to a higher glucose response compared to the morning (21, 22). This acute metabolic disruption of night time eating has been confirmed in the meta-analysis performed in Chapter 2, indicating that food intake during the night (anytime between 2000h and 0400h) leads to an increased glucose and insulin response compared to the day (anytime between 0700h to 1600h). These advances in research suggest that a fasting period implemented anytime during the dark hours are likely to have metabolic benefits, as it will reduce the exposure to glucose excursions. Therefore, in any potential future efficacy trial, the criterion relating to night shift work hours may become more flexible.

Similarly, criteria relating to the shift work schedule and history could also become less restrictive. Results from this Chapter showed that the largest proportion of ineligible individuals were those who worked less than three night shifts per fortnight or had night shift work experience of less than one year. As explained above, results from Chapter 2 showed acute metabolic consequences from a single meal consumed during the night. As such, night shift workers who work less than three night shifts per fortnight may also benefit from an intervention such as 'Shifting the Risk', because any reduction in exposure to glucose excursion is favourable. However, the intervention's effect on this group of shift workers may be more subtle, compared to those who work night shifts frequently. This hypothesis needs to be validated in future studies, ideally one with a sufficient sample size to allow for subgroup or dose-response analysis, based on number of night shifts worked. Furthermore, a high

number of interest was received from night shift workers with less than one year experience. This may be an indication of a lack of guidance in supporting transition to shift work. In an attempt to adjust to the shift work lifestyle, those in their first year of shift work may experience significant disruptions to their eating and sleeping habits. Described in an Australian study, nurses who have had 3 to 6 months of shift work experience reported an increase in unhealthy eating habits and changes to their temporal eating patterns (23). Furthermore, a group of newly employed Brazilian physicians, who have worked shift work for at least 7 months, have reported observing substantial weight gain since starting shift work (24). Referring to the Health Belief Model, individuals newly engaged in shift work may have an increase in 'perceived severity' and 'perceived susceptibility' of the detriments of shift work whilst adapting to the shift work lifestyle (18). This increases their motivation to adopt health behaviour changes and hence the seeking of dietary interventions. Future dietary interventions involving night shift workers should consider harnessing the increased demand and interest from this group of shift workers, and address the gap in nutrition advice that supports shift work transition.

3b.4.1.3 Can free-living night shift workers follow the fasting intervention?

A high intervention adherence rate was recorded from the dietary recalls, indicating that participants were able to follow the fasting intervention during the specified 4-week period of the study. However, long term sustainability of the fasting intervention is unclear, as indicated through findings from participant interviews. Participants reported that they were unlikely to continue the intervention after study completion, due to the lack of tangible health improvements observed. Studies have indicated 'receiving feedback on the outcome of behaviour' as an important motivator for long term (≥ 12 months) maintenance of dietary interventions in overweight and obese adults (25). Moreover, participants of 'Shifting the Risk' may have had low incentives to continue the intervention, as it did not address the health concerns that prompted them to participate. At study completion, participants indicated weight gain as their major health concern and reason for participation, despite being informed at enrolment that this was not the study's aim. The Theoretical Framework of Acceptability suggests that after partaking in a healthcare intervention, participants evaluate its acceptability and value of maintenance based on seven constructs, one of which is whether the expected purpose was achieved (26). Observational studies have shown an increase in the prevalence and risk of overweight and abdominal obesity in night shift workers compared to day workers (27, 28). Therefore, weight loss may be an ideal objective for future dietary interventions involving night shift workers, as it aligns with this population's demand and interest.

3b.4.2 Will it work?

As 'Shifting the Risk' was conducted in free-living night shift workers, it provided a unique opportunity to examine whether meal timing interventions can be translated into real workplace settings. Although the dietary strategy recommended in 'Shifting the Risk' was simple in theory, i.e. maintain a fasting period between 0100h and 0600h, participants still experienced challenges during implementation. As indicated by findings from the interviews, inflexibility of meal break schedule was a common challenge faced by participants, which hindered their ability to arrange meal breaks to prepare for the fasting period. Facing barriers that cannot be overcome by individual efforts reduces participants' self-efficacy, which in turn affects their perceptions of intervention acceptability (26). This finding indicates the need for support from workplace management, in order to optimise success in dietary interventions involving night shift workers. In a Scientific Statement on population approaches to improve eating habits, the American Heart Association recommended that effective workplace interventions require changes at the environmental level, while the provision of nutrition information alone is unlikely to improve dietary habits (29). If organisational support in manipulation of meal break scheduling cannot be obtained, flexibility would need to be introduced into the fasting protocol of 'Shifting the Risk' to suit individual work schedules, to optimise acceptability and feasibility. As discussed in the previous section, current evidence suggests that a fasting period implemented during any time of the night have potential to improve metabolic health. Introducing flexibility into the fasting period may also increase recruitment rate, as it eliminates the exclusion criterion on requiring work hours to encompass 0100h to 0600h.

Another advantage of workplace interventions is the provision of a targeted audience for recruitment. Previous workplace interventions in shift work prevalent settings such as manufacturing plants (30, 31) and hospitals (32, 33) have attained sample sizes of 110 to 850 participants. These interventions also included group-based components, such as team challenges and group education sessions. This may have led to higher levels of participation, compared to the direct engagement with ESTA established in the current study, which only utilised this shift work organisation as a recruitment site. As observed in previous workplace interventions, engagement with industry is difficult to establish, with majority of employers expressing a lack of interest in healthcare interventions, due to the absence of direct benefits (34). If cost-benefit analysis is incorporated into dietary interventions, work place engagement may be forthcoming. Studies have shown that workplace nutrition and physical activity interventions are able to elicit positive influences on work-related outcomes. In a U.S study, male manufacturing workers (n= 4189) were offered a selection of health promotion programs, including health screening, on-site wellness programs and telephone health coaching, during a 6-year period (35). Those who did not participate in any programs during the 6-year period had a higher

increase in absent days per year (1.2 days/participant), compared to those who participated in one or more programs. Considering the number of program participants (n= 2596) and the daily wage ($200), this attributed to an annual saving of $623,400. In a smaller study with U.S. aged care workers (n= 72), results showed that the 28-week weight loss intervention led to an increase of work productivity of 10 to 30% (36). Research in this area that specifically involves night shift workers are scant but emerging. As observed in an Australian intervention of manufacturing shift workers, in addition to achieving the primary outcome of weight loss, a reduction of absenteeism was also reported after the 14-week intervention (37). Evidence seems to also suggest potential improvements in work safety, with the maintenance of an overnight fast, under simulated shift work conditions, resulting in a lower number of crashes in driving simulations conducted during the night (38). In addition to being beneficial to employers, improvements in these work-related outcomes may also be recognised by night shift workers as tangible health benefits, which our participants indicate as important motivators to the participation of dietary interventions and maintenance of behaviour change.

3b.4.3 Strengths and limitations

Examination of feasibility is a key purpose of pilot studies (10, 12, 39). Not only can it inform the protocol changes required in any potential future efficacy trial, it can also provide valuable considerations to research groups who are conducting similar work. To the author's knowledge, there has been no exploration of feasibility in real-world shift work interventions, despite a general consensus that this population is difficult to engage in health research (40, 41). A combination of quantitative and qualitative assessment methods were used, providing an in-depth exploration. While quantitative assessment generated objective data on the intervention's acceptability and demand, participant interview data were able to provide context and potential explanations to these observations.

Two limitations should be acknowledged for this feasibility exploration. The number of interests generated by each recruitment strategy was an estimate based on the start and end dates of strategy implementation. Only approximations could be derived, as there were overlaps in start and end dates of some recruitment strategies (outlined in Appendix L). We were unable to ascertain the exact strategy from which each interest was generated, as not all interested individuals completed the online screening questionnaire. Secondly, participants who withdrew during the study (n= 4) were invited to complete the interview, but none agreed to partake. One participant withdrew because they could not maintain the fasting intervention and another could not complete the study procedures. They are likely to have a different perspective of the study and the fasting intervention's feasibility, which we were unable to capture.

3b.5 Conclusion

This Chapter is the first to explore the feasibility of a dietary intervention conducted in free-living night shift workers. The recruitment strategies successfully attained the target sample size, with strategies that provided a targeted audience engaging the most number of eligible participants, including social media Facebook paid advertisement and direct engagement with shift work organisation ESTA. Future research should consider capitalising on the increased interest in health-related interventions from individuals who are new to shift work, or engaged in night shifts infrequently. While our participants showed that they were able to follow the fasting intervention during the study period, its sustainability is currently unclear and likely requires workplace support and observation of tangible health improvements to motivate long term maintenance. An incorporation of cost-benefit analysis into dietary interventions may promote engagement of workplace management. If workplace support cannot be obtained, researchers need to consider the influence of workplace barriers on participants' self-efficacy of implementation. Weight loss was identified as the preferred outcome of interventions; therefore, future dietary interventions should consider incorporating this component and promoting studies from this angle. This Chapter has informed important learnings that should be considered in future real-world night shift worker interventions, in order to optimise participant acceptability and chances of trial completion.

Acknowledgements: We would like to acknowledge Monash University nutrition students Jessica Jong and Sophie Page for their assistance with data analysis of interviews.

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Chapter 4 . Australian night shift workers' experience with food: A photovoice study

Preamble

Findings from cross-sectional studies have shown that night shift workers have a poor diet quality, compared to day workers. An Australian study involving 118 shift workers across multiple industries reported that permanent night shift workers have the highest saturated fat intake amongst all types of shift workers (permanent morning, permanent night, rotating) (72). Moreover, studies involving nurses (93) and fire fighters (68) suggest that this may be related to increase snacking of discretionary foods during night shift. Based on findings from Chapter 2, eating during this time has additional metabolic implications, such as increased postprandial glucose and insulin response. Further, shift workers' intake of core foods such as vegetables are also lower than that of day workers, as reported in both Brazilian bus drivers (73) and physicians (105).

In order to develop effective health promotion strategies that improve the diet quality and temporal eating pattern of night shift workers, there is a need to understand the drivers of their food choices and eating habits. A small number of qualitative studies have indicated that social and environmental factors, such as colleagues' influence and meal break schedules at work, have a substantial impact on workers' food choices and eating habits (23, 68, 94). This was reflected in findings of Chapter 3b, whereby inflexibility of meal break schedule led to challenges in implementation of the meal timing intervention (i.e. fasting from 0100h to 0600h). This Chapter reports on a qualitative study, which further explores the influences of night shift workers' food choices and eating habits. Evidence on this is minimal in the Australian context; and is required to help researchers understand elements of effective and sustainable dietary interventions and health promotion strategies targeting Australian night shift workers.

As this is a qualitative study, an elaborated statement of researcher positioning is presented below, which describes the researcher's rationale for conducting this research. This Chapter has been prepared as a manuscript for submission to *Health and Place*.

Researcher positioning

The researcher's epistemological positioning is grounded in pragmatism. Research centred in this paradigm focuses on finding practical solutions to societal problems (106). The problem examined in this Thesis is the increased risk of metabolic diseases experienced by night shift workers. Through the lens of pragmatism, the value of knowledge is determined by its ability to create useful actions to solve the problem. Therefore, knowledge constructed through quantitative and qualitative methods are equally valuable, as long as they are able to address the problem (106, 107). This worldview aligns

with the aim of this Thesis, which is to address the problem of increased metabolic disease risks in night shift workers, through identifying practical strategies that will mitigate the metabolic impact of eating during the night. Quantitative methods (Chapter 2 and Chapter 3a) were used to determine dietary strategies that are of metabolic benefit. Building upon this, the addition of qualitative methods in Chapter 3b and the current Chapter supports development of dietary interventions and health promotion strategies that are feasible and practical.

The researcher is professionally trained as a dietitian and has been engaged in shift work research for the past six years. The research question of this qualitative study arose from recounts and stories told by night shift workers involved in the 'Shifting the Risk' study (Chapter 3). Each participant was involved in the study for approximately three months. During this time, the researcher made frequent contacts with participants, including the collection of weekly 24-hour dietary recalls and outcome assessment sessions at our research facility. When participants described their food intake, it was usually followed by reasons for their food choices. The researcher recognised that for the majority, these reasons were unrelated to health concerns, but rather, a myriad of social and environmental factors such as influence of workplace culture and food availability at work. During the completion interviews of 'Shifting the Risk', participants also reported a range of individual, social and environmental factors, which influenced the ease of intervention implementation (Chapter 3b). Reflecting on personal experience from working shifts in hospitality for nine years, the researcher recognised that she often engaged in unhealthy eating practices while at work, despite having adequate nutrition knowledge from her training as a dietitian. This was due to influences of the workplace environment, such as discretionary food items being readily available, the lack of facilities and time to prepare food during meal breaks. It seemed apparent that night shift workers' dietary habits are influenced by various unique individual and external factors, which need to be considered in order to develop effective dietary interventions and health promotion strategies for this population.

4.1 Introduction

Epidemiological studies have shown that shift workers are at a 20 to 40% increased risk of metabolic diseases such as CVD, T2DM and obesity (1-7). The definition of 'shift work' varies across countries, but is typically used to describe work that encompasses hours outside of the usual daytime range of 0800h to 1700h (8). Amongst industrialised countries, approximately 20% of employed individuals are engaged in this type of work (8). In Australia, 1.4 million individuals are classified as shift workers, who work across multiple industries and occupations, including healthcare, hospitality and transport (9).

The atypical working hours of shift work leads to a temporal eating pattern, that is misaligned with the regulation of our circadian clock system (10). As with many living organisms, humans have internal body clocks, which govern behavioural and physiological processes. As diurnal animals, humans are programmed to eat and work during the day, while night time is reserved for rest and fasting (11). However, night shift workers often adopt an irregular eating pattern, with frequent small meals or snacks throughout the 24-hour day, rather than the typical pattern of three main meals a day (12-14). Snacking during night shift is common, characterised by foods that are high in sugar and fat, such as discretionary snacks and sugar-sweetened caffeinated beverages (12, 15). It has been speculated that the combination of eating the 'wrong food' and at the 'wrong time' is one of the causal factors to shift workers' increased risk of metabolic disease. This is supported by findings from a meta-analysis, reporting that shift workers' overall daily energy intake is similar to that of their day-working counterparts (16).

It is evident that night shift workers' eating habits are different to that of day workers, likely contributing to their increased risk of disease. Eating at night causes circadian misalignment, leading to a reduction in glucose tolerance (17, Chapter 2) and an increase in postprandial lipid response (18). Yet, there are currently no tailored dietary guidance for this population. In order to develop guidance that is practical and achievable, it is important to understand the drivers behind what and when they eat. A recent narrative review collated studies involving night shift workers across different industries, and found that eating to stay alert, to maintain good health and with colleagues as a social function were the three key influences of workers' eating habits during night shifts (12). In Australia, there has been a few qualitative studies exploring factors that shape night shift workers' eating habits at work, with each involving a discrete occupation (15, 19-21). Across these studies, work structure and workplace environment related to the specific occupation were the key influences of eating habits. For example, Queensland paramedics reported that their work environment (i.e. the ambulance) limited their food choices during shift to those that were transportable and non-perishable (19). Moreover, interrupted meal breaks were often observed in healthcare professionals such as nurses

(21), leading to consumption of unhealthy foods and overeating when they were given an opportunity to take a break. Lastly, in occupations with well-established group cultures, the attitudes and food choices of co-workers' could also influence individuals' food choices (15, 22). A group of Australian fire fighters have previously reported that, the dishes chosen for communal cooking and the decision to purchase take-away foods during shift were usually determined by group consensus (15). In addition, in a qualitative study of Irish shift workers (n= 109), participants working in healthcare reported that using discretionary food as a reward is part of the night shift culture, and some felt that they adopted this habit in order to conform (22). These drivers of food choices are closely related to the specific work environments; therefore, it is unknown whether these factors also apply to night shift workers of other occupations.

Furthermore, cross-sectional studies have reported that night shift workers' overall dietary intake is less healthy, compared to day workers. In an Australian study involving 118 shift workers, permanent night shift workers had the highest saturated fat intake, compared to permanent day workers and rotating shift workers (23). In a Brazilian study with 150 male bus drivers, a higher proportion of night drivers failed to meet the recommended daily vegetable intake, compared to day drivers (24). It is currently unknown whether such poor diet quality is fully attributed to workers' eating habits during their night shifts, as there has been little investigation on what, when and why night shift workers eat while not at work. To date, a small number of published studies have alluded to the impacts of night shift beyond the time spent at work. For example, Irish healthcare shift workers and Australian fire fighters have indicated a preference for convenient foods following their night shift, as they are too tired to prepare a meal (15, 25). If night shift work is confirmed to have a substantial impact on night shift workers' eating habits during their rostered days-off, health promotion strategies for this population may need to expand the focus beyond changing habits at work.

Therefore, this qualitative study aims to explore Australian night shift workers' experience with food and eating, both at work and outside of work, the latter of which has not been adequately described in the literature. The photovoice method will be utilised in combination with a semi-structured interview, to gain a deeper understanding into factors that shape night shift workers' food choices and eating habits. The photovoice method has several advantages. Firstly, it empowers participants by perceiving them as the 'knowledge owners' (26). Through capturing photos in their daily lives, they are able to convey stories related to the research question that are important to them (26). The photo-taking activity also increases participants' awareness of their day-to-day surroundings and experiences (27), which could be useful in capturing eating habits. The incorporation of photos in interviews is able to assist reflections, prompting memory and description of experiences that are related indirectly to the photos (28). These stories often remain untold in explorations that only utilise

interviews (28). This photovoice study will generate insight into night shift workers' overall food experience, providing important points of consideration in developing practical guidance for this population. Furthermore, it will suggest potential target areas for health promotion strategies, which may take place within or beyond the workplace context.

4.2 Methods

This qualitative study was undertaken to explore the food and eating experiences of night shift workers in Melbourne, Australia; recruitment commenced in July 2019 and data collection was completed in January 2020. This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving research study participants were approved by a University Human Research Ethics Committee (project ID: 19509).

This research is grounded in pragmatism, which focuses on finding practical solutions to the societal problems (29). Through exploring Australian night shift workers' experience with food and eating, this research will inform practical dietary guidance and health promotion strategies for this population. This contributes to the solution of the nutrition problem identified, which is the increased risk of metabolic diseases observed in night shift workers.

The first author (G.K.W.L) is professionally trained as a dietitian and has been engaged in shift work research for the past six years. Critical reflexivity was employed throughout the study, whereby the researcher acknowledged her background as a nutrition professional, and reflected on how that would affect the decisions made during the research process and data interpretation (30).

4.2.1 Participants and sampling

A purposive sampling strategy was utilised, aiming for maximum variation in gender, type of shift schedule and occupation. We aimed to attain sufficient information power rather than data saturation (31). This was achieved by recruiting shift workers with variation in aforementioned characteristics, whom provided unique opinions and recounts of experience. Eligible participants were night shift workers above 18 years of age, who had at least 6 months of night shift work experience and were working at least 3 overnight shifts per month. These work characteristics were chosen in an attempt to recruit participants who were regularly engaged in night shift work. Participants were recruited through paid Facebook advertisements, word of mouth and a registry of interested individuals from the 'Shifting the Risk' study (Chapter 3a).

Participants expressed interest through completing an online screening questionnaire (Qualtrics, Utah, United States). Researcher G.K.W.L contacted eligible participants via phone call, to explain the aims and procedures of the study. For those who agreed to participate, a briefing of the photo-taking activity was provided and the semi-structured interview was scheduled. Written informed consent was obtained prior to the interview, via email for those who were interviewed online and in hardcopy for those who were interviewed in-person.

4.2.2 Data collection

This photovoice study included three data sources; (i) photos taken by the participants, (ii) demographics questionnaire and (iii) semi-structured interview.

4.2.2.1 Photo-taking activity

The photo-taking activity was explained to participants via phone call. Participants were asked to take photos that conveyed, '*What are shift worker's food choices and eating habits at work and outside of work?*' No limit was set on the number of photos to take. However, participants were asked to take photos on days when they were working night shift and also on rostered days-off. Participants were asked to complete this activity in the subsequent two to three weeks. Additional prompt questions and photo examples were provided to participants in a hand-out (Appendix M). Photos were taken on smart phones and sent to the researcher prior to the semi-structured interview.

4.2.2.2 Demographics questionnaire

Participants were asked to complete a demographics questionnaire (Appendix N) via an online platform (Qualtrics, Utah, United States) prior to the semi-structured interview. This questionnaire gathered information on demographics (e.g. age, level of education and household composition) and shift work status (e.g. occupation, shift schedule and years in shift work).

4.2.2.3 Semi-structured interview

In order to understand the breadth of night shift workers' experience with food and eating, the semi-structured interview was designed to explore three key areas using photos: (i) description of food choices and eating habits on night shifts and rostered days-off, (ii) factors influencing food choices and eating habits on night shifts and rostered days-off, and (iii) perceptions and enablers to healthy eating, with a focus on workplace influence (interview guide in Table 4-1). With such study design, interview responses and photos taken are equally valued as data sources, eliciting data richness and allowing for data triangulation. This was preferred over the SHOWED mnemonic method typically used in photovoice studies (32), which relies on participants to take photos that encapsulate and convey experiences largely on their own.

During the interview, all photos that the participant took were displayed to them either in hardcopy or on a PowerPoint Presentation. These were categorised into night shifts and rostered days-off, if that information was provided, and also into day shifts if relevant. When responding to interview questions, participants were asked to select photos, and use them in their descriptions of experiences and reflections. No guidance was provided in regard to which and how many photos to use.

The semi-structured interview format was adopted, whereby the main questions asked were based on the three key areas, followed by a series of prompting questions based on participant's photos, their responses in the interview and the demographics questionnaire. The interviews took place in-person (in a meeting room at our research facility) or online (via Zoom (Zoom Video Communications, California, United States)). All interviews were conducted by the first author (G.K.W.L), whom was introduced to the participants as a PhD student or a researcher, rather than a dietitian. The researcher's interest and experience in this field of research was also mentioned. During the interview, it was emphasised to participants that the photos would not be used as dietary assessments. As such, the researcher refrained from commenting on the nutrition value of participants' food choices or their perceptions of the constituents of a healthy diet. When participants persisted in obtaining the researcher's opinion on their dietary choices, the researcher would reiterate that this was not the focus of the interview, and that general healthy eating information could be provided after the interview if requested. As part of critical reflexivity, the researcher took field notes prior to and after each interview, documenting assumptions, rationale for prompting interview questions and tentative codes. All interviews were audio-recorded and subsequently transcribed.

4.2.3 Data analysis

The NVivo software (QSR International, Version Pro 12, Melbourne, Australia) was used to manage and support data analysis. Interview transcripts were uploaded to the software, with photos linked to the sections of dialogue where they were discussed. The thematic data analysis approach was taken, via an iterative inductive coding process. Prior to coding each specific transcript, the researchers reviewed the field notes that were taken after the interview, which provided tentative codes and context of the interview. Authors G.K.W.L and S.K cross-checked two interviews to develop the initial codebook. Author G.K.W.L then coded remaining interviews, meeting with S.K to refine the codebook after every three interviews. The codes were grouped into categories and then allocated to themes and subthemes, which were refined and verified by authors G.K.W.L and SK. Four themes with corresponding subthemes, illustrative quotes and photos are presented in the Results. All participants were provided with a pseudonym to preserve confidentiality.

Table 4-1. Interview guide for semi-structured interview.

Section 1: Description of food choices and eating habits
Q1. Let's start off by talking about what a typical day looks like. Using your photos, can you please tell me what a typical day looks like when you have to work night shift? **Q2**. Okay, now let's take a focus on food. Again using your photos, tell me about your food choices and eating habits, on a day when you have to work night shift. **Q3**. Now let's look at a typical day-off. Which of these photos look like your food choices and eating habits on a typical day off? **Q4**. Are there any differences or similarities in your food choices and eating habits between the two (a day when you are working night shift and a day off)? - Could you share with me why this is the case? - (for those who work other types of shifts) Did you take any photos on your day/ evening shift? Could you tell me about those?
Section 2: factors that influence food choices and eating habits
Q5. (Refer back to photos) What influenced your decision to choose this meal/snack? **Q6**. Comparing a day when you are working night shift to a day when you have a day-off. Are there any similarities or differences in the factors that influence your food choices and eating habits?
Section 3: Perceptions and enablers of healthy eating
With your experience as a shift worker, I would like to know your opinion on ways that can help shift workers practice healthy eating. **Q7**. But "healthy eating" can mean a whole range of different things to us. Using your photos, can you please show me what it means to you? **Q8**. Is this meaning different when you are at work compared to not at work? Can you explain this to me using your photos? **Q9**. Can you please share with me, what are some of the enablers that help you/ will help you make these choices or maintain these habits? **Q10**. (PICK PHOTOS that are relating to workplace – discussed or new) All of these photos here relates to your workplace. Several workplaces that employ shift workers have said that they would like to support healthy eating. - Do you have any suggestions for them, on how to do this?

4.3 Results

During the recruitment period, 39 individuals expressed interest in the study. Eight were ineligible because they did not work overnight shifts, contact was lost with 20 individuals after two attempts to follow-up via their preferred method of contact. One individual agreed to participate but withdrew, before providing consent or completing any of the activities. Ten participants consented to participate and completed the study; all were located in Victoria, except one who was located in Queensland. Participant demographic characteristics are described in Table 4-2. Number of photos taken by participants ranged from 4 to 29. Most photos were of food items consumed during shifts or on rostered days-off, with a small number representing their eating environment. Six interviews were conducted via video call and four were conducted in-person. Interview length ranged from 20 to 82 minutes.

Table 4-2. Demographic characteristics of participants in the Photovoice Study (n= 10).

Demographics	**Frequency** [a]
Age (years), range	19 – 66
Gender	
Female	4
Male	6
Occupational fields	
Healthcare	2
Emergency services and support	3
Security	3
Hospitality and food service	1
Avionic maintenance	1
Form of shift work	
Permanent night shift	4
Rotating shifts	6
Years in shift work, range	2 – 25
Household composition	
Living alone	3
Living with others	7

[a] Frequency presented unless otherwise stated.

The majority of participants indicated their lifestyle had been negatively affected by night shift work in some way, such as their sleeping or eating habits, maintenance of their social life or hobbies. Data analysis yielded four interactive themes, each with two subthemes, describing factors that influenced night shift workers' food choices and eating habits: 1) Supportive workplace management contributes to enabling workplace food and eating environments; 2) Social support, network and opportunities are essential to shift workers; 3) Constant battle with fatigue and 4) Food literacy knowledge and skills as enablers. Relationships between subthemes are illustrated in a mind map (Figure 4-1). Themes and subthemes have been mapped onto the Social Ecological Model (33) in Figure 4-1. This Model categorises influences of behaviour into intrapersonal, interpersonal and organisational levels; and will be referred to in the discussion of findings.

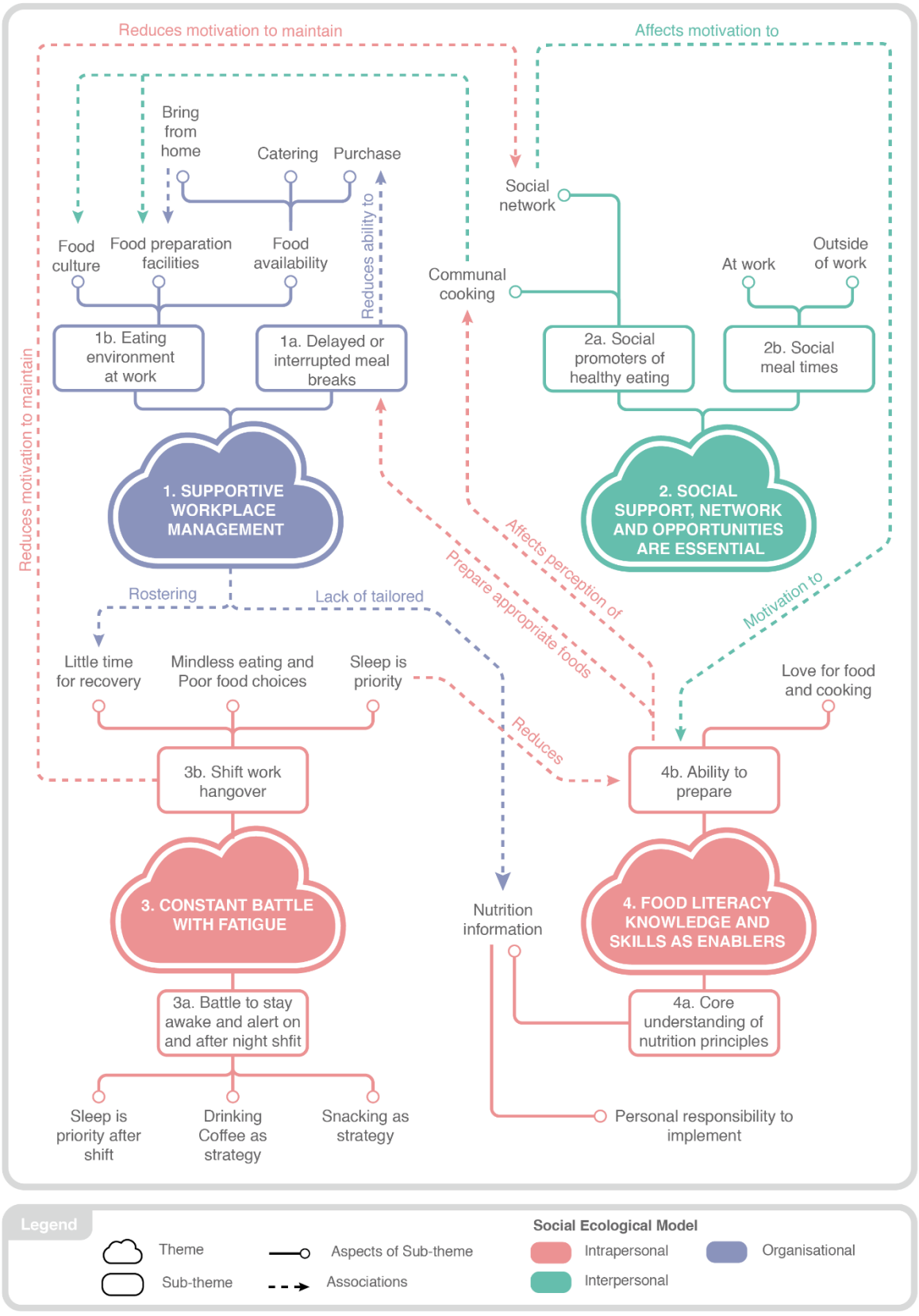

Figure 4-1. Mind map illustrating relationships between influences of night shift workers' food choices and eating habits (themes and subthemes).
Themes and subthemes have been mapped onto the Social Ecological Model [33], which categorises influences of behaviour into intrapersonal, interpersonal or organisational levels.

4.3.1 Theme 1: Supportive workplace management contributes to enabling workplace food and eating environments

Participants perceived some aspects of the meal break structure and workplace food environment as impractical, and that it was a reflection of management's lack of consideration for their health and wellbeing. Numerous participants expressed a lack of recognition from their workplace management, for their contributions to the company or the value of their profession. This was particularly evident for permanent night shift workers, who indicated they "*don't really have a voice*", as staff meetings and health initiatives were often scheduled at times convenient for day-time staff.

4.3.1.1 Subtheme 1a: Delayed or interrupted meal breaks

Most participants did not have designated break times, during both night and day shifts. Fulfilling work responsibilities was their priority; therefore, meal breaks were often delayed, missed or interrupted. Many would "*front load*" meals and caffeine, as Nathan reflected on his choice of a croissant (Figure 4-2a) during his afternoon shift:

"In that situation you're not like really hungry coz you've already eaten [lunch]. But you're worried that you will get tied up for the rest of your shift."

This habit was not limited to night shifts only, and was instead, dependent on the expected workload. A food item that was more "*substantial*" would tend to be chosen, to remain satisfied for longer. On night shifts, delays in meal breaks particularly affected those who relied on purchasing foods, as this would limit their options to fast foods only.

If interruptions to meal breaks were expected during a shift, some participants would prepare foods that could be "*put down and finished later*", such as a vegetable pasty which could be eaten cold (Figure 4-2b), rather than a main meal that requires reheating (Figure 4-2c). Some participants were able to describe their rationale in planning, selecting and preparing these foods suitable for their shifts.

4.3.1.2 Subtheme 1b: Eating environment at work

Availability of food options and food preparation facilities varied depending on the workplace physical environment and culture. For participants who relied on purchasing food while at work, unhealthy food options were more common during night shifts compared to day shifts, due to reduced availability of food outlets. Belinda described that on a day shift:

"Your options are better, because if you don't bring your food, you can just go and get something that's – I mean that's a tuna patty, it's not too bad?"

In comparison, all cafés near her workplace are closed during the night, therefore:

"By the time I could go and get something to eat, it was KFC."

Some participants were not permitted to leave their worksite during shift, therefore relied on food brought from home. This required a level of food literacy from the individual or supportive family members to plan and prepare food, and the availability of food storage and preparation facilities in the workplace. As described by Cassey, who could prepare convenience foods in her current workplace, as she had access to a fridge, microwave and kettle. In contrast to her previous workplaces*, "where my [her] break room has been an alley way."*

Participants perceived the lack of these facilities as a reflection of management's indifference to their employee's health. As Samuel detailed, a new cooktop was installed in his workplace kitchen after many years, but it was not permitted for use, as it was not approved in the building plan:

"So it's very disorganised, and they don't seem to understand or <u>care</u> to be honest, about healthy eating."

Catering was provided by some workplaces, but options were not necessarily healthy or desired. The culture of sharing discretionary foods was common across multiple workplaces and especially prominent during night shifts, when workload is reduced or a mood boost was required. As Belinda narrated around the Halal Snack Pack (Figure 4-2d) that her colleague organised, which she considered as unhealthy due to its high fat and carbohydrate content.

(a) "*Front loading*" food and caffeine was common, if meal breaks were expected to be delayed. (Nathan)

(b) Richard prepared foods that can be "*put down and finished later*", during a shift when meal break interruptions were expected.

(c) Richard chose a main meal, during a shift where a light workload was expected and with minimal meal break interruptions.

(d) Halal Snack Pack (chips with kebab meat, topped with cheese and sauce). Culture of sharing discretionary foods during night shift. (Belinda)

Figure 4-2. Participant photos related to Theme 1: Supportive workplace management contributes to enabling workplace food and eating environments.

4.3.2 Theme 2: Social support, network and opportunities are essential to shift workers

Being on night shifts was described as "*isolating*" by both permanent and rotating shift workers. Lingering fatigue associated with night shift reduced participants' motivation to engage in social activities:

"You're so mentally and emotionally and like physically drained, you don't have any time for it. You just don't have the effort." (Nellie)

This had a negative flow-on effect on workers' eating habits, in that they were less likely to engage in healthy eating practices in the absence of social networks. Support from family and significant others were crucial, in order to cope with the disruptions of night shift work.

4.3.2.1 Subtheme 2a: Social meal time

Outside of work, participants used meal times as opportunities for socialisation, in order to maintain relationships with family and friends. These opportunities were especially valued due to the lack of socialisation during night shifts. Having company at meal times also prevented participants from skipping meals or choosing convenience foods. This was exemplified in Nellie's experience:

"Whereas if it's just me I'll probably just sleep in and then have one meal, [then] go to work."

Meal breaks during night shifts also became opportunities for social interaction.

"We go upstairs and we have coffee when we have chances. And we try to do it every night, it's just more of a social thing as well." (Samuel)

Nellie described the contrasting food environment between day and night shifts in a hospital setting. During night shifts, a grazing table (Figure 4-3a) would often be set up at the main nurse's station, if those working were friendly with each other. In contrast, they tend to take official breaks during day shifts and reside to the staff room. Nellie reflected:

"At night there's less of you [staff], and so you kinda (sic) gravitate towards each other. Coz (sic) it's – A lot of the time the only social interaction on night duty is the other staff."

4.3.2.2 Subtheme 2b: Social promoters of healthy eating

Having a strong social network was closely associated with participant's motivation to prepare food. Belinda reflected that it is difficult to be organised with meal preparation because she is single. She noticed that mothers at her workplace would often bring plated meals to work, which were prepared because they had to cook for their children. This was evident in one participant Susan, who prepared

dinner (Figure 4-3b) for her family and set aside a serving to be brought to her night shift. Samuel reflected that he prepared meals at home more frequently when he was living with others:

"Coz (sic) ah there was more of a reason to cook that way. And I enjoy cooking, it's just that um for one person, it's yeah, it's a bit hard to motivate yourself to do it."

Communal cooking was reported in some workplaces, but relied on the availability of food preparation facilities in the workplace. Otherwise, it was dependent on particular motivated individuals, who would bring prepared dishes or cooking appliances to work. The activity was staff initiated, and typically included dishes that could be prepared in bulk, such as burgers and pasta. Being part of this experience was enjoyable for some but stressful for others, which was related to their level of food literacy and the size of the team at work.

(a) Eating is a social activity during night shifts. In the hospital setting, staff would set up grazing platters at the main nurses' station, where they would gather during their breaks. (Nellie)

(b) Having social network was associated with participant's motivation to prepare food. Susan prepared dinner for her family and set aside a serving to be brought to work.

Figure 4-3. Participant photos related to Theme 2: Social support, network and opportunities are essential to shift workers.

4.3.3 Theme 3: Constant battle with fatigue

Participants expressed experiencing a constant state of fatigue caused by shift work, in particular night shift work. Food was used as a means to fight fatigue during night shifts. However, the lingering impact of fatigue from night shift work affected eating habits negatively.

4.3.3.1 Subtheme 3a: Battle to stay awake and alert on night shift

During night shifts, snacking was used as a measure to stay awake and focussed.

"You're not even hungry, but you just need that something to do to keep you awake… That's why you can't skip chips…" (Nellie)

Coffee was also used as a measure to stay awake. Participants who drank coffee regularly ensured that they had a sufficient caffeine intake during night shifts, as they were concerned about falling asleep during work. The importance of staying awake during the commute home after a night shift was also highlighted:

"It becomes like a safety issue. You need – you need the caffeine." (Nathan)

Participants expressed being exhausted after night shifts and would often eat something that was quick and convenient (for example Figure 4-4a) or skip meals, so they could get to bed as soon as possible and sleep for as long as possible. If they skipped breakfast, it was likely that they would wake up being really hungry, and "*over compensate"* by consuming foods that were viewed as unhealthy, such as fast food and deep fried foods.

4.3.3.2 Subtheme 3b: Shift work hangover

Night shifts led to fatigue that lingered in between and after consecutive night shifts, creating the experience of a 'hangover', which had an impact on food choices and preparation. During a block of night shifts, getting enough sleep in preparation for the next shift was considered priority over eating.

"I'm not as interested in healthy eating on my days-on, as I am on my days-off… You're just more focused on sleeping, rather than going to the shops and getting food and things like that… When I'm at work, it's really just work and sleep." (Samuel)

Participants reported having little time or motivation in between night shifts for food preparation tasks such as grocery shopping or cooking. Therefore, if they were not organised prior to a block of night shifts, they would resort to convenience foods. Even if they had groceries prepared, the decision of whether they would cook before going to their night shift was largely dependent on whether there was time to get enough sleep.

Fatigue accumulated during a block of night shifts would often extend into participants' rostered days-off. Participants reported requiring the first couple of days-off to recover; their eating habits were subsequently affected.

"My first few days after a lot of night duties, my body clock is still out-of-whack… Even during my days off after night duties, it takes a little while for me to get back into an eating pattern." (Nellie)

This lingering fatigue experienced after a block of night shifts led to poor food choices or reduced motivation to cook despite the best intentions. As Samuel described, he made an effort to do grocery shopping on his first day-off and was prepared to make dinner. However, he "*hit a wall*" in the afternoon and resorted to food delivery.

Tiredness affected participant's ability to make rational food choices, which led to mindless eating or choosing unhealthy options. This was evident in both during (Figure 4-4b) and after night shifts:

"Coming off a night shift and going onto a day off… I'll find that those days, I will be buying the custard tart, or buying the tub of ice cream, not just the one." (Susan)

The effect of 'shift work hangover' was more apparent in rotating shift workers compared to permanent night workers. This was likely due to constant changes in shift types and structure of rosters, which allowed little time for recovery in between and after a block of shifts.

"The way our roster is structured, the long break is at the wrong time. We have a long break before our night shift. It should be after night shift… So you don't get enough recover time." (Richard)

(a) Samuel chose a convenient food option, consumed during the commute home after a night shift, so that he could get to bed as soon as possible.

(b) Fatigued during night shifts is associated with mindless eating, for example, ordering two meals instead of one: *"Because you're (I'm) so exhausted, I ended up getting poke as well, which… I don't, still don't understand why I did that."* (Nathan)

Figure 4-4. Participant photos related to Theme 3: Constant battle with fatigue.

4.3.4 Theme 4: Food literacy knowledge and skills as enablers

It was evident that participants had an adequate understanding of core nutrition principles. However, this did not reduce the difficulties they faced in maintaining healthy eating habits, with numerous external factors, such as limited food availability during night shifts, inappropriate meal break structure and minimal social networks, acting as barriers. The shift work lifestyle had significant impacts on participants' ability to prepare food, however, the enjoyment for food and cooking seemed to act as a motivator for some.

4.3.4.1 Subtheme 4a: Core understanding of nutrition principles

All participants could describe the principles of healthy eating. When asked about their interpretation of healthy eating, majority identified the importance of "*balanced*" meals or diet (Figure 4-5a, Figure 4-5b), with a focus on vegetables, but also including meat and grains (e.g. toast, rice, pasta).

Participants had their own way of planning meal times around their shift schedule, based on hunger, workload and break times. Some workplaces attempted to meet occupational health and safety requirements by providing nutrition information to employees. However, this was described as *"dry training"* that was *"forced upon"* them and were not tailored to their needs. As exemplified in Belinda's description of the information boards, outlining advice for healthy eating and physical activity, which were displayed on the back of toilet doors at her workplace:

"And there was one there for you know how to keep healthy. But they're all general, you know, they're like get up in the morning and go and do this. Well my morning it's dark when I get up you know?"

Participants voiced that the management's inability to provide tailored nutrition information accentuated the employer's indifference to their health and wellbeing.

Maintaining or practising healthy eating habits was described as a "*battle*" by participants. When reflecting on their photos, participants were quick to identify eating habits that they perceived as unhealthy. Through the narration of their experiences, participants seemed to be aware of how their eating habits were affected by external factors, such as short or delayed meal breaks, the workplace food environment and culture. As explained by Marcus, who works in an entertainment venue as a security guard:

"It doesn't matter how much you can empower or give information to your staff. When your [workplace is] probably the leading venue space of caffeine and cigarettes [consumption]." (Marcus)

Despite recognising these factors, when asked explicitly about factors that help them practise healthy eating, participants perceived this as largely their responsibility. They stated needing to be *"disciplined", "more organised"* and be more *"conscious"* about their food choices.

4.3.4.2 Subtheme 4b: Having the ability to prepare is crucial

Participants reported having little time for grocery shopping and cooking, especially during a block of night shifts. This was mainly due to the shift work hangover described previously and hence participants often sought convenient food options. However, perceptions of "*convenient*" food options varied amongst participants, and was related to their enjoyment of food and cooking. Those who "*loved being in the kitchen*" were more likely to view having a proper meal as a "*necessity*". They would therefore, know how to use cooking appliances and ingredients to create "*shortcuts*" and reduce food preparation time.

"That [Figure 4-5c] would be done in a slow cooker at the start of the day, all the ingredients thrown in and then served when I get home for dinner." (Susan)

(a) "*Meal prep*" done prior to night shifts, using grocery delivery service. Nellie added extra vegetables into the dish, quoting the importance of a "*balanced*" meal.

(b) Samuel's perception of a "*healthy meal*", with vegetables, meat and grain foods.

(c) Dinner that Susan prepared using a slow cooker. Those who enjoyed cooking had knowledge of "*shortcuts"* that could be used to reduce food preparation time.

Figure 4-5. Participant photos related to Theme 4: Food literacy knowledge and skills as enablers.

Participants who expressed an enjoyment for food and cooking would also tend to "*meal prep*", cooking a batch of meals on their days-off, in preparation for their night shifts (Figure 4-5a). In comparison, participants who did not express this enjoyment engaged in food planning and preparation activities less often, as exemplified in Samuel's experience:

"So if I was getting hungry… I wouldn't organise myself beforehand. And I'd just either be lying at home, 'Ah I'm starving, starving… Ah I'll go get something to eat.' So then you'd just go and you'll just get take away." (Samuel)

As a result, they did not have planned meal times and consumed convenience foods more often.

4.4 Discussion

This qualitative study is the first to use the photovoice enquiry method to explore the food experience of Australian night shift workers from different occupations. Four main themes emerged from the data, describing key influences of workers' food choices and eating habits at work and also outside of work, the latter of which was a novel aspect of the study. Our findings indicate that night shift workers' food choices and eating habits are influenced by a complex interplay between multiple individual, social and environment factors. Workplace management plays a crucial role in supporting healthy eating practices within the workplace, through ensuring food availability, providing food preparation facilities and appropriate meal break scheduling. In addition, their arrangement of shift rosters had an indirect impact on workers' eating habits outside of work. Shift rosters provided little time for recovery between shifts, leading to the experience of shift work hangover, which was evident in many participants' stories. This affected workers' abilities and motivations to engage in healthy eating practices both on days with work and on days-off, despite their best intentions. In this context, the presence of a supportive social network and being equipped with adequate food literacy skills are essential, to reduce the perceived efforts required to engage in healthy eating.

Our findings indicate that night shift workers' food choices and eating habits are determined by both individual and broader environmental factors, as such, the key findings will be discussed in the context of the Social Ecological Model (SE Model) (33, 34). This Model recognises that human behaviour is shaped by, and has effects on, both individual and environmental factors (33). In the SE Model presented by McLeroy et al, environmental influences are further divided into four levels including: interpersonal, organisational, community and public policy (33). Perceived from the SE Model, the cumulative impact of these environmental factors influences the physical, emotional and social wellbeing of individuals (34). However, this Model also recognises the role of intrapersonal factors, such that the same environment may affect an individual's health differently, depending on their genetic disposition, personality, behaviour and perceptions of environmental controllability (34). This complex and dynamic interplay between individual and environmental influences on human behaviour described by the SE model fittingly describes the factors that shape night shift workers' eating habits found in the current study (illustrated in Figure 4-1). The SE Model has previously been used to conceptualise influences of eating habits in population groups such as Nigerian nurses (35), U.S. college students (36) and formerly homeless youths (37). This Model provides a novel lens in interpreting influences of night shift workers' food choices and eating habits, as previous studies have considered individual and environmental factors separately.

As ‘work’ is the characteristic that defines our target population, it is not surprising that many influences of food choices and eating habits discussed by our participants are related to their workplace. Consistent with previous qualitative research on nurses (38) and paramedics (19), our findings indicate that workers’ choice of convenience food during night shifts was prompted by limited food availability at work and impractical meal break scheduling. A cross-sectional study of Irish shift workers (n= 1300) has shown that this can have a direct influence on workers’ dietary intake, whereby workers who have access to vending machines in their workplace were 64% more likely to consume soft drinks at least once per week (39). Participants in our study expressed that their workplace management did not acknowledge these workplace barriers to healthy eating and failed to provide supportive measures accordingly. As a result, workers may feel undervalued, leading to low self-efficacy and the adoption of a ‘why bother’ attitude towards making positive lifestyle changes (25). These findings indicate the importance of a supportive workplace management. As suggested by our data, the workplace not only influences workers’ ability to practise healthy eating through the provision of a healthy setting, but the employee recognition that they provide also has an indirect influence on workers’ perceived need and capacity to make positive dietary changes.

As outlined by the World Health Organisation, in order for a workplace to be a healthy setting, it should aim to protect and promote health at work. This can be achieved through changes in the physical environment and organisational structure of workplaces (40). Behaviour change initiated by environmental enablers is more likely to be sustained, as it does not require continuous voluntary efforts from the individual (34, 41). In the published literature, there are only a few reports of worksite health promotion programs with environmental modifications, which specifically target night shift workers. However, health promotion programs conducted in manufacturing companies, which typically employ shift workers, have indicated the effectiveness of worksite environmental modifications on improving workers’ dietary intake. The Well-Works Study implemented strategies targeting multiple levels of the SE Model, including health education programs (intrapersonal level), changes to food items available in canteens and vending machines (organisational level) and food catering policies (local policy level) (42). The study included 24 manufacturing worksites (total 2658 participants) in the U.S., and reported an increase in fruit and vegetable intake and a reduction in fat intake (as percentage energy) at the end of the 2-year intervention. Furthermore, the potential effectiveness of strategies targeting both individual and environmental factors was highlighted in the Food Choice at Work trial conducted in manufacturing worksites in Ireland (43). This 7-month trial involved four worksites including a Control site (no intervention), an Education site (individual and group education on healthy eating), an Environmental site (menu modifications in canteen items to reduce saturated fat, salt and increase fibre, strategic positioning of healthier alternatives) and a

Combined site (Education and Environmental). Their findings showed that while a significant reduction in participants' BMI, dietary saturated fat and salt intake was observed in the Combined intervention, the Education and Environmental interventions had minimal effects by themselves.

Fatigue has been reported by previous literature as one of the main detriments of night shift work, affecting workers' food choices and eating habits during their shifts (21, 25, 44). In the current study, participants consistently reported that this feeling of tiredness lingered beyond their shifts. This had an impact on their ability to make healthy food choices and motivation to engage in food preparation activities not only during and immediately after their night shifts, but also on their rostered days-off. A cross-sectional study including 118 Australian shift workers has shown that increased levels of fatigue is associated with increased daily fat intake (as percentage energy) (23). Moreover, a simulated night shift study reported that sleep restriction (5.5 hours per night) increased snack consumption, in particular sweet snacks, compared to regular sleep duration of 8.5 hours per night (45). These unhealthy eating habits are likely to be maintained in night shift workers' daily lives, as they find it difficult to recover from fatigue caused by night shift work. As such, health promotion strategies should also aim to improve night shift workers' eating habits beyond that observed within the workplace. Moreover, the experience of shift work hangover reduced workers' motivations in planning and preparing meals, an observation that has been previously reported in Irish shift workers (25) and Australian fire fighters (15). While the company of family and friends promotes healthy eating practices, the experience of shift work hangover poses challenges in the maintenance of these social networks. This shows the interconnection between intrapersonal (fatigue), interpersonal (social networks) and organisation (workplace) factors that affect night shift workers' eating habits, as illustrated in Figure 4-1.

An individual's 'ability to prepare food' is an intrapersonal influence of food choices and eating habits, regardless of the type of work they are engaged in. Conceptualised by Vidgen et al, the 'ability to prepare food' is one of four food literacy components, along with the ability to plan and manage, select and eat food for health (46). Participants in the current study demonstrated an understanding of the basic principles of healthy eating. However, their motivations to translate this nutrition knowledge into action were often compounded by the lack of social networks and experience of shift work hangover, experiences attributed to the night shift work schedule. Due to the experience of constant fatigue, participants typically prioritised sleep over the maintenance of healthy eating habits, both during and after a block of night shifts. This is consistent with findings from a study with Australian train drivers, where health issues related to sleep (fatigue, lack of sleep, sleep patterns) were the most frequently reported and highly prioritised health concern (20). Participants often expressed a lack of time for food preparation, as a significant proportion of time outside of work was

spent on rest and recovery. Considering this, nutrition education that improves food literacy skills required for efficient food preparation is especially important for night shift workers. This may include improving their knowledge in recipe adaptations and capacity at utilising cooking appliances, so that a healthy meal can be prepared easily and quickly given their time constraints. Due to night shift workers' experience of constant fatigue, it is important that healthy eating practices recommended do not create additional time stressors or encroach on their sleep opportunities.

It is evident that night shift workers' food choices and eating habits are shaped by interacting factors situated in various levels of the SE Model. Hence, the most effective health promotion programs are those that utilise a combination of strategies, targeting multiple levels of influences (34). This was observed in both the Well-Works and Food Choice at Work studies described above (42, 43). In the context of our findings, fatigue caused by night shift work had multiple direct and indirect impacts on workers' eating habits. Constant fatigue is closely related to inappropriate rostering, allowing little time for workers to recover in between and after night shifts. This is a factor that needs to be addressed at a workplace organisation level and/or instigated from a public policy level, through mandatory occupational health and safety regulations. A recent discussion paper prepared by a group of experienced shift work researchers suggested that night shift rosters should have no more than three consecutive night shifts and intervals between two shifts should be at least 11 hours, in order to prevent fatigue-related injuries and possibly breast cancer (47). If implemented, a policy such as this is likely to have a flow-on effect on enabling healthy eating habits, an inherent advantage of environmental health promotion strategies (48). Workplace management can also consider harnessing existing workplace cultures such as communal cooking. Healthy eating habits could be promoted through this avenue, by creating supportive environments such as the provision of appropriate food preparation facilities, ingredients and recipes. A secondary effect of this is the cultivation of social networks between colleagues, an interpersonal element of wellbeing that is affected by night shift work. Participants in the current study described that communal cooking is often staff-initiated, with staff members bringing cooking appliances and utensils to the workplace, which may pose occupational and safety hazards. This emphasises the importance for such activities to be organised and overseen by workplace management. Lastly, strategies targeted at the intrapersonal level typically aim to improve eating habits through some form of nutrition education. For night shift workers, it is important to recognise the multi-level influences of their eating habits, and nutrition education needs to include food literacy knowledge and skills to overcome these environmental barriers.

4.4.1 Strengths and limitations

Method triangulation was achieved through the combined use of multiple data collection methods, including photo-taking, semi-structured interviews, field notes and demographic questionnaires. This generated complementary data sources, allowing researchers to verify research findings and thereby increasing its validity (49). Furthermore, the incorporation of photos in the interview prompted participants to reflect on factors that affected their daily eating habits, allowing researchers to gain a deeper and broader understanding of their experiences. For example, one participant had a photo of a nicely plated home-cooked meal, which was distinctively different to other photos of frozen meals prepared from bulk-cooking. This photo drew the participant's attention during the interview, which led them to explain that having company during meal times served as a motivation to cook and enjoy food. As exemplified, the photos encouraged participants to discuss experiences that were important to them, without further verbal prompts or interview questions. Moreover, participants reported that the photo-taking activity itself helped them notice and recognise their daily eating habits; a notable advantage of the photovoice method that has previously been reported (27). Another strength of this study is the exploration of night shift workers' experiences outside of work, a component that has not been adequately examined in previous literature. Although participants were not able to clearly distinguish their food experiences on days-off from days at work, they often referred to the burdens of night shift work when describing their eating habits on days-off. This suggested that impacts of night shift work extend beyond that within the workplace.

To the authors' knowledge, the current study is one of few qualitative studies that explored food experiences of night shift workers across multiple occupations. This provided insights into common burdens of night shift work experienced by Australian employees, which are evident regardless of occupations and industries. To further strengthen the findings of this study, future qualitative explorations could involve multiple Australian night shift workers across different occupational fields, which would provide opportunities to explore similarities and differences between occupations. Lastly, our findings showed the importance of a supportive workplace environment in enabling healthy food choices and eating practices. As such, further qualitative investigation focussing on this area is warranted, as it has the potential to inform workplace and public policies such as occupational health and safety regulations.

4.5 Conclusion

This study aimed to explore Australian night shift workers' experience with food and eating not only at work, but also in their daily lives. The combination of photovoice enquiry and interviewing methods allowed for an in-depth exploration. Our findings confirmed that workers' food choices and eating habits are shaped by a complex interplay of individual and environmental factors, which need to be considered in order to develop practical dietary guidance for this population. Workplace management play a crucial role in creating a physical environment that supports healthy eating habits. Moreover, workplace and public policies regulating meal break and roster structures exacerbate intrapersonal barriers to healthy eating, such as constant fatigue and reduced motivation for food preparation. These factors not only hindered workers' ability to make healthy food choices at work, but also continued to affect their eating habits on rostered days-off. As such, health promotion strategies for this population should consider targeting dietary behaviours within and external to the workplace setting. More importantly, health promotion for this population should have a multi-strategy approach and target multiple levels of the Social Ecological Model, ranging from interventions targeted at the individual, to strategies that involve workplace-settings based approaches and organisation and/or public policy reforms.

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Chapter 5 . Thesis Discussion

The program of research in this Thesis has advanced our knowledge of the ways in which night time eating affects night shift workers' metabolic health. Firstly, we confirmed that eating at night, as a form of circadian disruption, leads to acute metabolic disturbances. Informed by this finding, we tested the metabolic effects of an overnight fasting period in a pilot study, which was the first meal timing intervention conducted in free-living night shift workers. Through examining the feasibility of this study protocol, we identified important areas of consideration for future dietary interventions involving manipulation of meal timing and/or night shift workers. Lastly, in a qualitative exploration, we described interconnecting individual, social and workplace factors, which influence night shift workers' food choices and eating habits. The learnings from this body of work have implications for the design and implementation of nutrition interventions and health promotion strategies for night shift workers. These are **urgently** required, to mitigate the harmful effects of shift work on cardiovascular and metabolic health.

5.1 Summary of key findings

The systematic literature review and meta-analysis pooled findings from diurnal studies, confirming that a meal eaten during the night leads to higher glucose and insulin responses compared to the day (Chapter 2). This indicates an acute metabolic disturbance caused by circadian misalignment (i.e. food intake mistimed to the regulation of our body clocks). It presents a physiological mechanism, explaining the increased risk of metabolic diseases observed in populations who habitually eat late at night, such as night shift workers. Whilst the time of day difference in postprandial glucose response was robust, such difference in postprandial insulin response was lost in a sensitivity analysis that compared afternoon to night clock times, rather than morning to night. This suggests the need to investigate the time of day difference in responses that contribute to insulin concentration in the bloodstream, such as insulin secretion and insulin sensitivity, in order to understand the time of day difference in insulin action. Overall, findings from this review emphasise the need for strategies to minimise frequency of glucose excursions experienced by night shift workers, supporting the rationale for the 'Shifting the Risk' study (Chapter 3).

A meta-analysis conducted by our research group has indicated no significant difference in overall energy intake between night shift workers and their day-working counterparts (64). This finding highlights the impact of meal timing, rather than energy intake, as a potential contributing factor to the increased metabolic disease risk observed in night shift workers. Supported by these findings and results from Chapter 2, the 'Shifting the Risk' study investigated the effect of a meal timing intervention on the metabolic health of night shift workers (Chapter 3a). This was the first meal timing

intervention conducted in free-living night shift workers, and investigated whether a 5-hour overnight fasting period (0100h to 0600h) without overt changes in daily energy intake, was able to improve CVD risk markers of workers with abdominal obesity. Although no difference was observed in postprandial TAG and glucose iAUC after the 4-week intervention period, a lower mean body weight was observed, compared to the control period. This warrants further investigation into the effect of meal timing on changes in energy expenditure and body weight. The pilot data generated allowed for sample size estimations, which can be used to verify these findings in a fully-powered manner. Critical to a pilot study, the appropriateness of selected outcome measures was evaluated. It was determined that postprandial TAG and glucose responses remain as the preferred outcome measures compared to fasting TAG and glucose concentrations, as they are stronger predictors of CVD and T2DM risks in healthy individuals. However, peak postprandial TAG concentration may be a better outcome measure compared to TAG iAUC, as it allows for comparisons with risk indicative reference ranges available in the literature, and requires a shorter assessment period thus reducing participant burden.

The feasibility of the 'Shifting the Risk' protocol was further evaluated, providing useful areas of consideration for any potential future efficacy trials (Chapter 3b). Targeted recruitment strategies such as social media Facebook paid advertisements and direct engagement with a shift work organisation reached the highest number of eligible participants. Participants were able to maintain the fasting intervention for the 4-week period during the study. However, long term sustainability remains unclear, with participants highlighting the need for more overt health improvements to generate motivation to sustain the behaviour change. Participants indicated shift-work related weight gain as their major health concern. Together with the small change in body weight observed in Chapter 3a, a weight loss intervention that incorporates manipulation of meal timing may be an effective and engaging dietary intervention for the shift working population.

The fasting intervention implemented in 'Shifting the Risk' was simple in theory. However, participants still encountered challenges during implementation, such as the reliance on caffeine to maintain alertness and inflexible meal break structure enforced by their workplace (Chapter 3b). If support from workplace organisation cannot be obtained to facilitate behaviour change, flexibility may be required within the intervention protocol, to optimise participant acceptability and long term maintenance of behaviour change. These factors should also be considered more broadly, in the design of dietary interventions and health promotion strategies for night shift workers, to ensure practicality and feasibility of implementation. As such, factors that influence night shift workers' eating habits were further explored in a qualitative study (Chapter 4). This study used a combination of photovoice enquiry and interviewing methods, to explore Australian night shift workers' experience with food at work and outside of work. It was revealed that night shift workers' food choices and eating habits are

shaped by a complex interplay of individual and environmental factors. A key example of such interplay is the impact of constant fatigue (individual factor) on night shift workers' motivations to engage in healthy eating habits (individual) and to maintain social networks in their daily lives (interpersonal), which can be partially attributed to inappropriate rostering (organisational). Moreover, the workplace eating environment, such as food availability and meal break structure, has a significant influence on what and when night shift workers choose to eat whilst at work. Acknowledging the interplay between drivers of night shift workers' food choices and eating habits, health promotion strategies for this population require a multi-strategy approach that targets multiple levels of influence. This may involve tailored individual education on food literacy, in combination with development of healthy workplace settings (108) and localised or public policy reforms, to create a supportive environment for implementation of healthy eating habits.

The findings from this research will be synthesised in this Chapter, to inform the development of practical dietary strategies aimed at improving the metabolic health of night shift workers.

5.2 Implications for future research and practice

This section will contain two parts. Firstly, future research directions investigating the effect of meal timing on night shift workers' metabolic health will be discussed. These are mainly informed by findings from Chapter 2 and 3a, which affirmed the metabolic consequences of night time eating. Secondly, potential health promotion strategies targeting night shift workers will be recommended. These are mainly based on findings from Chapter 3b and 4, which revealed that night shift workers' food choices and eating habits (i.e. what and when they eat) are influenced by an interplay of individual and environmental factors.

5.2.1 Implications for future research: effect of meal timing on metabolic health

5.2.1.1 Additional outcome measures recommended for meal timing interventions

Findings from the 'Shifting the Risk' study (Chapter 3b) showed that it was feasible for free-living night shift workers to maintain a short period of fasting during the night. The acute metabolic benefit of this intervention can be speculated based on the systematic synthesis of data from diurnal studies (Chapter 2 and (39)), in that an overnight fasting period is able to reduce the frequency of postprandial rises in glucose and TAG response caused by night time eating. Furthermore, the reduction of energy intake during the night may facilitate weight maintenance, through positive changes in TEF (Chapter 3a). Therefore, the long term effects of an overnight fasting period should be further investigated in a fully-powered manner, informed by the pilot data generated in Chapter 3a. Findings generated from this Thesis have indicated additional outcomes of interest. Firstly, results in Chapter 2

showed that the time of day difference in postprandial insulin response was lost, in the sensitivity analysis which substituted morning clock times with afternoon clock times. This indicates variability in postprandial insulin response, which can be attributed to the individual variation in physiological mechanisms such as insulin sensitivity, insulin production rate and β-cell responsiveness (109, 110). The postprandial plasma insulin concentration is a composite effect of these mechanisms, hence may not be the most representative indicator of insulin action. Markers of insulin resistance and insulin production rate (e.g. HOMA-IR and plasma c-peptide concentration respectively) should therefore be considered as secondary outcome measures in future meal timing interventions (111, 112), which will be able to provide a clearer indication of insulin action and glucose tolerance. Secondly, a recent simulated night shift study has shown that consuming a meal at 0130h led to a higher number of car crashes in a simulated drive, compared to participants who maintained a period of fasting during the night (113). Such tangible health and safety outcomes are highly valued by night shift workers and their employers, when considering the benefits of implementing health-related interventions (Chapter 3b). As such, future meal timing interventions should also consider the inclusion of these safety outcomes, in addition to metabolic markers.

5.2.1.2 What should workers eat during night shift?

Frequent snacking during night shifts is a typical eating habit reported by night shift workers (68, 70, 93, 114), as observed in participants of 'Shifting the Risk' at baseline (100). Evidenced by findings from Chapter 2, such a habit has negative impacts on workers' metabolic health, as food intake during any hours of the night (2000h to 0400h) confers acute metabolic disturbances. However, it is impractical and unsafe for night shift workers to avoid eating during this entire time, as shift workers have indicated that an extended period without food intake can affect their judgement and work performance (Chapter 3b, (68, 93, 94)). Furthermore, eating occasions during night shifts have a profound social meaning to workers, such that they facilitate the building and maintenance of social networks (Chapter 4), hence it is difficult and inappropriate to eliminate entirely. As such, recommendations on meal and snack options that are more appropriate for the night would be valuable. Such recommendations would describe food options with nutrient compositions that are able to minimise postprandial glucose and TAG excursions observed during the night (Chapter 2 and (39)). In the current literature, only a limited number of studies have compared the night time metabolic response to meals of varying nutrient compositions. The majority of these studies are conducted in simulated shift work settings; however, considered inferences can be made to provide future research directions.

Based on its ability to reduce the magnitude and duration of postprandial glucose response during the day (115-117), previous research has speculated that a low glycaemic index (GI) meal may attenuate postprandial hyperglycaemia observed during the night. Contrary to this hypothesis, we previously showed in a diurnal study that the glucose lowering effect of a low GI meal observed in the morning was not replicated at night (32). Findings from this study showed that the postprandial glucose response after a low GI meal consumed at 2000h and midnight was six to nine times higher than that observed at 0800h. Moreover, whilst the postprandial glucose concentration after a low GI meal consumed at 0800h returned to fasting levels after 45 minutes, it remained elevated for 3-hours postprandially after the same meal consumed at midnight. Despite this, two studies have examined whether a low GI meal is at least a better option than a high GI meal for the night (98, 118). This was confirmed in a study by Haldar et al, which showed that a low GI meal consumed at 1830h led to a lower postprandial glucose and insulin response compared to a high GI meal (118). However, this was considered as a proof-of-concept study, utilising high and low GI rice as the test meals, which is unlikely to be representative of a typical meal consumed during night shifts (Chapter 4). In comparison, a study by Gibbs et al utilised core foods as test meals (cereal, dairy foods and fruit), and reported no difference in postprandial glucose and insulin response between the low and high GI meals consumed at 2000h (98). This small number of studies suggest that due to the robustness of the diurnal variation in glucose tolerance, carbohydrates may not be the best macronutrient to consume during the night, regardless of its glycaemic index.

As such, research groups have investigated whether increasing proportion of protein in meals, thereby reducing proportion of carbohydrate, could attenuate the postprandial hyperglycaemia observed during the night. This hypothesis is supported by acute postprandial studies conducted during the day, which showed that the co-ingestion of protein within a meal containing carbohydrate can effectively lower postprandial glucose response (119, 120), by increasing insulin sensitivity (121). This hypothesis was confirmed in a study conducted by our research group, which reported that a high protein meal was able to produce a significantly lower glucose response at night, compared to a standard protein meal (median iAUC: 59.6 mmol/l.3h vs. 208.8 mmol/l.3h) (122). Although results are promising, the high protein test meal utilised (pasta and milkshake) was fortified with protein supplements to achieve the high protein composition (41% of energy (% E)), limiting its transferability to the real shift work setting. In a more recent study by Cunha et al, a high protein meal (35% E) was achieved using core foods, which was compared to a high carbohydrate meal in a free-living shift work setting for the first time (123). Consumed at 0100h, the high protein meal led to a lower postprandial glucose response compared to the high carbohydrate meal, confirming the findings reported by our research group.

The findings summarised above support further investigation into whether replacing carbohydrate with protein in meals consumed at night can mitigate postprandial hyperglycaemia. Future studies are recommended to confirm findings by Davis et al and Cunha et al in larger sample sizes, and to determine the amount or percentage of protein required to elicit a beneficial response. To enhance transferability to the shift working population, these studies should be conducted in real night shift work settings, utilising test meals that workers are able to store and prepare in their workplace (Chapter 4). Cunha et al were able to implement their study within the workplace, as they utilised glucometers to measure capillary blood response postprandially (123). The use of continuous glucose monitoring may be another feasible outcome assessment method, which may further reduce participant burden, as they are not required to take any measurements themselves after the device has been fitted (124). A measurement of physical activity level via an activity monitor is also necessary, as it cannot be controlled in the real work setting, but has an influence on postprandial glucose response (125, 126). Guidance on the optimal meal and snack to consume during night shifts is imperative to night shift workers, which in combination with a short fasting period, would further improve their metabolic health.

5.2.1.3 Effect of meal timing manipulation on energy expenditure

Results from 'Shifting the Risk' showed that participants' mean body weight was lower at the end of intervention period, compared to the end of the control period, in the absence of changes in physical activity energy expenditure. Whilst the pilot nature of the study precludes detection of statistical significant changes, recent evidence on diurnal variation of energy expenditure (40) suggests that the intervention may have promoted changes in energy expenditure, leading to a small amount of weight loss. Discussed in Chapter 3a, this observation may be attributed to the reduced TEF observed during the night, i.e. the body is 'burning' less energy after food intake during this time (43, 44). In addition to this, a simulated night shift study by McHill et al. has shown that daily total energy expenditure was lower during a night shift, compared to a day shift, independent of changes in physical activity levels and energy intake (127). Changes in TEF were not observed, suggesting that the difference in total energy expenditure may be attributed to changes in RMR (indicator of basal metabolic rate). This speculation is supported by findings from constant routine studies, which report a natural lull in total energy expenditure during the biological night, when food intake and sleep were kept constant (53, 56). Although it could not be determined if the observation reported by McHill et al was caused by the independent or combined effects of mistimed sleep and mistimed food intake (127), future interventions should verify whether an overnight fasting period is able to prevent the reduction of daily energy expenditure associated with night shift work, and thereby facilitating weight management in night shift workers.

Considering that working night shifts may change RMR (127) and that it accounts for the largest proportion of total energy expenditure (42), the assessment of RMR can be prioritised in future interventions investigating the effect of an overnight fasting period. This could be assessed in fasting conditions, at the start and end of intervention. It should be noted that this assessment cannot determine whether the intervention is able to shift the circadian or diurnal rhythms of RMR. This would require a constant routine study, which may be impractical for free-living night shift workers to attend. A few meal timing interventions have previously included an assessment of RMR (128-130). However, all interventions examined the effect of breakfast skipping compared to breakfast consumption and reported no differences between the two conditions. This further substantiates the need to further investigate the effect of manipulating meal timing at night on energy expenditure and body weight.

Whilst the potential benefit of an overnight fasting period on prevention of postprandial hyperglycaemia and lowering in energy expenditure are exciting propositions, participants from 'Shifting the Risk' expressed a desire for health-related interventions with tangible health benefits, in particular weight loss (Chapter 3b). The provision of tangible health benefits are likely to motivate individuals to sustain behaviour change. A systematic review of 48 physical activity and dietary interventions have shown that 'feedback on outcome of behaviour', is an important driver for individuals with overweight and obesity to maintain behaviour change in the long-term (>12 months) (131). Shift workers, in particular, may be more interested in weight loss interventions, as they have a high perceived susceptibility to weight gain, through personal or observations of colleagues' experience (Chapter 3b). Such perceived risk has been substantiated by evidence from epidemiological studies. In a cohort study of Japanese male workers (n= 7254), rotating shift workers had 13% higher odds of experiencing increase in BMI of ≥5% and ≥10% at 14-year follow up, compared to day workers (63). Furthermore, in a 5-year cohort study (n= 7332), female night shift workers had a 37% increased odds of experiencing major weight gain (i.e. >5 kg), compared to day workers (132). To date, only one study has specifically targeted weight loss in night shift workers (133). Although this 14-week intervention resulted in a significant amount of weight loss (mean, 95% CI = 4.0, 2.9 – 5.1), the intervention focused on education of healthy eating principles and physical activity guidelines. Given that we can now confirm the causal relationship between night time eating and increased postprandial glucose and insulin responses (Chapter 2), an intervention combining meal timing and weight loss strategies may be a novel approach, which simultaneously targets T2DM, CVD risk and weight loss in night shift workers.

An intervention combining meal timing and weight loss has since been developed by A/Prof Bonham and colleagues (http://anzctr.org.au/Trial/Registration/TrialReview.aspx?id=377429&isReview=true).

This trial ('SWIFt' trial) is funded by the National Health and Medical Research Council of Australia, comparing the effectiveness of three different weight loss strategies in night shift workers, (i) intermittent fasting on two days with night shift, (ii) intermittent fasting on two days with day shift or day-off and (iii) continuous energy restriction. The 'SWIFt' trial is a fully-powered study, and its design was supported by the findings from this Thesis. Firstly, the 'SWIFt' trial's hypothesis is that compared with continuous energy restriction, participants randomised to the intermittent fasting condition on night shift (i.e. restricted to 2.1 to 2.5 MJ in the 24-hour period that includes a night shift) will have reduced HOMA-IR, thereby optimising glycaemic control. This hypothesis is supported by the time of day difference in postprandial insulin response affirmed in Chapter 2. The conclusions from Chapter 2 also emphasised the need to include a measure of insulin resistance in future interventions, due to the variability of postprandial insulin response observed across included studies. Secondly, rather than specifying the clock hours of the fasting period, the 'SWIFt' trial anticipates participants randomised to the intermittent fasting on night shift condition to inadvertently include a fasting period during the night, by restricting daily energy intake to 2.1 to 2.5 MJ on fasting days. This arrangement reduces the likelihood of conflicting meal breaks, which were reported by participants in 'Shifting the Risk' (Chapter 3b).

5.2.2 Implications for practice: a multi-strategy approach to health promotion is necessary for night shift workers

Chapter 3b and 4 described Australian night shift workers' perspectives and experiences with food, identifying facilitators and barriers to not only eating at the 'right time', but also making healthy food choices. Findings from these chapters stipulated the need for a multi-strategy approach to health promotion, to facilitate night shift workers in making healthy food choices and eating habits, and consequently improving and protecting their metabolic health. A multi-strategy approach refers to one that includes a range of interventions, simultaneously targeting multiple levels of the Social Ecological Model. In light of findings generated in this Thesis and evidence from the current literature, this section will provide recommendations for health promotion strategies intervening at the intrapersonal, organisational and public policy level. Intrapersonal and organisational level strategies suggested below can be described as 'nutrition-specific', as they aim to directly influence night shift workers' food choices and eating habits (134). In comparison, public policy level strategies can be 'nutrition-specific' or 'nutrition-sensitive', with 'nutrition-sensitive' approaches referring to those that involve changes outside of the health and food sectors, indirectly influencing eating behaviour by addressing social, environmental and economical determinants of food consumption (134). It should be noted that although recommendations for the different levels will be discussed separately,

evidence suggests that the combination of strategies targeting all levels are most effective in cultivating healthy eating habits (135-137).

5.2.2.1 Intrapersonal level

A key finding from Chapter 4 is the dominating effect of fatigue, which not only affected workers' eating habits during their night shifts, but also continued into their days-off, reducing their motivations to engage in healthy eating practices despite their best intentions. Participants described having little energy and/or time to plan, purchase and cook healthy meals even on their days-off, due to lingering fatigue from night shifts. Therefore, nutrition education may be a suitable intrapersonal level strategy for night shift workers, but it needs to be focused on building food literacy skills; enabling quick and convenient meal planning, grocery shopping and meal preparation. Whilst individual food literacy skills also influence eating habits of the general population (138), night shift workers specifically require skills and knowledge on efficient food preparation, due to the unique burdens of constant fatigue that they experience (Chapter 4, (23, 92, 95)). Examples of tailored nutrition education for this population may therefore include, ways to prepare healthy meals using a microwave or pressure cooker, preparing convenient and non-perishable healthy snacks for night shifts and strategies to do bulk-cooking efficiently. In addition to individual nutrition education, public dietary guidelines for night shift workers should also focus on these nutrition topics. Evidently, the limited dietary recommendations currently available for shift workers have not considered the burdens of fatigue, as they continue to advise workers to adopt healthy eating habits (e.g. cooking a main meal to take to their shift), without providing guidance on how to do so (87, 88).

When considering the delivery mode of nutrition education to shift workers, group-based workplace education may be the most appropriate. Compared to individual-based education, group-based health education provides an opportunity for participants to relate to each other and normalise their experiences (139). Evidenced in a study evaluating diabetes healthcare education, the feeling of group empathy was observed, whereby participants described themselves as "being equal" and their shared experiences of diabetes management promoted the creation of interpersonal bonds (140). Such connections between group members promote peer support (139), which facilitate the development of practical health behaviour changes, through discussion of common barriers and enablers to change (140). Specifically in an occupation involving shift workers, a U.S. study have shown greater dietary social support when nutrition education was delivered to fire fighters in a team-based setting, compared to individual counselling using motivational interviewing (141). Social support is especially important in promoting healthy eating habits in night shift workers, as colleague's influence has been recognised as a key influence of food choices in the workplace (23, 68).

Undoubtedly, fatigue experienced by night shift workers is closely related to the lack of sleep. Consistent with findings from Chapter 4, health issues related to sleep (e.g. fatigue, lack of sleep, sleep patterns) are main health concerns of night shift workers (95, 142). As a result, workers would use the majority of their time off-work attempting to obtain sufficient sleep, rather than engaging in healthy eating practices (Chapter 4, (23, 142)). It is important for nutrition professionals to respect this prioritisation of sleep, when providing individual nutrition education to night shift workers. Dietary recommendations should then be devised accordingly, and not create additional burdens or time stressors for night shift workers. This is because lack of sleep is an additional risk factor for metabolic disturbances. In laboratory studies with healthy men, five nights of sleep restriction (4 hours in bed) led to increased fasting insulin levels and reduced glucose tolerance, compared to usual sleep conditions (8 or 10 hours in bed) (143, 144). Furthermore, laboratory studies have shown that sleep restriction is associated with increased subjective hunger (145) and voluntary energy intake, in particular snacks high in carbohydrates (146, 147). In the long term, a meta-analysis of 14 observational studies have shown that sleep duration of ≤5 hours/day and ≤6 hours/day is associated with a 48% and 18% increased risk of developing diabetes respectively (148).

Intrapersonal health promotion strategies such as nutrition education often rely on the individual to be ready and motivated to work against environmental constraints, when trying to adopt and maintain health behaviour changes (149). Furthermore, whilst strengthening night shift workers' food literacy skills via nutrition education may encourage them to adopt healthy eating habits, it does not overcome the constant fatigue that they experience. This intrapersonal barrier to healthy eating is attributed to environmental factors, such as inappropriate shift rostering. As such, even though intrapersonal level strategies have their merits, organisational and public policy modifications are required to overcome environmental barriers, in order to promote sustained improvements in food choices and eating habits (136, 149).

5.2.2.2 Organisational (workplace) level

'Creating supportive environments' is one of the key actions stipulated in the 1986 Ottawa Charter for Health Promotion (150). According to the WHO, the workplace is one of the major settings within which supportive environments should be created (108, 150). From a nutrition perspective, a healthy workplace setting should have minimal barriers to healthy eating, and enables healthy eating by making it the easy and convenient choice. Informed by findings from Chapter 3b and Chapter 4, a workplace that promotes healthy eating in night shift workers could be created in a number of ways. Firstly, healthy food options should be made readily available in the workplace. This could be in the form of catering or for purchase, but needs to be affordable and quick and convenient to eat.

Workplaces could consider the provision of healthy frozen meals and shelf-stable snacks such as nuts and tinned tuna with crackers at low-cost. These options have a long shelf-life, thus preventing food wastage and reducing the costs to the company, but are also healthy to the employees. Whilst limited food availability within the workplace also affects day workers' food choices, its impact on night shift workers is compounded by reduced availability of food outlets surrounding the workplace during the night (Chapter 4). Secondly, food preparation equipment and facilities should be provided at the workplace, allowing workers to re-heat or prepare meals that are brought from home. Having access to food preparation facilities at work may also encourage communal cooking and social meal times. Although meals prepared during communal cooking are not always healthy (Chapter 4, (68)), communal cooking contributes to individual wellbeing by fostering social networks, which night shift workers value but struggle to maintain outside of work (Chapter 3b, 4, (142)). Workplace management could also utilise this platform to promote healthy eating, by providing commercially available grocery and cooking kits. Lastly, provision of a break-time area away from food would be beneficial, reducing food temptations for those who are adopting dietary change such as that in 'Shifting the Risk' and reducing eating occasions overnight. The development of supportive food and break-time environments should be central to future workplace interventions targeting night shift workers, as they facilitate the adoption of healthy eating practices at work, by making it the easy and convenient option.

Currently, there are no published studies of workplace interventions, which aim to improve night shift workers' eating habits through modification of the workplace food environment. In a recent systematic review by Lassen et al, four workplace interventions that involved a nutrition component were identified (151). Two of these interventions were education-based, aiming to improve eating habits and physical activity through delivery of information (individual or group), facilitated goal setting and prompting self-monitoring (133, 141). Whilst the method of delivery was evidence-based, the content delivered did not consider the metabolic implications of circadian disruption and did not include any recommendations on meal timing. The third study identified by Lassen et al provided shift workers with a probiotic dairy drink and aimed to reduce common infections, which is unrelated to the prevention of metabolic diseases discussed here (152). A limitation amongst these studies is that rather than modifying the workplace environment to support healthy eating habits, the workplace setting was merely used as a recruitment site. In comparison, the last nutrition workplace intervention identified by Lassen et al provided healthy food options (salad or sandwich, a bottle of water and a snack) to hospital workers (n= 59) during every shift for 4-weeks (153). Whilst the analysis for the entire sample found that the intervention led to an increase in fibre intake and a reduction in fat intake, these improvements were not observed in the subgroup analysis of shift workers (n= 16, worked

evening, night and/or weekend shifts). The reason for this was not investigated, overlooking the feasibility of such intervention for shift workers. Based on the study protocol, it can be speculated that night shift workers had low uptake of this intervention, as meals were only delivered during the day time and on weekdays. This discussion demonstrates the need for approaches that modify the workplace environment of night shift workers. Moreover, it highlights that effectiveness of nutrition interventions for day workers may not necessarily translate to the night shift work environment; stressing the need for workplace interventions that address the unique barriers to healthy eating embedded in the night shift work environment.

In order to successfully implement interventions that involve changes to the workplace environment, engagement with workplace management is essential (154). The perspectives of management personnel in workplaces employing night shift workers have not been specifically explored. Insights elicited from participants in Chapter 4 indicate that workplace managements express little effort or concern over the workplace food environment, consistent with findings from a qualitative study involving 109 Irish shift workers (23). It is currently unknown whether this lack of interest arises from the unawareness that workers' night time eating habits have negative health impacts, or a perception that workers' health is unrelated to the benefits of the company. This perception could be altered by including economic evaluations in nutrition interventions, demonstrating direct financial benefits associated with the intervention. For example, an American study that aimed to improve eating habits and physical activity levels of fire fighters found that after the 1-year intervention, workers' compensation claims and medical costs were significantly reduced in participating fire departments, compared to non-participating departments within the state (155). Moreover, an Australian intervention with manufacturing shift workers indicated that in addition to weight loss (primary outcome), a reduction in absenteeism, workplace injuries and productivity loss was also observed after the 14-week intervention (156). These work and health outcomes are not only important to employers, but also to employees, and are likely to be perceived by night shift workers as tangible health outcomes (Chapter 3b). Therefore, the inclusion of these outcomes in future health promotion strategies will promote interest and participation from both the night shift workers and their workplaces.

5.2.2.3 Public policy level

As with 'creating supportive environments', 'building healthy public policy' is another key action area outlined in the 1986 Ottawa Charter for Health Promotion (150). According to the WHO, 'public policy' encompasses policies in all sectors and at all levels, including those within local organisations (e.g. workplaces), state and national government legislations (150). From the nutrition perspective, public

policy reforms could be a form of 'nutrition-specific' or 'nutrition sensitive' strategy (134). Public policy reforms that are 'nutrition sensitive' often involve sectors outside of food and health, but are able to instigate a flow-on effect and indirectly promote healthy eating practices (157). Development of healthy public policies is a crucial element of health promotion, as it has a wide reach and induces health behaviour changes without voluntary efforts from individuals (150, 157).

At the localised workplace level, policies that will promote healthy eating practices during night shifts may include regulation on provision of healthy food options (nutrition-specific), appropriate meal break scheduling, shift rosters and provision of food preparation facilities in the workplace (nutrition-sensitive). Implementation of these policies has direct financial costs for the employer. As such, workplace managements are unlikely to act on these, unless the financial gains outweigh the costs, or that they are mandated by government legislations. However, current Australian Government legislations seem to lack regulations that protect the metabolic health of night shift workers. For example, based on national legislation on nurses' employee entitlements, employers are only required to allow for a ten-hour interval between two shifts (158), leaving little time for recovery in between (Chapter 4). A report prepared by the Australian Nursing Federation has indicated that increased levels of fatigue in nurses are associated with increased medical errors (159), a consequence that is detrimental to both the employer and the patients. Furthermore, regulations around meal break scheduling state that, the time at which workers receive a meal break is spontaneous and dependent on the workload during the shift (158, 160, 161). This arrangement does not take into account the metabolic disruptions associated with night time eating (Chapter 2), and encourages night shift workers to 'front-load' their food intake, in case of missed meal breaks (Chapter 4). Such regulations apply to shift workers employed across multiple industries, including nurses, fire fighters and manufacturing workers (158, 160, 161). Lastly, the current Australian Work Health and Safety Regulations have not recognised the health implications of night shift work, failing to specify requirements for a safe night shift work environment (162). Australian policy makers need to distinguish night shift work from typical day-time work, in that the work hours cause circadian disruption, which increases risk of metabolic diseases in night shift workers ((17, 19, 21) Chapter 2).

Moreover, the night shift work environment poses unique barriers (Chapter 3b, 4), impeding workers' efforts and motivations to adopt healthy eating habits. Therefore, it is especially important to provide night shift workers with a workplace environment and roster scheduling that support healthy eating practices, in order to protect their metabolic health. In order to bring policy makers' attention to these work safe issues, advocacy driven by unions of shift working industries may be required (163). A focus on cost-benefit analysis is favoured, such as fatigue related medical errors in nursing, to highlight the significance of this issue. Evidently, public policy reforms are required in the Australian setting, to

protect the metabolic health of night shift workers. Whilst not all proposed reforms are nutrition-specific, they have potential flow-on effects to improve eating habits of Australian night shift workers.

Figure 5-1 below presents a summary of the health promotion strategies discussed in this section and recommendations for future research directions proposed in Section 5.2.1. These recommendations are informed by the body of research generated in this Thesis, and are tailored to improving the metabolic health of night shift workers.

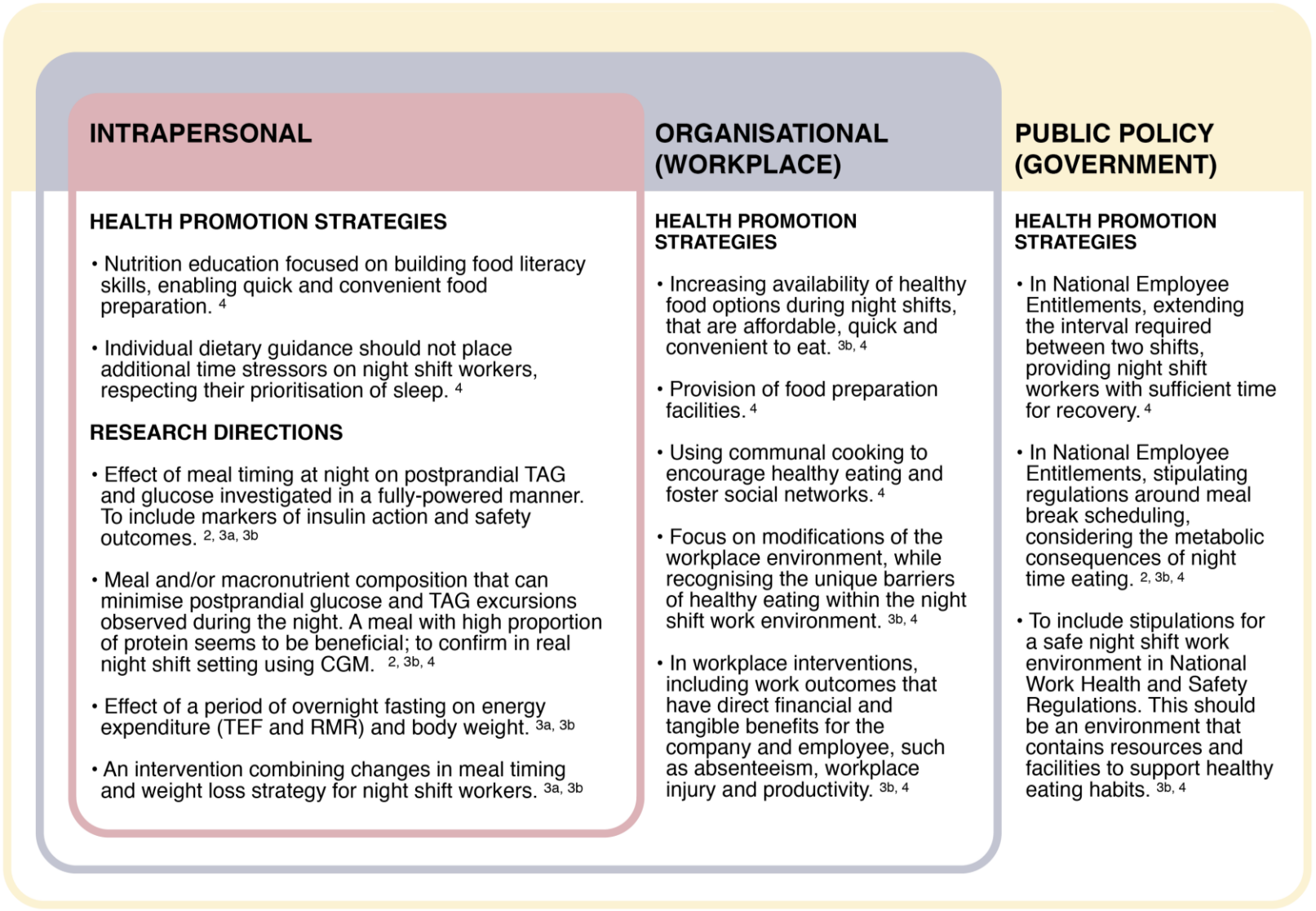

Figure 5-1. Summary of future research directions and health promotion strategies for night shift workers, generated from the findings of this Thesis. Recommendations are grouped into intrapersonal, organisation and public policy levels, as described in the Social Ecological Model (157).

Superscript represent Chapter of which recommendations are based on. *Abbreviations: CGM, continuous glucose monitoring; TEF, thermic effect of food; RMR, resting metabolic rate.*

5.2.3 Overall strengths and limitations

The integrated approach of quantitative and qualitative research methods is a major strength of this Thesis, as it provided a comprehensive understanding of ways in which night shift work affects individuals' metabolic health. Using quantitative methods, Chapter 2 (meta-analysis) and Chapter 3a ('Shifting the Risk': CVD risk factors) extended our understanding on the effect of meal timing, specifically night time eating, on CVD and T2DM risk factors. Using qualitative methods, Chapter 3b ('Shifting the Risk': feasibility) and Chapter 4 (Photovoice study) provided insights into night shift workers' perceptions of a meal timing intervention and experiences with food. The complementary effect of quantitative and qualitative research methods is exemplified in Chapter 3 ('Shifting the Risk'). Whilst quantitative investigations indicated that free-living shift workers are able to maintain a short 5-hour fasting period during night shifts, and that it may lead to a small amount of weight loss, data from the study completion interviews explained 'how' this was achieved and 'why' participants were interested in the study. Both sets of data were used to guide the development of the 'SWIFt' study protocol, a fully-powered weight loss intervention in night shift workers, demonstrating its merit.

The choice of pragmatism as the epistemological underpinning of Chapter 4 (Photovoice) was a considered approach, taking into account the composite use of qualitative and quantitative methods in this Thesis. Research centred in this paradigm values both types of data, as long as they contribute to the solution of the problem identified (106). Therefore, even though data in Chapter 4 were analysed using an inductive coding approach, the discussion of findings is focused on recommendations for health promotion strategies targeting night shift workers. This was further elaborated in this Chapter, in the context of findings from all studies in this Thesis.

The limitations of each study are reported in the relevant Chapters. In this Chapter discussing the overall findings of the Thesis, the following important limitations should be considered. Night shift workers who participated in 'Shifting the Risk' had different occupations, shift work schedules and years of shift work experience. Due to this, they may have been experiencing varying levels of circadian misalignment. A protocol for collection of urinary 6-sulfatoxymelatonin was included in 'Shifting the Risk', in order to assess participants' melatonin levels over a 48-hour period, which would provide an indication of the participants' circadian phase (164). However, participants reported the collection protocol to be difficult and burdensome, and therefore seldom provided the full set of sample required for analysis. As a result, we were unable to assess the influence of or adjust for the degree of circadian misalignment in the data analysis of 'Shifting the Risk'.

Similar to ‘Shifting the Risk’, participants from the Photovoice study (Chapter 4) also had varied occupations, shift work schedules and years of shift work experience. In contrast to ‘Shifting the Risk’, this was an intentional sampling approach of the Photovoice study, aiming for maximum variation within the sample, in order to elicit uniquely different experiences from participants (165). However, the limited number of participants within each of these shift work characteristics precluded formal comparisons during data analysis.

5.3 Thesis Conclusion

This Thesis has substantiated the need to consider meal timing and food choices during the night, in the development of nutrition interventions and health promotion strategies for night shift workers. The causal relationship between night time eating and poor glucose tolerance has been confirmed, indicating a physiological response that explains, in part, the increased risk of metabolic diseases observed in night shift workers. This knowledge was translated into a meal timing intervention, which for the first time, showed that night shift workers are able to maintain a short overnight fasting period within their work environment. A small loss of body weight was also observed. Future research is required to verify this finding, through examining the effect of meal timing during the night on energy expenditure and body weight changes. Through reflecting on their experience, participants expressed that flexibility of meal break structure during their shift and evidence of overt health benefits would promote long term maintenance of dietary interventions. This finding stresses the need to consider workplace influences in future interventions involving night shift workers, which was echoed in the qualitative exploration of Australian night shift workers' experience with food. Furthermore, this exploration explained that night shift workers' food choices and eating habits are shaped by a complex interplay of individual, social and environmental factors. As such, health promotion strategies for this population require a multi-strategy approach, simultaneously targeting multiple levels of influence. Further, this presents a unique opportunity to improve night shift workers' eating habits through nutrition-sensitive approaches, such as workplace or government policy reforms on shift rostering. In conclusion, this Thesis presented multiple strategies in which to improve when and what night shift workers eat. There is an urgent need to test the efficacy of strategies recommended, in order to protect the metabolic health of this essential workforce.

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Appendices

Appendix A: Published manuscript included in Thesis.

Time of day difference in postprandial glucose and insulin responses: systematic review and meta-analysis of acute postprandial studies

Leung GKW, Huggins CE, Ware RS, Bonham MP. Time of day difference in postprandial glucose and insulin responses: systematic review and meta-analysis of acute postprandial studies. *Chronobiology International*. 2020;37(3):311-26. Doi: 10.1080/07420528.2019.1683856.

CHRONOBIOLOGY INTERNATIONAL
https://doi.org/10.1080/07420528.2019.1683856

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Time of day difference in postprandial glucose and insulin responses: Systematic review and meta-analysis of acute postprandial studies

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ABSTRACT

Current dietary trends show that humans consume up to 40% of their energy intake during the night. Those who habitually eat during the night are observed to have an increased risk of metabolic conditions such as type-2 diabetes and cardiovascular disease. Increasing evidence suggest that a biological consequence of eating during the night is a larger postprandial glucose response, compared to meals eaten earlier in the day. However, findings from individual acute postprandial studies have been inconsistent, due to variations in protocols. Therefore, this review aimed to systematically summarize findings from acute postprandial studies and investigate whether postprandial glucose and insulin response at night differs to during the day in healthy adults. This would indicate a possible physiological mechanism linking habitual nighttime eating and increased risk of metabolic conditions. Seven electronic databases were searched in February 2018. Included studies met the following criteria: had a day-time test between 0700 – 1600h, a nighttime test between 2000 and 0400h, the test meals were identical and consumed by the same participant at both day and night time points, preceded by a 3-h fast (minimum). Primary outcome measures were postprandial glucose and insulin incremental area under the curve (iAUC) or area under the curve (AUC). Studies that reported numerical data were included in the meta-analyses, conducted using Stata statistical software (version 13.0, StataCorp, College Station, TX, USA). For eligible studies that did not report numerical data, their authors' conclusions on the effect of time of day on the primary outcome measures were summarized qualitatively. Full text of 172 articles were assessed for eligibility. Fifteen studies met the eligibility criteria, ten of which were included in the meta-analyses. Meta-analysis for glucose showed a lower postprandial glucose response in the day compared to during the night, after an identical meal (SMD = −1.66; 95% CI, −1.97 to −1.36; $p < .001$). This was supported by the findings from included studies ineligible for meta-analysis. Meta-analysis also showed a lower postprandial insulin response in the day compared to during the night (SMD = −0.35; 95% CI, −0.63 to −0.06; $p = .016$). However, findings from included studies ineligible for meta-analysis were inconsistent. Our results suggest poor glucose tolerance at night compared to the day. This may be a contributing factor to the increased risk of metabolic diseases observed in those who habitually eat during the night, such as shift workers.

ARTICLE HISTORY
Received 26 July 2019
Revised 4 October 2019
Accepted 20 October 2019

KEYWORDS
Circadian rhythm; diurnal; diurnal rhythm; meal time; shift work; night; chrononutrition; time of day

Introduction

In Westernized countries, there is an observed trend of humans consuming more energy during the latter hours of the 24-h day. Surveys from the UK and Germany report that dinner accounts for ~40% of daily energy intake, while breakfast only accounts for half of that observed during dinner (Almoosawi et al. 2016). This habitual nighttime eating behavior is in conflict with metabolic activities that follow a circadian rhythm. As a consequence, nutrient metabolism is inefficient during this time, which may result in increased risks of obesity and metabolic syndrome (St-Onge et al. 2017).

The circadian clock system, or "body clock," receives light cues from the external environment (i.e. sunlight) and informs our body whether it is daytime (favoring activity and feeding) or nighttime (favoring sleep and fasting) (Johnston 2014; Kalsbeek et al. 2014). Experimental studies that remove the influence of daylight, bouts of sleep and nutrient loads are used to examine endogenous (natural) rhythms (Qian and Scheer 2016). These "constant routine" studies show that endogenous

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Supplemental data for this article can be accessed here.

rhythms promote a steady increase in blood glucose and insulin concentration through the 24-h day and peak during the biological dark hours (Morgan et al. 1998; Shea et al. 2005).

Acute postprandial studies have examined metabolic responses to larger nutrient loads at different times of the day, in free-living individuals whose circadian rhythms are synchronized with the external light cues. This was firstly conducted by Bowen et al. in the 1960s, who reported a higher glucose response in the afternoon compared to the same meal consumed in the morning (Bowen and Reeves 1967). In recent years, other postprandial studies have reported similar findings with higher postprandial glucose response during the night compared to the day, however results have been conflicting for insulin (Al-Naimi et al. 2004; Biston et al. 1996; Knutsson et al. 2002; Santos et al. 2006). Prolonged postprandial hyperglycemia and reduced insulin sensitivity are both independent risk factors for type-2 diabetes mellitus (T2DM) and cardiovascular disease (CVD) (Dali-Youcef et al. 2013; Gao et al. 2004; Nilsson et al. 2003; Onat et al. 2013). As a consequence, it is important to understand the impact of eating at night on metabolic responses, as this may be a modifiable risk factor.

This systematic literature review and meta-analysis aims to synthesize results from acute postprandial studies, and determine whether the postprandial plasma glucose and insulin concentration differ between daytime (0700–1600h) and nighttime (2000–0400h) in healthy adults. If nighttime eating is confirmed to be associated with dysregulated glucose metabolism, it will justify the importance to consider meal timing, when designing dietary interventions or recommendations to protect the metabolic health of those who habitually eat late at night.

Method

This review is reported according to the Preferred Reporting Items for Systematic reviews and Meta-Analysis (PRISMA) Statement (Liberati et al. 2009).

Search strategy

A systematic search was conducted in February 2018, to identify articles investigating the impact of nighttime eating (food intake after 2000h) on postprandial glucose and insulin concentration in healthy participants. Electronic databases searched were CINAHL plus, Cochrane Library, EMBASE, Ovid MEDLINE, Informit, Psych Info, and Scopus. The search was limited to English language articles and human studies only. An example of the full search strategy from Ovid MEDLINE is presented in Appendix 1.

A keyword search was conducted by combining three sets of search terms with "and" (Table 1). Truncations appropriate for each database were used. A subject term search was also conducted where available, using the terms listed in Table 1.

All titles and abstracts retrieved from the literature search were screened for eligibility by two authors independently. Full-texts of accepted abstracts were reviewed independently by authors GKWL and MPB. Any discrepancies were discussed with author CEH before a final decision on inclusion was made.

Selection criteria

The PICOS (Participants, Intervention, Comparator, Outcomes, Study Design) criteria for study selection are presented in Table 2. Briefly, acute postprandial studies where the same participant consumed a test meal during the day (0700h to 1600h) and during the night (2000h to 0400h) were included. The limits of 1600h and 0400h were chosen, as these were the latest times that participants could be given a meal, before the 3-h postprandial period would become "night" or "day," respectively. "Test meal" was defined as macronutrient administered orally in any form, i.e. as mixed-meal or single

Table 1. Systematic literature review search terms.

SET 1		SET 2		SET 3
Night		Food		Postprandial
Nighttime		"Meal"		"post prandial"
"night time"		Diet*		Postmeal
Nocturnal		Eat*		"post meal"
Diurnal	**AND**	"Nutrient"	**AND**	"tolerance test"
Evening		Energy		"postprandial period"
"night work"		Energy intake		
Shiftwork		Feed*		
"shift worker"		Snack*		
Circadian rhythm		Nutrition		
		"beverage"		
		Drink*		
		glucose		

Table 2. PICOS criteria for inclusion and exclusion of studies.

Parameter	Inclusion criteria	Exclusion criteria
Participants	Age: 18 to 50 years BMI: 18.5 to 30 kg/m^2 "Healthy" participants	Had conditions that affected glucose or lipid metabolism (impaired glucose tolerance, impaired fasting glucose, T2DM, CVD or metabolic syndrome). Used oral hypoglycemic agents, insulin and/or lipid lowering medication.
Intervention	A meal during the day (0700h – 1600h) - preceded by a 3-hour fast (minimum)	
Comparators	A meal during the night (2000h – 0400h) - preceded by a 3-hour fast (minimum) Energy and macronutrient composition identical to day meal	
Outcomes	Postprandial glucose AUC or iAUC Postprandial insulin AUC or iAUC	Did not report numerical value and overall conclusion on primary outcome measures
Study design	Acute postprandial studies Each participant completed both the day and night session	Postprandial blood samples were collected hourly or bihourly

Abbreviations: AUC, area under the curve; BMI, Body Mass Index; CVD, cardiovascular disease, iAUC, incremental area under the curve; T2DM, type-2 diabetes mellitus.

macronutrient load. Studies that administered nutrients intravenously were excluded. Test meals were identical and isocaloric, and preceded by a three-hour fasting period (minimum), which is typically long enough for glucose and/or insulin to return to basal levels. Healthy adult participants were included. Studies that reported participants to be "healthy", but did not explicitly state exclusion of participants based on BMI or conditions listed as exclusion criteria in Table 2 were included.

The primary outcome measures were postprandial plasma glucose and insulin area under the curve (AUC) or incremental area under the curve (iAUC). Studies were excluded if postprandial blood samples were collected hourly or bihourly; as AUC and iAUC could not be calculated using infrequent measurements. Studies that did not provide numerical data nor an overall conclusion regarding the effect of time of day on the primary outcome measures (after contacting the authors on two occasions) were excluded.

Data extraction

A data extraction table was developed by author GKWL, based on a data extraction tool from the Dietitians Association of Australia (Williams et al. 2011). Data were extracted by two authors and included study design, location/setting, sample size, sample characteristics, intervention (pre-intervention fasting period, consumption time and macronutrient composition of test meal), blood collection protocol (time points and route) and data on the primary outcome measures (postprandial glucose and insulin AUC or iAUC). Attempts were made to contact corresponding authors if numerical data on the primary outcome measures were omitted from the article. Authors of three studies dating from 1973 to 1979 could not be contacted as contact information were unavailable (Aparicio et al. 1974; Terpstra et al. 1978, 1979). Eight authors were contacted; three provided data (Gil-Lozano et al. 2016; Holmback et al. 2002; Holmbäck et al. 2003), four did not respond (Lund et al. 2001; Morris et al. 2016, 2015; Sato et al. 2011) and one responded but data were no longer available (King et al. 1994). The conclusions made by the authors of each article, regarding the effect of time of day on postprandial glucose and insulin responses were identified when available.

Quality assessment

Quality assessment of each study was completed by two authors independently, using the *American Dietetic Association (ADA) Quality Criteria Checklist: Primary Research* (American Dietetic Association 2003). This checklist includes four questions relating to the relevance of the study to the target population and 10 questions on validity of the study, which includes questions assessing bias.

Item two, which assesses participant selection bias, was awarded with a "yes" if health status of participants were sufficiently described. As all included studies had participants completing all intervention arms, it was assumed that study groups were comparable and item three was awarded with a "yes" for all studies. Item four was assessed as unclear, if it could not be determined whether all enrolled participants were accounted for; this formed a "no" when scoring the overall rating of the study quality. As primary outcome measures were determined from objective tests (i.e. plasma glucose and insulin concentrations), it was assumed that data collectors were blinded for outcome assessment and item five was awarded with a "yes" for all included studies.

Each study was designated with an overall rating of positive, neutral or negative. To be designated with a positive rating, the study must be free from selection bias; have comparable groups; describe the intervention in sufficient detail; and clearly define outcomes that were obtained from valid and reliable measurements. Studies awarded with positive ratings indicate that they have high validity. Studies that were designated with a negative rating were not included in the synthesis of results.

Meta-analysis

A meta-analysis was conducted for each of the primary outcome measures (postprandial glucose AUC/iAUC and insulin AUC/iAUC). Some studies included multiple time points, however only one day versus night comparison was selected for the meta-analysis. The first time point that occurred within the specified day-time bracket in the eligibility criteria (0700–1600h) was chosen, as this is typically the first meal after waking. The nighttime point chosen was 11 to 12 h apart from the day-time point. Primary outcome measures were reported using different units in included studies; hence, the standardized mean difference (SMD) and associated 95% confidence interval were reported.

Where a study included multiple intervention arms, each was included in the meta-analysis separately, given that each arm had different test meal compositions and was conducted on a separate day. The participants in the study by Gil-Lozano et al. (2016) completed three protocols, each with different sleep conditions. Data chosen for meta-analysis were from sessions without sleep deprivation implemented. The first day-time point included in the Holmback et al. studies were 0800h (Holmback et al. 2002; Holmbäck et al. 2003). However, this time point was not chosen as it was preceded by different pre-test conditions compared to the nighttime comparison (i.e. longer fast and absence of standard pre-test meal). The next day-time point (1200h), which had similar pre-test conditions as the nighttime point was chosen. The two studies by Morris et al. (2015, 2016) exposed participants to a circadian alignment and a circadian misalignment condition and pooled data across the conditions for analysis. Raw data were not available from authors upon request, hence these studies were not included in the meta-analysis.

Heterogeneity was calculated using the I-squared (I^2) statistic. If I^2 was less than 50%, a fixed effects model was used, otherwise a random effects model was used. As participant level differences in glucose/insulin concentration between day and night were not provided by the included studies, when calculating within-participant differences, the correlation coefficient was estimated (Higgins et al. 2008) using raw glucose data provided by Holmback et al. (2002) and raw insulin data provided by Holmbäck et al. (2003). The within-person correlation coefficient was estimated to be 0.4 and 0.3, for glucose and insulin, respectively. These coefficients were used to calculate standard deviations of the difference between day and night for all included studies.

Publication bias was assessed by visual interpretation of funnel plots. Two types of sensitivity analyses were conducted. Firstly, the meta-analyses were re-run, excluding one study at a time, in order to determine whether the summary effect was overly influenced by a particular study. Secondly, several studies included a later time point that still fell within the day-time bracket specified in the eligibility criteria. Therefore, we substituted the original day versus night comparisons with these (where available) as another form of sensitivity analysis. When testing the overall effect of time of day, a p-value of ≤ 0.05 was considered significant. Analyses were conducted using Stata statistical software (version 13.0, StataCorp, College Station, TX, USA).

Data synthesis

Studies that did not report numerical data on primary outcome measures could not be included in the meta-analyses. Instead, the conclusions made by each study's authors, on the effect of time of day on primary outcome measures, were summarized qualitatively. Participant characteristics and descriptions of the intervention are also presented for each study.

Results

Study selection

Electronic searches identified 7297 articles (Figure 1). Following removal of duplicates and screening of titles and abstracts, 172 articles remained, for which full text were retrieved and assessed for eligibility. Overall, 15 studies met the eligibility criteria and were included. Data from 10 studies were included in the meta-analysis. Thirteen comparisons were included in the glucose meta-analysis. Eleven comparisons were included in the insulin meta-analysis.

Characteristics of included studies

Characteristics of the 10 studies included in the meta-analyses (Table 3 (Al-Naimi et al. 2004; Biston et al. 1996; Bo et al. 2017; Gibbs et al. 2014; Gil-Lozano et al. 2016; Holmback et al. 2002; Holmbäck et al. 2003; Leung et al. 2019; Owens et al. 1996; Van Cauter et al. 1992)) and five studies in the qualitative synthesis (Table 4 (Aparicio et al. 1974; King et al. 1994; Lund et al. 2001; Morris et al. 2015, 2016)) are presented. Each of the included studies had the same participant complete both day and night test sessions. The order to complete day or night session was randomized in four studies, and hence were classified as randomized crossover trials (Al-Naimi et al. 2004; Bo et al. 2017; Gibbs et al. 2014; Gil-Lozano et al. 2016). The order was not randomized in others, hence were classified as the following study design: two were pseudo-randomized controlled trials (Morris et al. 2016,

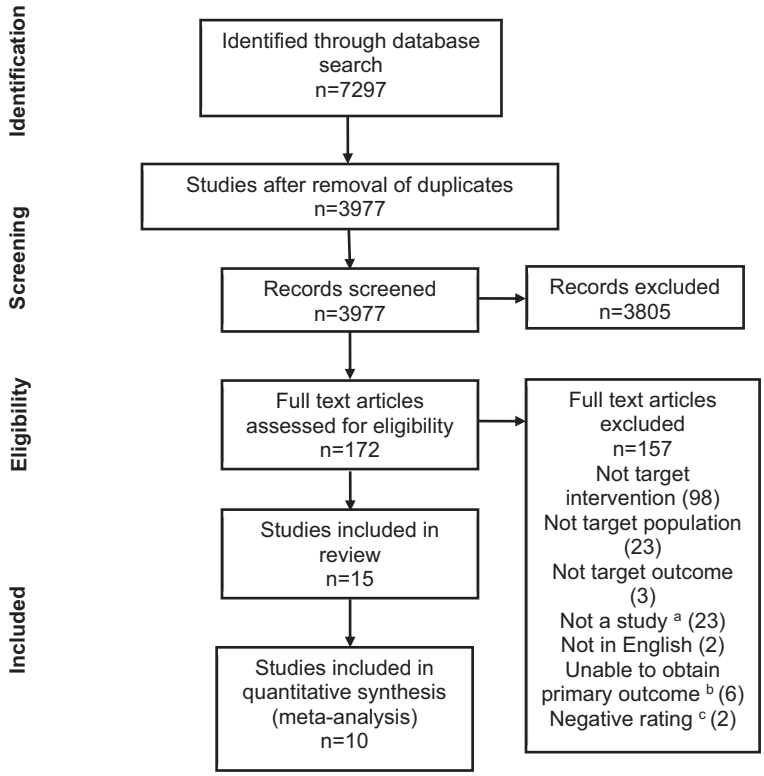

Figure 1. Selection process of included studies of systematic review.

Note: [a] Includes narrative reviews, conference abstracts, news articles. [b] Studies that collected blood samples hourly or bi-hourly; did not provide numerical data and did not make overall conclusion on primary outcome measures. [c] Studies rated as "negative" in quality assessment, using the ADA quality criteria checklist: Primary research.

Table 3. Characteristics of studies included in meta-analyses.

		Participant characteristics			Intervention				
Reference	Study Design	Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (daytime, nighttime) [a]	Fast period	Pre-test meal (MJ)	Energy/test meal (MJ)
Al-Naimi et al. (2004)	Randomized crossover trial	M (8)	R: 20 – 33	R: 20 – 25	2 occasions, each with 3 meals (1: 1300, 1600, 1900 h; 2: 0100, 0400, 0700 h)	a. 1300, 0100 h b. 1600, 0400 h	3 hrs 3 hrs	1.0 3.2	3.2 1.0
Biston et al. (1996)	Non-randomized experimental trial	M (6) F (3)	24.5 ± 2.5 ($\bar{x}$ ± SEM)	22.7 ± 2.1 ($\bar{x}$ ± SEM)	1 occasion, 4 identical meals over 24 hrs: 0830 (pre-test meal), 1400 (test 1), 2000 (test 2), 0830 h next day (test 3)	a. 0830, 2000 h b. 1400, 2000 h	– 12.5 hrs for 0830h arm, 5.5 hrs for 1400 h arm, 6 hrs for 2000 h arm	2.9	2.9
Bo et al. (2017)	Randomized crossover trial	M (10) F (10)	27.6 ± 3.4 ($\bar{x}$ ± SD)	23.4 ± 3.2 ($\bar{x}$ ± SD)	2 occasions, each with 1 meal (0800, 2000 h)	0800, 2000h	8 hrs	4.5	4.9
Gibbs et al. (2014)	Randomized crossover trial	M (1) F (9) n = 9 included in original insulin analysis	25.5 ± 8.8 ($\bar{x}$ ± SD)	21.9 ± 1.7 ($\bar{x}$ ± SD)	1. low GI 2. high GI Each protocol with: 2 occasions, each occasion with 1 meal (0800, 2000 h)	0800, 2000 h	10 hrs for 0800 h arm 8 hrs for 2000 h arm	2.6	1.3 (both low GI and high GI)
Gil-Lozano et al. (2016)	Randomized crossover trial	M (6)	21.1 ± 0.9 ($\bar{x}$ ± SEM)	23.9 ± 1.0 ($\bar{x}$ ± SEM)	2 occasions, each with 1 meal (1100, 2300 h)	1100, 2300 h	5 hrs	1.3	3.6
Holmback et al. (2002)	Non-randomized experimental trial	M (7)	32 (26–43) ($\bar{x}$ (R))	23.8 (19.9–26.6) ($\bar{x}$ (R))	1. high carbohydrate (65% energy) meal [b] 2. high fat (45% energy) meal [b] Each protocol with: 1 occasion, 6 identical meals over 24 hrs (0800, 1200, 1600, 2000, 0000, 0400 h)	a. 1200, 0000 h b. 1600, 0400 h	4 hrs	~ 1.9 [c]	~ 1.9 [c]
Holmbäck et al. (2003)	Non-randomized experimental trial	M (7)	32 (26–43) ($\bar{x}$ (R))	23.8 (19.9–26.6) ($\bar{x}$ (R))	1. high carbohydrate (65% energy) meal [b] 2. high fat (45% energy) meal [b] Each protocol with: 1 occasion, 6 identical meals over 24 hrs (0800, 1200, 1600, 2000, 0000, 0400 h)	a. 1200, 0000 h b. 1600, 0400 h	4 hrs	~ 1.9 [c]	~ 1.9 [c]
(Leung et al. 2019)	Non-randomized experimental trial	M (2) F (8)	23 (4) (med(IQR))	22.6 (3.0) (med(IQR))	1. Oral glucose tolerance test 2 occasions, each with 1 meal (0800, 2000 h)	0800, 2000 h	10 hrs	2.7	1.3
		M (2) F (7)	26 (15) (med(IQR))	23.6 (3.9) (med(IQR))	2. Low GI meal 3 occasions, each with 1 meal (0800, 2000, 0000 h)	0800, 2000 h	10 hrs	2.7	3.3
Owens et al. (1996)	Non-randomized experimental trial	F (9)	R: 19 – 20	20.2 ± 3.0 ($\bar{x}$ ± SD)	4 occasions, each with 1 meal (0200, 0800, 1400, 2000 h)	a. 0800, 2000 h b. 1400, 0200 h	10.5 hrs	n/a	2.4

(*Continued*)

Table 3. (Continued).

		Participant characteristics			Intervention				
Reference	Study Design	Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (daytime, nighttime) [a]	Fast period	Pre-test meal (MJ)	Energy/test meal (MJ)
Van Cauter et al. (1992)	Non-randomized experimental trial	M (4) F (4)	R: 22–35	21.5 ± 2.2 ($\bar{x}$ ± SD)	1. 1 occasion, 5 identical meals over 34 hrs: 2000, 0200, 0800 (test 1), 1400, 2000 h (test 2) 2. 1 occasion, 3 identical meals over 34hrs: 2000, 0800 (test 1), 2000 h (test 2)	0800, 2000 h	1. 6 hrs 2. 12 hrs	2.1 2.1 [d]	2.1 2.1 [d]

[a]Comparison (a) used in main meta-analysis. Comparison (a) was replaced with (b) in sensitivity analysis.
[b]Participants followed the assigned diet for 7 days, data for day and night comparison collected on the last day.
[c]Energy content of test meal was matched to each participant's energy requirement. Approximate energy content of each test meal was estimated by dividing mean daily energy intake of participants (11.3MJ/24 h.) by 6 (meals).
[d]Did not specify energy content, assumed to be the same as protocol 1.
Abbreviations: F, female; GI, glycemic index; hrs, hours; M, male; med, median; R, range; SEM, standard error of means; SD, standard deviation; $\bar{x}$, mean; yrs., years.

2015) and nine were non-randomized experimental trials (Aparicio et al. 1974; Biston et al. 1996; Holmback et al. 2002; Holmbäck et al. 2003; King et al. 1994; Leung et al. 2019; Lund et al. 2001; Owens et al. 1996; Van Cauter et al. 1992).

Included studies had 6 to 20 participants, aged from 19 to 43 years. Five studies included males only (Al-Naimi et al. 2004; Aparicio et al. 1974; Gil-Lozano et al. 2016; Holmback et al. 2002; Holmbäck et al. 2003), two included females only (King et al. 1994; Owens et al. 1996) and eight included both genders (Biston et al. 1996; Bo et al. 2017; Gibbs et al. 2014; Leung et al. 2019; Lund et al. 2001; Morris et al. 2016, 2015; Van Cauter et al. 1992). Eleven studies included healthy weight participants (Al-Naimi et al. 2004; Biston et al. 1996; Bo et al. 2017; Gibbs et al. 2014; Gil-Lozano et al. 2016; Holmback et al. 2002; Holmbäck et al. 2003; Leung et al. 2019; Morris et al. 2016; Owens et al. 1996; Van Cauter et al. 1992), as assessed by mean BMI (or range when mean was not reported), and only one study reported a mean BMI in the overweight category (Morris et al. 2015). Participants' BMI were not reported in three studies (Aparicio et al. 1974; King et al. 1994; Lund et al. 2001), but were stated to be "healthy", hence were assumed to be non-obese. Participants included in studies by Lund et al. (2001) and Morris et al. (2016) were shift workers. The two studies by Holmback et al. (2002, 2003) appeared to have the same sample population, as they had identical participant characteristics, however one reported on glucose response (Holmback et al. 2002) and the other reported on insulin response (Holmbäck et al. 2003).

Included studies had varying protocols (Tables 3 and 4). Five studies administered multiple test meals at set intervals on a single occasion (e.g. four meals over a 24-h period) (Biston et al. 1996; Holmback et al. 2002; Holmbäck et al. 2003; King et al. 1994; Van Cauter et al. 1992). Similarly, three studies administered multiple test meals per occasion, however, the protocol was carried out multiple times (Al-Naimi et al. 2004; Morris et al. 2016, 2015). The remainder of the studies administered one test meal per occasion, over two or more occasions (Aparicio et al. 1974; Bo et al. 2017; Gibbs et al. 2014; Gil-Lozano et al. 2016; Leung et al. 2019; Lund et al. 2001; Owens et al. 1996).

Five of the studies included two arms. Both studies by Holmback et al. (2002, 2003) had a high fat and a high carbohydrate intervention.

Table 4. Characteristics of studies included in qualitative synthesis.

		Participant characteristics			Intervention				
Reference	Study design	Gender (n)	Age (yrs.)	BMI (kg/m^2)	Protocol	Comparison (daytime, nighttime)	Fast period	Pre-test meal (MJ)	Energy/test meal (MJ)
Aparicio et al. (1974)	Non-randomized experimental trial	M (6)	31 ± 7 ($\bar{x}$ ± SEM)	n/s	4 occasions, each with 1 meal (0600, 1200, 1800, 0000 h)	1200, 0000h	10 hrs	Consumed, but energy content not reported	1.7
(King et al. 1994)	Non-randomized experimental trial	F (10)	R: 19–42	n/s	1 occasion, 7 identical meals over 42 hrs: Day 1–1200, 1800, 2400h, Day 2–0600 (start of blood sampling), 1200, 1800, 2400 h, Day 3 – 0600 h (end of blood sampling)	n/s	6 hrs	2.0	2.0
Lund et al. (2001)	Non-randomized experimental trial	M (10) F (2) Shift workers	28 ± 1.9 ($\bar{x}$ ± SEM)	n/s	3 occasions, each with 1 meal (1st (of 7) day shift – 1330h, 2nd (of 7) night shift – 0130 h, 2nd day after return to day shift – 1330 h)	1330, 0130h	5 hrs	2.1	3.3
Morris et al. (2015)	Pseudo-randomized controlled trial	M (8) F (6) n = 10 included in analysis	28 ± 9 ($\bar{x}$ ± SD)	25.4 ± 2.6 ($\bar{x}$ ± SD)	Circadian alignment: 2 occasions, each with 4 meals over 27 hrs: 2000, 0800 (test), 1130, 2000 h (test) Circadian misalignment: 2 occasions, each with 4 meals over 27 hrs: 0800, 2000 (test), 2330, 0800 h (test)	0800, 2000h	Alignment: 12 hrs for 0800 h arm; 8.5 hrs for 2000 h arm Misalignment: 12 hrs for 2000 h arm; 8.5 hrs for 0800 h arm	Consumed, but energy content not reported	33% energy requirements
Morris et al. (2016)	Pseudo-randomized controlled trial	M (3) F (6) Shift workers; n = 7 included in analysis	34 ± 8 ($\bar{x}$ ± SD)	24.2 ± 3.4 ($\bar{x}$ ± SD)	Circadian alignment protocol: 1 occasion, 4 meals over 27 hrs: 2000, 0800 (test), 1130, 2000 h (test) Circadian misalignment protocol: 1 occasion, 4 meals over 27 hrs: 0800, 2000 (test), 2330, 0800 h (test)	0800, 2000h	Alignment: 12 hrs for 0800 h arm; 8.5 hrs for 2000 h arm Misalignment: 12hrs for 2000 h arm; 8.5 hrs for 0800 h arm	33% energy requirements	33% energy requirements

Abbreviations: F, female; GI, glycemic index; hrs, hours; M, male; n/s, not stated; R, range; $\bar{x}$, mean; SEM, standard error of means; SD, standard deviation; yrs., years.

Gibbs et al. (2014) had a low glycemic index (GI) meal intervention and a high GI meal intervention. Van Cauter et al. (1992) had one arm which administered meals at 6-h intervals and another at 12-h intervals. Leung et al. (2019) had an oral glucose tolerance test (OGTT) and a low GI meal intervention. Each arm was included as a separate comparison in the meta-analysis. Aparicio et al. (1974) also administered its test meal as an OGTT, all other included studies administered mixed-meals.

Difference in postprandial glucose response between day and night

Meta-analysis

Thirteen comparisons, from nine studies, were included in the meta-analysis for postprandial glucose response. Two sets of comparisons were included from studies by Gibbs et al. (2014), Holmback et al. (2002), Leung et al. (2019) and Van Cauter et al. (1992), as each included two intervention arms. The daytime points included in the meta-analysis were within the morning hours (0800h to noon), except one study, which had 1300h (Al-Naimi et al. 2004). The meta-analysis showed that postprandial glucose response was lower during the day compared to that observed in the night, after an identical meal (SMD = −1.66; 95% CI, −1.97 to −1.36; $p < .001$) (Figure 2). Heterogeneity was low ($I^2 = 33.0\%$, $p = .12$). Data used for meta-analysis are presented in Appendix 3.

Qualitative synthesis

Five studies did not report numerical AUC/iAUC and overall study conclusions were summarized qualitatively (Table 5) (Aparicio et al. 1974; King et al. 1994; Lund et al. 2001; Morris et al. 2015, 2016). All reported a lower response during the day compared with at night; this was confirmed by statistical tests in four studies (King et al. 1994; Lund et al. 2001; Morris et al. 2015, 2016) while one study did not perform statistical tests (Aparicio et al. 1974).

Difference in postprandial insulin response between day and night

Meta-analysis

Eleven comparisons, from eight studies, were included in the meta-analysis for postprandial insulin response. Two sets of comparisons were

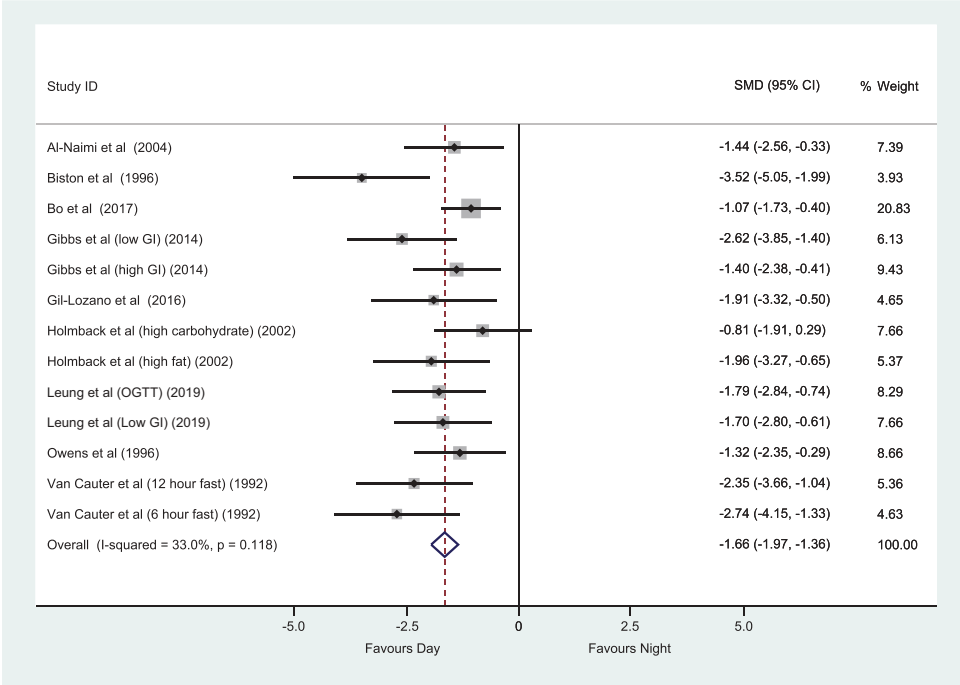

Figure 2. Glucose response meta-analysis: comparison of postprandial glucose response between day and night. Fixed effects model was used.

Table 5. Qualitative synthesis of studies not included in meta-analyses.

Reference	Conclusion on postprandial glucose response	Conclusion on postprandial insulin response
Aparicio et al. (1974)	Postprandial glucose response was affected by time of day: lower levels in the morning (0600 h) and higher levels at 1800 h and midnight (p= n/p).	Postprandial insulin levels were higher in the morning (0600 h) compared to 1800 h and midnight (p= n/p).
King et al. (1994)	In the period of 0000 to 0600 h, postprandial glucose rise was greater compared to the other periods of intervention ($p < .001$).	Postprandial insulin response was affected by time of day ($p < .01$), which reflected the slower rate of decline after meal consumption at 0000 h, compared to the other periods.
Lund et al. (2001)	Postprandial blood glucose response was affected by time of day: higher values during night shift compared with 1st day shift ($p < .01$).	Postprandial blood insulin response was affected by time of day: higher values during night shift compared with 1st day shift ($p < .01$).
Morris et al. (2015)	There was a circadian effect on postprandial glucose response, independent of behavioral effects. Two-hour postprandial glucose AUC was 12% higher at night compared to morning ($p < .0001$).	Early-phase postprandial insulin AUC was 27% lower at night compared to morning ($p < .0001$). Late-phase postprandial insulin AUC was not affected by circadian phase ($p = .26$).
Morris et al. (2016)	There was a circadian effect on postprandial glucose response, independent of behavioral effects. Postprandial glucose was 6.5% higher at night compared to morning ($p = .0041$).	There was a circadian effect on postprandial insulin response, independent of behavioral effects. Both early-phase and late-phase postprandial insulin were 18% lower at night compared to morning ($p = .011$, $p < .0001$ respectively).

AUC, area under the curve; n/p, not provided. Conclusions made by each study's authors on the effect of time of day on postprandial glucose and insulin response.

included from studies by Gibbs et al. (2014), Holmbäck et al. (2003) and Van Cauter et al. (1992), as each included two intervention arms. The daytime points included in the meta-analysis were within the morning hours (0800h to noon), except one study, which had 1300h (Al-Naimi et al. 2004). The meta-analysis showed that postprandial insulin response was lower during the day compared to that observed in the night, after an identical meal (SMD = −0.35; 95% CI, −0.63 to −0.06; p = .016) (Figure 3). Low heterogeneity was detected (I^2 = 31.0%, p = .15). Data used for meta-analysis are presented in Appendix 4.

Qualitative synthesis

Five studies did not report AUC/iAUC and overall study conclusions were summarized qualitatively (Table 5), results varied amongst studies. Three studies reported lower response at night compared to the day (Aparicio et al. 1974; Morris et al. 2015, 2016). This was not confirmed by statistical tests in the study by Aparicio et al. (1974). Both studies by Morris et al. (2015, 2016) analyzed early-phase and late-phase insulin separately. Both reported lower early-phase insulin response at night (defined as 0–30-min postprandial (Morris et al. 2015) or 10–30-min postprandial (Morris et al. 2016)). Morris et al. (2016) found that late-phase insulin response (40–90 min postprandial) was also lower at night, but included permanent shift workers as participants. In contrast, Morris et al. (2015) reported that late-phase insulin response (30–120 min postprandial) was not affected by time of day, but included day workers as participants.

Lund et al. (2001) reported significantly greater postprandial insulin response at night compared to the day, and included shift workers based in Antarctica as participants. King et al. (1994) concluded that postprandial insulin response was significantly affected by time of day, however, no magnitude of response was reported.

Risk of bias within studies

Based on the ADA quality assessment checklist (American Dietetic Association 2003), seven of the included studies were rated positive (Biston et al. 1996; Bo et al. 2017; Gibbs et al. 2014; Gil-Lozano et al. 2016; Holmback et al. 2002; Holmbäck et al. 2003; Leung et al. 2019), eight were rated neutral (Al-Naimi et al. 2004; Aparicio et al. 1974; King et al. 1994; Lund et al. 2001; Morris et al. 2016, 2015; Owens et al. 1996; Van Cauter et al. 1992)

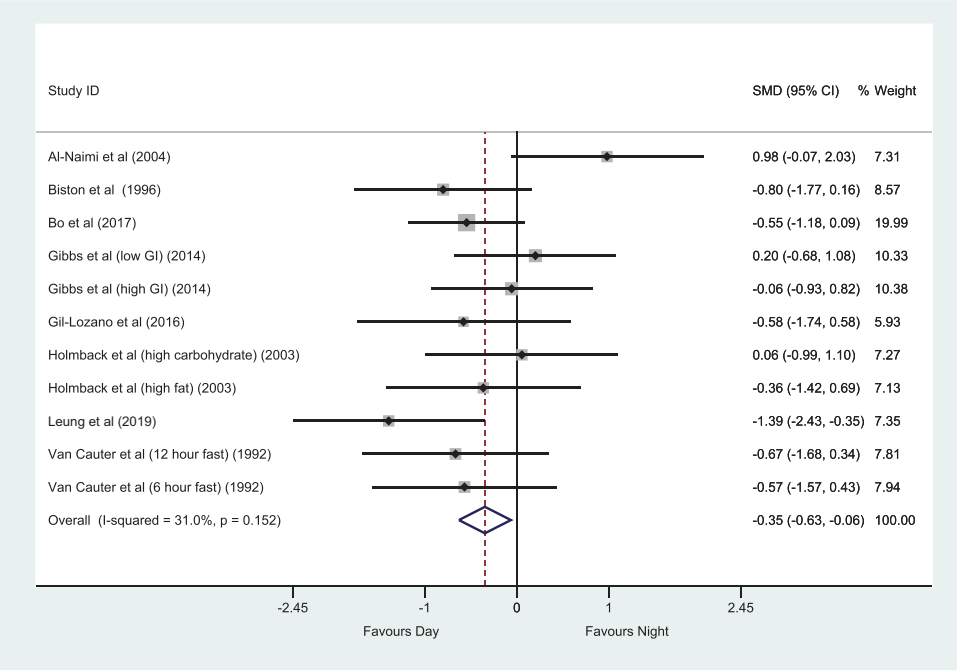

Figure 3. Insulin response meta-analysis: comparison of postprandial insulin response between day and night. Fixed effects model was used.

and two were rated negative (Terpstra et al. 1979; van Gent et al. 1979). The main difference between the positive and neutral studies was that those rated positive were free from participant selection bias. Studies rated neutral did not specify the exclusion of participants based on metabolic conditions that are likely to affect primary outcome measures of the current review, such as T2DM and CVD. The reporting of weight status was also omitted in some studies rated neutral. Studies rated negative were excluded in the synthesis of results. See Appendix 2a and 2b for checklist for each individual study.

Publication bias and sensitivity analysis

Assessment of funnel plots (Appendix 5) showed no evidence of publication bias in glucose and insulin meta-analyses. Take-one-out sensitivity analyses did not lead to any notable changes in the meta-analysis results for both primary outcome measures (Appendix 6).

Four studies examined glucose response at additional time points during the day, all of which were in the afternoon (1400h or 1600h). One study included two arms, therefore provided five afternoon versus night comparisons that were substituted into the original metal-analysis as a form of sensitivity analysis. This did not change the results of the original meta-analysis; postprandial glucose response remained lower during the day compared to the night (SMD = −1.03; 95% CI, −1.32 to −0.74; $p < .001$) (Appendix 7a). However, heterogeneity was higher ($I^2 = 79.2\%$, $p < .001$).

Similarly, three studies examined insulin response at additional time points during the day. This provided four afternoon (1400h or 1600h) versus night comparisons that were substituted into the original meta-analysis as a form of sensitivity analysis. This changed the results of the original meta-analysis; postprandial insulin response was no longer different between day and night (SMD = −0.23; 95% CI, −0.51 to 0.05; $p = .11$) (Appendix 7b). However, low heterogeneity was detected ($I^2 = 28.5\%$, $p = .17$).

Discussion

Summary of findings

This systematic review and meta-analysis of 15 studies, which included paired day/night data from the same participant, found that both postprandial glucose and insulin responses are

significantly higher at night compared to the day. This was important to establish as modern lifestyles have led individuals to extend their eating period during the 24-h day and eat later into the night (Gill and Panda 2015; Shaw et al. 2019). The results of this review further substantiate the recommendations by the American Heart Association (AHA) Statement on meal timing and CVD prevention, that meal timing can form the basis of dietary strategies to protect metabolic health (St-Onge et al. 2017).

A propensity for raised plasma glucose following meal consumption later in the 24-h day and evening, may negatively impact the metabolic health of individuals who habitually consume large proportions of their energy intake at these times. Observational studies have reported that eating late at night is associated with increased risk of hyperglycemia, metabolic syndrome and obesity (Kutsuma et al. 2014; Nakajima and Suwa 2015; Wang et al. 2014). The shift working population is a classic example of those who habitually eat at night. Meta-analyses have shown that shift workers experience up to 40% increased risks of T2DM and CVD compared to those who have never been exposed to shift work (Gan et al. 2015; Tenkanen et al. 1997; Vyas et al. 2012). Higher odds of weight gain have also been reported (Lee et al. 2016; Suwazono et al. 2008), but without differences in daily energy intake compared to day workers (Bonham et al. 2016). This indicates that timing of energy intake may be a key contributor to increased metabolic disturbances observed in this population.

The higher postprandial glucose response at night (compared to the day) reported in the current meta-analysis may be caused by a reduction in insulin sensitivity (Lee et al. 1992; Morgan et al. 1999; Saad et al. 2012). A reduced responsiveness of the liver and peripheral tissues to insulin stimulation at night would reduce glucose uptake by these tissues. Concomitantly, at night the body is under circadian rhythm regulation that dampens anabolic processes (as shown in constant routine studies) (Morgan et al. 1998) and increases endogenous glucose production through the breakdown of stores (e.g. glycogen) (Saad et al. 2012).

The current meta-analysis also showed higher postprandial insulin concentrations at night compared to the day, which is likely to be a counteractive response to the postprandial glucose elevation observed. Whilst this is probably a normal response for the healthy participants included in these acute postprandial studies, frequent hyperinsulinemia is observed in the development of T2DM (Kahn et al. 2006).

The daytime points included in the main meta-analysis were all during the morning hours (0800h to noon), except for one comparison (Al-Naimi et al. 2004). In a sensitivity analysis, morning time points were substituted with afternoon time points provided by the included studies. Whilst this did not alter the results of the glucose meta-analysis, it resulted in a loss of significance for insulin. The postprandial plasma insulin concentration is a composite effect of numerous physiological mechanisms, including β-cell responsiveness and insulin sensitivity. These have influences on insulin secretion and insulin action/clearance, respectively, and together will alter the amount of insulin present in the circulation (Cobelli et al. 2007; Polidori et al. 2016). Aforementioned studies have reported a reduction in insulin sensitivity and β-cell responsiveness at night (Lee et al. 1992; Saad et al. 2012). There is great inter-individual variability in these mechanisms (Clausen et al. 1996) and hence are likely to decline at different times and rates during the 24-h day. This may have contributed to the loss of statistical significance in the insulin sensitivity analysis. Therefore, time of the day differences in β-cell responsiveness, insulin secretion, sensitivity and clearance should be further examined specifically, to provide a better idea of the changes in insulin function throughout the 24-h day.

Strengths and limitations of the review

Strengths of this study include the comprehensive literature search of seven databases, and the inclusion of only studies with paired data from each participant. Excluding hours in the afternoon/evening when defining "day" and "night" in the eligibility criteria reduced heterogeneity and allowed us to conduct meta-analyses that made clear time of day comparisons. There is considerable variation in individuals' circadian rhythms (Baehr et al. 2001; Kantermann and Eastman 2017), therefore it is difficult to pinpoint the specific hours of the night at

which glucose tolerance and insulin sensitivity begin to be less favorable. Nonetheless, the meta-analyses conducted confirms that there is a time of day difference in postprandial glucose and insulin response. These findings support the need for further work in this area; as this is a potential mechanism for increased risks of metabolic disorders.

Although the eligibility criteria of the current review included participants who were overweight (BMI up to 30 kg/m^2), participants of all included studies were actually within the healthy weight range, which reduces the effects of confounding factors such as adiposity (Snijder et al. 2004), however this may also reduce the generalizability of these findings. We were unable to explore gender differences in the meta-analyses, as included studies had a combination of single- and mix-gender samples. However, as all included studies made within-participant comparisons, gender differences should have minimal influence on primary outcomes examined.

Future research directions

Whilst the AHA Statement suggests the maintenance of consistent overnight fast periods, this can be challenging for specific populations who work and eat at atypical times, such as shift workers. The feasibility and effectiveness of overnight fasts should be specifically examined in such populations. Moreover, investigation into dietary strategies to attenuate postprandial hyperglycemia observed during the night is recommended. Manipulation of macronutrient composition (to avoid carbohydrates) may be effective, as meals with higher proportions of protein and/or fat have been shown to reduce postprandial glucose and insulin response, at least in the day (Holmbäck et al. 2003; Moghaddam et al. 2006).

The actions of other glucoregulatory hormones such as glucagon and GLP-1 (Aronoff et al. 2004) should be examined, as some studies have shown that they also exhibit circadian rhythms. Glucagon, a pancreatic hormone, stimulates endogenous glucose production by the liver, thereby increasing blood glucose concentration. Results from a hypoglycemic clamp study showed that fasting and peak glucagon concentration is higher at night (2300h) compared to day (0900h) (Merl et al. 2004). GLP-1, an intestinal hormone, promotes glucose-dependent insulin secretion and suppresses glucagon secretion after food intake (Aronoff et al. 2004; Brubaker and Gil-Lozano 2016). Gil-Lozano et al. (2016) showed that postprandial GLP-1 concentration was higher at night (2300h) compared to the day (1100h). In addition to the postprandial glucose elevation that occurs, this may also contribute to the increased postprandial insulin response observed at night.

Conclusion

The current review highlights the importance of meal timing, to all those who eat at night habitually, such as shift workers. Our meta-analyses showed that postprandial glucose and insulin responses are higher during the night compared to the day. This may be a physiological mechanism causing the increased risks of T2DM and CVD observed in shift workers, and also metabolic syndrome and hyperglycemia observed in late-night eaters. Future intervention studies should investigate the benefits of food or carbohydrate avoidance at night, especially in at-risk populations such as shift workers.

Acknowledgements

The authors would like to express thanks to placement student Hazel Windsor-Aubrey and research assistant Claire Bristow who assisted with the study selection process of this review.

Declaration of interest

The authors of the review report no conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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Appendix B: Published manuscript included in Thesis.

Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers.

Leung GKW, Davis R, Huggins CE, Ware RS, Bonham MP. Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers. *Nutrition, Metabolism and Cardiovascular Diseases*. 2021;31(6):1890-1902. Doi: 10.1016/j.numecd.2021.03.008.

Nutrition, Metabolism & Cardiovascular Diseases (2021) **31**, 1890–1902

Available online at www.sciencedirect.com

Nutrition, Metabolism & Cardiovascular Diseases

journal homepage: www.elsevier.com/locate/nmcd

Does rearranging meal times at night improve cardiovascular risk factors? An Australian pilot randomised trial in night shift workers

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Received 5 November 2020; received in revised form 22 February 2021; accepted 9 March 2021
Handling Editor: A. Siani
Available online 19 March 2021

KEYWORDS
Meal timing;
Shift work;
Circadian misalignment;
Night eating;
Postprandial triglyceride;
Postprandial glucose

Abstract *Background and aims:* Shift workers face an increased risk of cardiovascular disease (CVD), type-2 diabetes and obesity. Eating during the night is a likely contributing factor, as it coincides with the time at which postprandial metabolism is least efficient. In this pilot randomised crossover trial, we examine the effects of a short overnight fast on CVD risk markers (primarily postprandial triglyceride and glucose response) of night shift workers.

Methods and results: Night shift workers with abdominal obesity underwent 4-week intervention and control periods, separated by ≥ 2 weeks washout. In the intervention period, an overnight fast (0100 h–0600 h) was implemented, by redistributing 24-h energy intake. Usual dietary habits were followed in the control period. Outcomes between intervention and control were compared using mixed effects linear regression models.

Nineteen adults completed the trial [13 females, mean (±SD) age 41 ± 10 years, BMI 30.7 ± 5.7 kg/m^2]. Postprandial triglyceride and glucose response post intervention were not different to post control. The overnight fast was well-tolerated by participants with an adherence rate of 95%, assessed by weekly 24-h dietary recalls. Exploratory analysis indicates lower mean body weight post intervention compared to post control (mean difference: −0.9 kg, 95% CI: −1.3 to −0.4).

Conclusions: Night shift workers who habitually ate during their night shifts were able to rearrange their meal times to maintain a small overnight fast, which may have promoted small weight changes. This warrants further investigation into the role of meal timing in mitigating the metabolic consequences of night shift work.

Trial registration: Australian New Zealand Clinical Trials Registry (http://anzctr.org.au/) registered on the 30th May 2017 (ACTRN12617000791336).

Introduction

Shift workers form a significant proportion of the workforce in industrialised countries [1]. Approximately 16% of the Australian workforce are shift workers [2], who work across multiple industries and are integral to the operations of the 'round-the-clock' modern society. Epidemiological studies have reported that shift workers are at an

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https://doi.org/10.1016/j.numecd.2021.03.008

increased risk of metabolic diseases compared to their day-working counterparts. Meta-analyses of observational studies have shown that the risk of shift workers developing type-2 diabetes (T2DM) is 10% higher [3], while the risk of developing cardiovascular disease (CVD) is 10 to 20% greater [4,5]. In a cohort of 1806 shift workers employed in manufacturing, the association between shift work and risk of CVD remained, after adjustment for traditional risk factors such as Body Mass Index (BMI), physical activity level and age [6]. Multiple observational studies have reported an increased risk of obesity in shift workers [7,8] that could not be explained by increases in energy intake. These findings are supported by a meta-analysis of 12 cross-sectional studies, which showed shift workers' daily energy intake was similar to that of their day-working counterparts [9].

Whilst shift workers' overall energy intake may be similar to that of day workers [9], the times at which they eat differ. Shift workers often eat during and throughout the night, typically fitting their meal times around their shift schedules [10–12]. However, as humans are diurnal animals, feeding is favoured during the day, whilst the night is reserved for rest and fast. This temporal separation of feed/fast activity is set by the circadian clock system [13]. This system regulates the timing of numerous physiological and metabolic processes, synchronising them with each other and to the anticipated feed/fast cycle. This mechanism evolved as a way to optimise metabolic efficiency [14]. Shift workers' habitual night time eating behaviour is therefore out of synchronisation with the timing set by the circadian clock, and its impact on metabolism is reflected in findings from diurnal studies. In these studies, postprandial lipaemic and glycaemic responses are measured at regular intervals, after participants consume identical meals at different times of the 24-h day. They are performed under controlled laboratory settings, with consistent energy intake and fast periods prior to the testing session, and restricted food intake and physical activity during the session. These studies have reported compelling evidence, showing increased postprandial glucose and insulin responses during the night compared to the day. These findings are supported by a recent meta-analysis of ten diurnal studies [15] and suggest a potential physiological mechanism contributing to the increased CVD and T2DM risks observed in shift workers. Similar diurnal variations have been observed in postprandial lipid metabolism. In a recent systematic review, all five included studies reported at least one parameter of postprandial triglyceride (TAG) response (e.g. total concentration or time course kinetics) that were different due to time of day [16]. However, findings are less convincing than for postprandial glycaemic response, as variations in study protocols precluded a meta-analysis. Recent evidence also suggests time of day differences in energy expenditure, with lowered thermic effect of food (TEF) and possibly resting metabolic rate during the night (summarised in Shaw et al. [17]). These findings provide plausible explanations for the comparatively rapid weight gain observed in shift working populations, in the absence of increases in energy intake. Together, these reviews suggest poor glucose tolerance, reduced insulin sensitivity, compromised lipid handling and energy expenditure during the night; all of which are independent risk factors for the development of CVD, T2DM and obesity [18–21]. Given that shift workers are typically awake working and eating during the night [22], their time of energy intake is a probable contributor to their increased risk of metabolic diseases.

Considering the exaggerated postprandial metabolic responses that occur at night, minimising energy intake during these hours by maintaining a small period of fasting, should induce metabolic benefits in night shift workers. In this pilot randomised crossover trial, we implemented a short overnight fast (0100 h–0600 h) in the participants' temporal eating pattern for four weeks, by redistributing energy intake to other hours of the 24-h day. We aimed to examine this meal timing intervention's effect on CVD risk markers of at-risk night shift workers (i.e. with abdominal obesity). This paper reports on biochemical (postprandial TAG, glucose and insulin response) and anthropometric (body weight) risk factors of CVD, before and after the intervention crossover. Adherence to the intervention is examined through dietary assessment. This is a novel study, translating effects of meal timing observed in acute laboratory studies, to a dietary intervention implemented in free-living night shift workers for a comparatively extended period. As such, sample size determination was precluded in this pilot; considerations and requirements for a future definitive randomised controlled trial (RCT) will be discussed.

Methods

Trial design

This pilot randomised crossover trial, referred to as the 'Shifting the Risk' Study, was conducted in Melbourne, Australia, between July 2017 and October 2018. The study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12617000791336) and the study protocol has been published [23]. This study was approved by the Monash University Human Research Ethics Committee (Project ID: 2017-8619) and was conducted in accordance with the Declaration of Helsinki for human studies.

Participants

We aimed to have 20 participants complete the study; an attrition rate of 30% was assumed, therefore we aimed to recruit and randomise 28 participants [23]. A formal sample size calculation prior to study commencement was not conducted, due to the pilot nature of the trial. Eligible participants were permanent or rotating night shift workers, involved in night shift work for a minimum of 12 months consecutively prior to study commencement and were expecting to work at least three night shifts per fortnight during the study's duration. They were aged

between 18 and 60 years and had abdominal obesity, as measured by researchers during the screening session prior to enrolment, and defined as waist circumference >94 cm (non-Asian men), >90 cm (Asian men) or >80 cm (all women) [24].

Night shift workers were ineligible for the study if they did not work or routinely eat between 0100 h and 0600 h whilst on night shift, had an existing diagnosis of CVD or T2DM, on medication for diabetes, hyperlipidaemia or medications known to alter metabolism (e.g. thyroxin, insulin sensitisers, glucocorticoids or anti-depressants). Further exclusion criteria are outlined in published study protocol [23].

Experimental protocol

The study was 11 weeks in duration; the experimental protocol is summarised in Fig. 1. In the run-in period, participants were asked to complete a 4-day food diary that included two night shifts, to capture their usual dietary intake on shift. Participants were then randomised to begin with either the intervention or control period (both four weeks minimum). These two test periods were separated by a wash-out period of two weeks minimum. Three acute meal challenge sessions were conducted at the Monash University Be Active Sleep Eat (BASE) Facility (Melbourne, Australia) for outcome assessment: at baseline, end of first period (challenge session 2) and end of second period (challenge session 3).

Test periods

During the intervention period, participants were asked to avoid energy intake for a fixed 5-h window during the night (0100 h–0600 h). They were instructed to do so for the entire 4-week period, including the non-night shift days. Participants were asked to change their meal times only, and not the types of foods and beverages consumed, with an aim to maintain usual total daily energy intake. Prior to starting the intervention period, each participant met with the study dietitian (G.K.W.L), who advised on strategies to redistribute meal times. Participants received SMS text messages on a night shift (at least once weekly), reminding them not to consume any food or beverages (besides water) during the designated fast window. During the control period, participants were asked to maintain (or return to) their usual dietary habits for four weeks.

The study was designed to keep participants weight stable during the study's duration. As such, participants were asked to avoid changing their food choices and physical activity levels. During both test periods, participants were asked to wear a SenseWear activity monitor (Armband Model MF-SW, Bodymedia, Pennsylvania, USA) for the 5-days prior to attending the challenge session. This monitor provides an estimate of physical activity level. Adherence to the dietary intervention was monitored by 24-h dietary recalls, which were conducted over the phone with a researcher. These were completed once weekly, after a night shift, during both test periods. Participants were asked to report their dietary intake from 0600 h the day prior to 0600 h of the current day.

Outcome measures

Primary outcome measures are postprandial TAG and glucose response, assessed by calculating incremental area under the curve (iAUC). The secondary outcome measure is postprandial insulin iAUC. Hourly concentrations from 0 to 6 h postprandially were used to calculate TAG iAUC, while concentrations from 0 to 3 h postprandially (eight time points) were used to calculate glucose and insulin iAUC. Other blood lipid and glucose outcomes examined include fasting total cholesterol, LDL-cholesterol (LDL-C) and HDL-

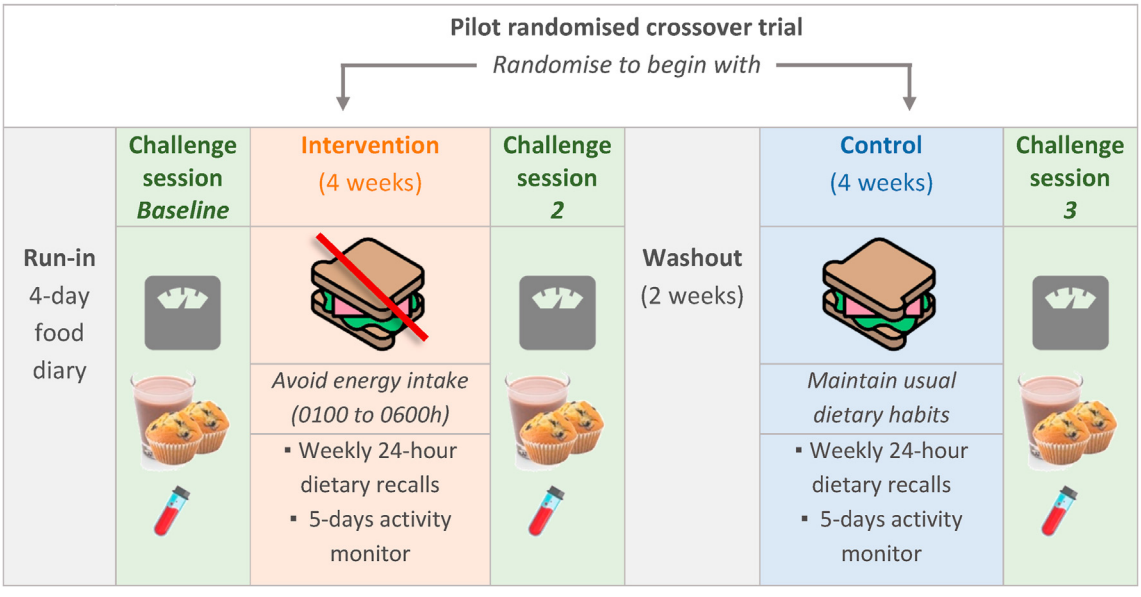

Figure 1 Experimental protocol of 'Shifting the Risk'. All challenge sessions were seven hours in duration, running from approximately 0730 h–1430 h. At each challenge session, participant's body weight was taken. A high fat test meal was then consumed and blood samples were taken for a 6-h postprandial period.

cholesterol (HDL-C) levels; lipid ratios; peak and time to peak postprandial TAG and glucose; postprandial TAG concentration at four hours; postprandial glucose concentration at two hours and HOMA-IR. Body weight and adherence to the dietary intervention were also recorded.

Outcome assessment

Acute meal challenge session (for biochemical and anthropometric measures)

Three challenge sessions were conducted: at baseline, end of first period (challenge session 2) and end of second period (challenge session 3). Outcome data, including postprandial TAG, glucose and insulin levels and body weight were collected during each session. Each session was scheduled on the second or third day after a night shift. Participants were asked to refrain from strenuous exercise and alcohol on the day prior to each session. They were given a standardised meal to consume between 1800 h and 2000 h the night prior (2810 kJ, 56% energy (E) from carbohydrate, 24% E fat, 17% E protein), then fast overnight, until arriving at the BASE facility for the session.

Each session ran for seven hours, from approximately 0730 h−1430 h. Upon arrival at the BASE facility, anthropometric measures were taken in the fasted state. Waist circumference was measured using standardised procedures, body weight and composition were measured using the SECA Bioelectrical Impedance Analyser (515/514, SECA Group, Hamburg, Germany). Blood pressure was taken in duplicate on participant's right arm, using an automated blood pressure monitor (ProBP 3400 Digital Blood Pressure Monitor, Welch Allyn, New York, USA), according to standardised procedures. A cannula was then fitted in the antecubital fossa or back of hand by a trained nurse or phlebotomist and a fasting blood sample was taken. Participants were provided with a test meal high in fat, to be consumed within 15 min. It consisted of two muffins and a milkshake, providing a total of 3779 kJ (56% E fat, 36% E carbohydrates, 8% E protein). A high fat load (58 g: 22% saturated, 30% monounsaturated; 48% polyunsaturated) was included to maximise the postprandial lipaemic response [25]. Blood samples were taken at regular intervals for six hours post-meal (15, 30, 45, 60, 90, 120, 180, 240, 300, 360 min). During the challenge session conducted at baseline, participants completed the Morningness and Eveningness questionnaire [26], to determine their chronotype. Participants were not permitted to consume any other food or drinks (except water) during the postprandial period and remained sedentary.

Adherence

Adherence to the dietary intervention was determined from weekly 24-h dietary recalls. It is reported as the percentage of dietary recalls collected in the intervention period, which documented nil energy intake between 0100 h and 0600 h. Estimates of usual daily physical activity energy expenditure (activity EE) during each test period were derived from the SenseWear activity monitor data.

Biochemical analysis

Serum lipid and plasma glucose concentrations were measured using the Indiko Clinical and Specialty Chemistry System (Thermo Fisher Scientific, Vantaa, Finland). Plasma insulin concentration was measured using the Human Insulin Specific RIA kit (HI−14 K, Merck Millipore, Massachusetts, USA) according to manufacturer's instructions and read on a Gamma Counter. HOMA-IR was calculated using the following formula: [fasting plasma insulin (μU/ml) x fasting plasma glucose (mmol/l)] ÷ 22.5.

Randomisation

The randomisation sequence was generated by a researcher who was not involved in study recruitment or baseline data collection (C.E.H), through a computer-generated random number sequence using a permuted block design. Researchers responsible for eligibility assessment and data collection (G.K.W.L and R.D) were provided with the treatment allocation in a sealed envelope after the enrolment of each participant.

Statistical analysis

Descriptive statistics are summarised as mean ± standard deviation (SD) for continuous data and as frequency (percentage) for categorical data. Outcome variables were visually tested for normality using residual plots. The association between test period (intervention/control) and biochemical outcome measures (challenge sessions 2 and 3) were assessed using mixed effects linear regression models, with test period (intervention/control) included as a fixed effect and participant included as a random effect. In each model, the individual participant's baseline challenge session outcome value was included as a fixed effect covariable, to adjust for any baseline differences between participants. For example, in the model comparing the difference in fasting glucose concentration between intervention and control, the fasting glucose concentration measured at the baseline challenge session was included as a covariate. Effect estimates are presented as mean difference (MD) and 95% confidence intervals (95% CI). A MD $>$ 0 indicates the outcome was greater post intervention compared to post control, and vice versa. A preliminary examination showed there was no detectable study period effect, that is, results did not depend on the order of the test sequence, and consequently, period was not included as a covariable in the analytic models. Sensitivity analyses investigated the effect of log-transforming outcome data. However, models were robust, therefore untransformed data and analyses are presented. Analysis was conducted using Stata statistical software (version 14.1, StataCorp, College Station, TX, USA).

Sample size estimation for future definitive RCT

Using results obtained from this pilot, we have estimated sample sizes required for fully-powered definitive RCTs, based on postprandial TAG and glucose iAUC (primary

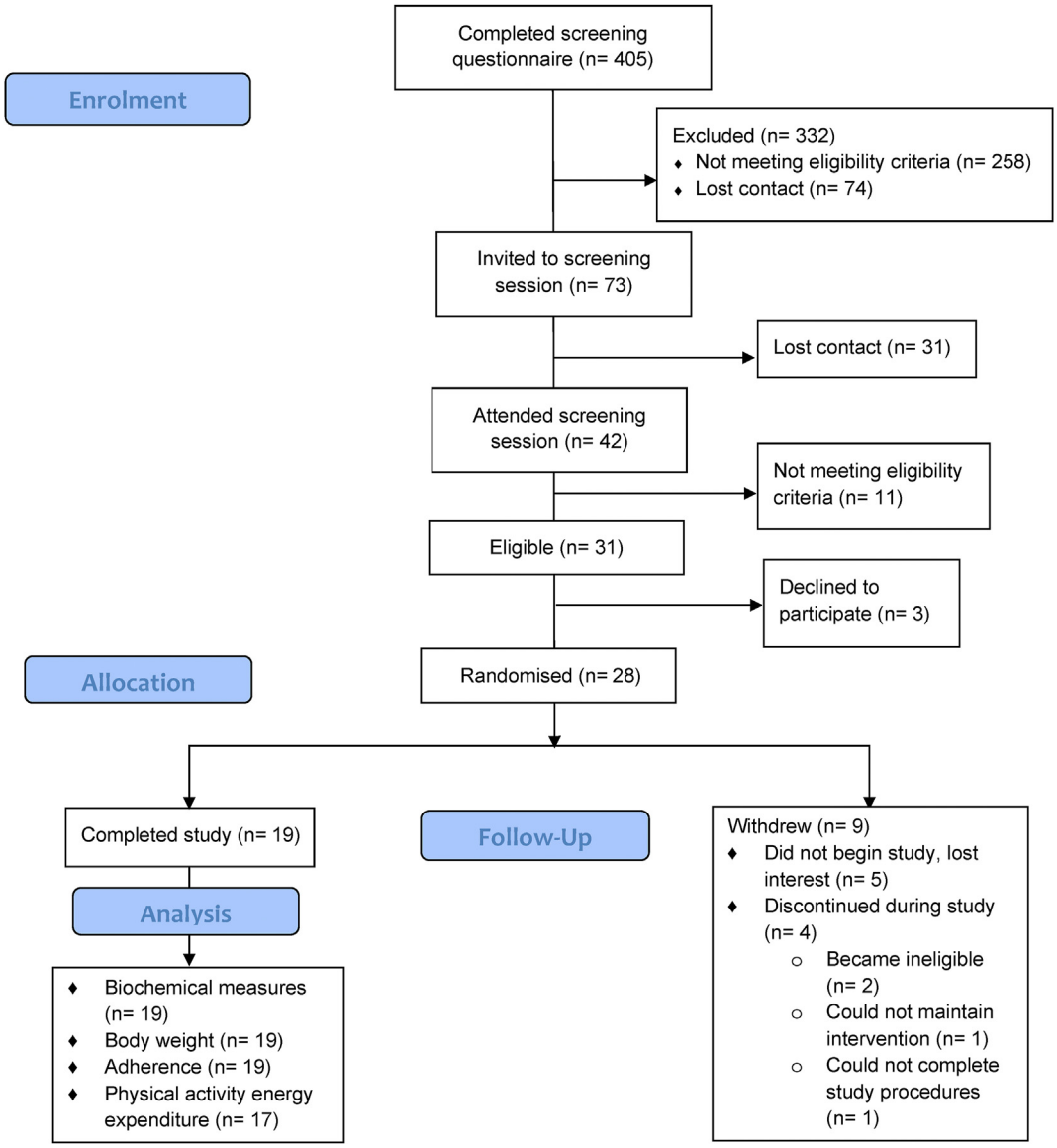

Figure 2 Study participant flow of 'Shifting the Risk'.

outcome measures), peak postprandial TAG concentration, glucose concentration at two hours postprandially and body weight. For each outcome, a sample size was estimated based on the observed SD of the difference in values between the two test periods from the same participant [27].

Results

Participant enrolment

There were 405 individuals who expressed interest in participating and completed the screening questionnaire. Thirty-one individuals were confirmed to be eligible from the screening session and 28 were randomised. Nineteen (68%) participants completed the study, giving an attrition rate of 32%. Amongst the nine participants who withdrew, four had commenced the study. One participant withdrew because they could not maintain the dietary intervention and another because they could not complete the outcome assessment procedures. All nine participants withdrew prior to completion of their first test period, and consequently did not contribute available data for analysis. Their age and anthropometric measures were taken during the screening session; and were not significantly different to those who completed the study (assessed by independent t-test, Supplementary Table 1). Fig. 2 details study participant flow.

Baseline characteristics

Baseline and demographic characteristics of participants were taken at the baseline challenge session and are presented in Table 1. Most participants were female (n = 13) and worked permanent night shifts (n = 12). The mean

Table 1 Demographic, employment and clinical characteristics of study participants in 'Shifting the Risk', measured at baseline (n = 19).

Demographics	Frequency (percentage)[a]		
Age (years), mean ± SD	41 ± 10		
Gender			
Female	13 (68)		
Male	6 (32)		
Occupational fields			
Healthcare and emergency services support	11 (58)		
Hospitality and food service	3 (16)		
Machinery operations and transport services	4 (21)		
Protective services	1 (5)		
Form of shift work			
Permanent night	12 (63)		
Rotating[b]	6 (32)		
Permanent morning[c]	1 (5)		
Years in shift work, median (range)	14 (1.2–28)		
Chronotype [d]			
Moderate morning	3 (16)		
Intermediate	14 (74)		
Moderate evening	2 (11)		
Non-smoker	17 (89)		
Body composition[e]	**Mean ± SD**		
	Total (n = 19)	Female (n = 13)	Male (n = 6)
Body weight (kg)	86.2 ± 17.2	84.7 ± 19.6	89.4 ± 11.1
BMI (kg/m^2)	30.7 ± 5.7	31.7 ± 6.6	28.6 ± 2.4
Waist circumference (cm)	97.3 ± 11.2	96.9 ± 13.4	98.2 ± 4.4
Fat mass (kg)	32.8 ± 12.8	36.6 ± 13.7	24.7 ± 5.0
Fat mass (%)	37.5 ± 9.0	42.1 ± 6.5	27.5 ± 3.3
Fat free mass (kg)	53.3 ± 10.8	48.1 ± 7.6	64.7 ± 7.4
Metabolic measures[e]	**Mean ± SD**		
Blood pressure (mmHg)	123 ± 13/79 ± 7		
Fasting biochemical measures[e]	**Mean ± SD**		
TAG (mmol/l)	1.1 ± 0.6		
Total cholesterol (mmol/l)	4.7 ± 0.8		
HDL-cholesterol (mmol/l)	1.4 ± 0.4		
LDL-cholesterol (mmol/l)	2.9 ± 0.8		
Glucose (mmol/l)	5.9 ± 0.5		
Insulin (μU/ml)	15.7 ± 5.9		
HOMA-IR	4.1 ± 1.8		
Postprandial biochemical measures[e]	**Mean ± SD**		
TAG iAUC (mmol/l.6 h)	145.8 ± 89.4		
Glucose iAUC (mmol/l.3 h)	89.5 ± 64.1		
Insulin iAUC (μU/ml.3 h)	7592.8 ± 4521.3		

Abbreviations: iAUC, incremental area under the curve; HOMA-IR, homeostatic model assessment of insulin resistance; SD, standard deviation; TAG, triglyceride.

[a] Unless otherwise stated.

[b] Rotating shift schedule included a combination of morning, afternoon and night shifts.

[c] Shift hours were from 0400 to 1200, participant met eligibility criteria.

[d] Assessed by Morningness and Eveningness Questionnaire [26].

[e] Measures taken at baseline acute meal challenge session.

(±SD) age was 41 ± 10 years; females had a mean waist circumference of 96.9 ± 13.4 cm, males' was 98.2 ± 4.4 cm and the sample's mean BMI was 30.7 ± 5.7 kg/m^2. The mean blood pressure, fasting TAG and glucose were within the healthy ranges [24,28]. The mean (±SD) postprandial TAG and glucose iAUC were 145.8 ± 89.4 mmol/l.6h and 89.5 ± 64.1 mmol/l.3h respectively, measured at baseline challenge session.

Comparison of outcome measures between intervention and control

Test periods

Within those who completed the study, ten participants began with the intervention period. The intervention period had a median (range) duration of 29 (24–49) days; and included 12 (7–24) night shifts. The control period

Table 2 Association between test period (intervention/control) and clinical outcomes for 'Shifting the Risk' participants (n = 19).

Outcome	Intervention (mean ± SD)[1]	Control (mean ± SD)[1]	Mean difference	95% CI	p-value
Fasting measures					
TAG (mmol/l)	1.3 ± 1.1	1.1 ± 0.5	0.2	−0.1–0.4	0.14
Total cholesterol (mmol/l)	4.8 ± 1.0	4.6 ± 0.7	0.1	−0.1–0.3	0.17
HDL-C (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	0.0	−0.1–0.0	0.45
LDL-C (mmol/l)	2.9 ± 0.8	2.8 ± 0.8	0.1	0.0–0.2	0.09
Glucose (mmol/l)	5.8 ± 0.6	5.8 ± 0.5	0.1	−0.1–0.3	0.39
Insulin (μU/ml)	16.6 ± 9.8	15.0 ± 7.2	1.5	−1.2–4.2	0.27
HOMA-IR	4.4 ± 3.3	3.9 ± 1.9	0.6	−0.3–1.4	0.17
Total cholesterol: HDL-C	3.6 ± 1.3	3.5 ± 1.2	0.1	−0.1–0.3	0.28
LDL-C: HDL-C	2.2 ± 0.8	2.2 ± 1.0	0.1	−0.1–0.2	0.43
Postprandial measures					
TAG response					
TAG iAUC (mmol/l.6 h)	174.5 ± 129.0	149.7 ± 109.7	24.8	−12.7–62.3	0.20
Peak TAG (mmol/l)	2.1 ± 1.6	1.9 ± 0.9	0.2	−0.1–0.6	0.20
Time to peak TAG (mins)	196 ± 77	202 ± 75	−6	−50–38	0.78
TAG concentration at 4 h (mmol/l)	1.9 ± 1.6	1.6 ± 0.9	0.3	0.0–0.7	0.06
Glucose response					
Glucose iAUC (mmol/l.3 h)	82.9 ± 71.1	93.9 ± 85.5	−11.0	−45.1–23.2	0.53
Peak glucose (mmol/l)	7.5 ± 1.3	7.4 ± 1.2	0.1	−0.2–0.5	0.41
Time to peak glucose (mins)	48 ± 30	44 ± 23	4	−9–17	0.54
Glucose concentration at 2 h (mmol/l)	5.6 ± 1.2	5.4 ± 1.4	0.2	−0.3–0.7	0.40
Insulin response					
Insulin iAUC (μU/ml.3 h)	7470.2 ± 5125.2	7378.2 ± 4516.8	92.1	−807.1–991.2	0.84
Body weight (kg)	86.2 ± 17.4	87.1 ± 17.7	−0.9	−1.3–−0.4	<0.001
Daily activity energy expenditure (kJ)	4983 ± 2329[2]	5254 ± 2324[2]	−188	−658–282	0.43

Associations between test period and outcomes assessed using mixed effects linear regression models. [1] Measures taken at acute meal challenge session 2 and 3. [2]Measures taken during test period. Abbreviations: CI, confidence interval; h, hour; HDL-C, HDL-cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; iAUC, incremental area under the curve; LDL-C, LDL-cholesterol; mins, minutes; SD, standard deviation; TAG, triglycerides.

had a median duration of 33 (26–48) days; and included 16 (7–29) night shifts. The washout period had a median duration of 15 (0–109) days. Participant whose washout period was 109 days completed the intervention period first.

Outcome measures taken at challenge sessions 2 and 3 were used, to compare the differences between intervention and control. Participants attended the challenge sessions between 2 and 6 days after their last night shift post intervention (84% met the requested 2–3 day timeframe) and between 2 and 11 days after their last night shift post control (74% met the requested 2–3 day timeframe).

Fasting TAG, glucose and insulin

Fasting TAG and glucose levels remained within healthy ranges after both test periods [24,28]. Comparing measures taken at the end of intervention to control, there were no differences in mean fasting TAG, glucose and insulin levels (Table 2).

Postprandial TAG response

During the challenge session post intervention, a steady increase in postprandial TAG concentration was observed, which reached a mean (± SD) peak of 2.1 ± 1.6 mmol/l between three and four hours postprandially (Fig. 3a). Visual examination of the time-course curve shows that TAG concentration did not return to fasting levels at the end of the session (mean: 1.6 ± 1.4 mmol/l at six hours postprandially). A similar postprandial response was observed at the challenge session post control, with no significant difference in postprandial TAG iAUC between the test periods (MD: 24.8 mmol/l.6 h; 95% CI: −12.7 − 62.3; p = 0.20; Table 2).

Postprandial glucose and insulin response

During the challenge session post intervention, postprandial glucose concentration reached a mean (±SD) peak of 7.5 ± 1.3 mmol/l at approximately 45 min postprandially (Fig. 3b). A steep decline was observed thereafter; and returned to fasting levels by approximately 90 min postprandially. Peak glucose concentration and time-to-peak were similar at challenge session post control (Table 2), however, visual examination shows that it took slightly longer to return to fasting levels (between 90 and 120 min postprandially). Overall, time-course curves observed during challenge sessions post intervention and control were similar, with no significant difference in postprandial glucose iAUC between the test periods (MD: −11.0 mmol/l.3 h; 95% CI: −45.1 − 23.2; p = 0.53; Table 2).

At the challenge session post intervention, insulin concentration rose concurrently with glucose, reaching a mean (±SD) peak of 100.7 ± 48.5 μU/ml at approximately 45 min postprandially (Fig. 3c). Visual inspection of time-course curve showed that insulin concentration at three hours postprandially did not return to fasting levels (mean: 38.1 ± 42.8 μU/ml), likely due to sustained postprandial TAG response. A similar postprandial response was observed at the challenge session post control, with

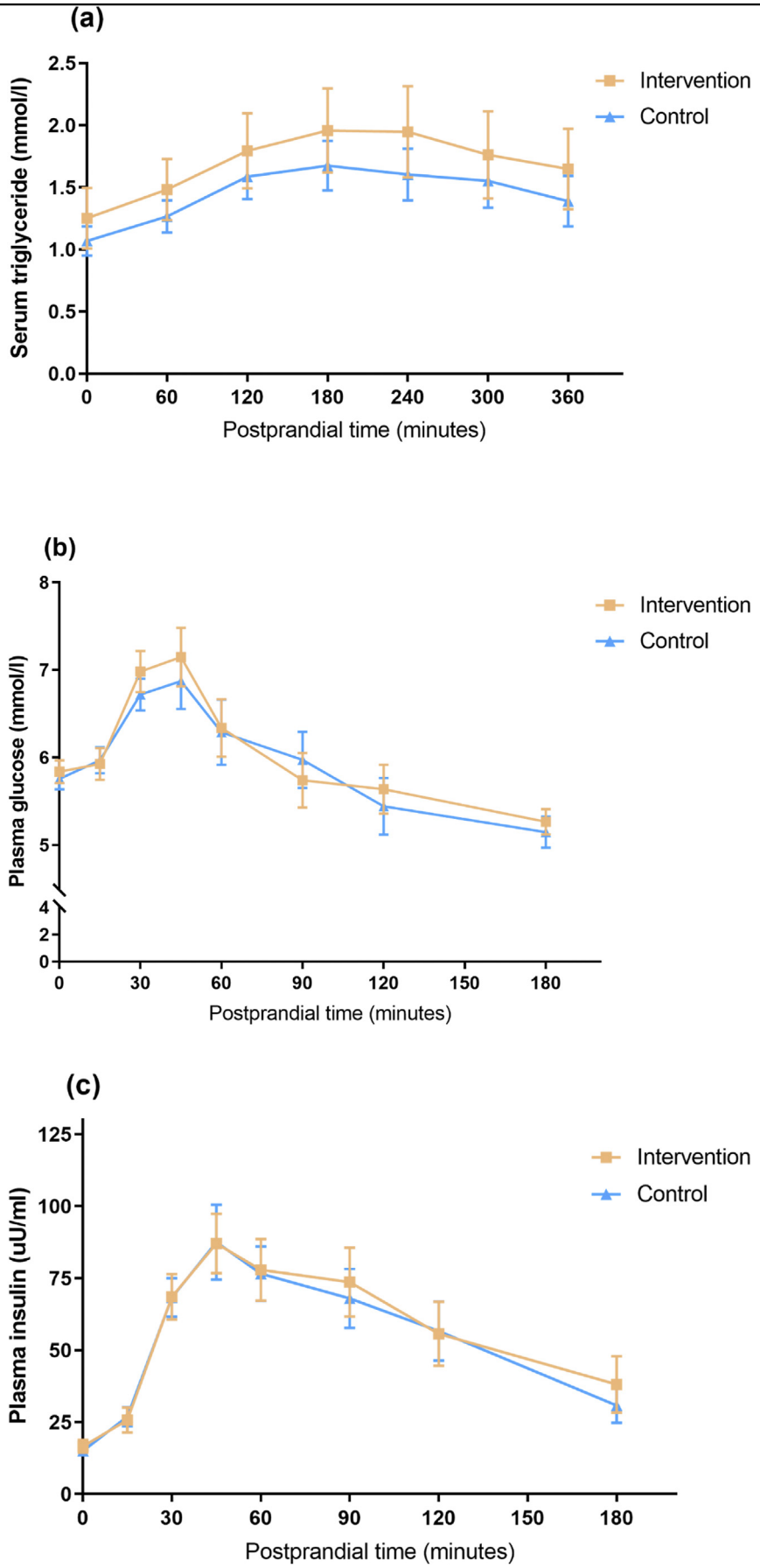

Figure 3 Postprandial time-course of triglyceride (a) glucose (b) and insulin (c) during acute meal challenge sessions conducted after intervention and control period. Values expressed as mean ± standard error of means; n = 19.

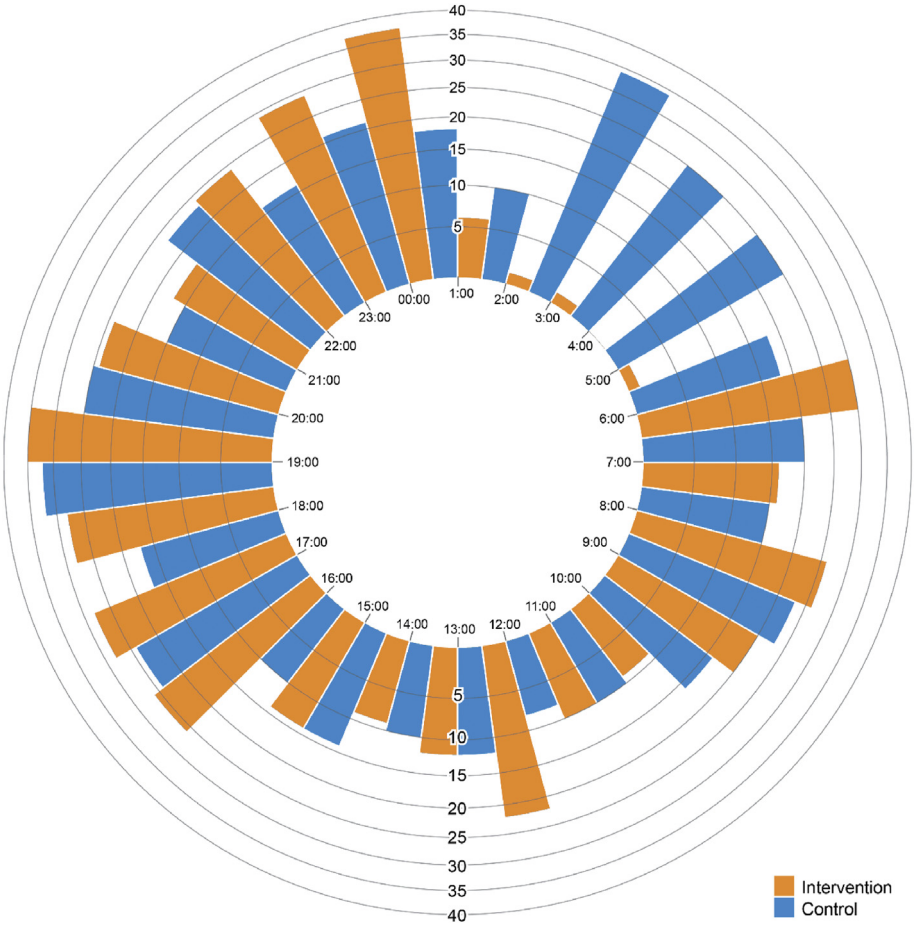

Figure 4 Radar plot of eating occasions (≥1 kJ) by time-of-day (24-h time) during intervention and control periods; n = 19 participants. Data collected via weekly 24-h dietary recalls, conducted after a night shift (71 recalls in intervention, 68 recalls in control). Circular axis of the plot represents the clock time of the 24-h day. Spoke axes indicate cumulative frequency of eating occasions during each hour (i.e. sum of eating occasions across all recalls at that time). For example, within all dietary recalls collected during the intervention period, 20 eating occasions were recorded at 0900 h.

no significant difference in postprandial insulin iAUC between the test periods (MD: 92.1 μU/ml.3 h; 95% CI: −807.1 − 991.2; p = 0.84; Table 2).

Body weight and physical activity energy expenditure

Mean (±SD) body weight at the end of intervention was slightly lower compared to the end of control (MD: −0.9 kg; 95% CI: −1.3 to −0.4; p < 0.001; Table 2).

Activity EE during each test period was estimated from the SenseWear activity monitor (n = 17 participants). Participants wore the monitor for 108.4 ± 22.5 h (approximately 4.5 days) in the intervention period and 92.1 ± 27.5 h (approximately 3.8 days) in the control period. There were no difference in daily activity EE between the two test periods (MD: −188 kJ; 95% CI: -658 − 282; p = 0.43; Table 2).

Adherence

During the control period, 68 dietary recalls were collected (89% of expected total). During the intervention period, 73 dietary recalls were collected (96% of expected total). These were used to assess adherence to the period of fasting (0100 h–0600 h). The overall adherence rate was 95%. Amongst dietary recalls collected during the control period, 112 eating occasions (≥1 kJ) were recorded between 0100 h and 0559 h; compared to nine during the intervention period (Fig. 4). Six of these nine occasions occurred at 0100 h (i.e. right at the start of the fasting period), the remaining three were non-adherence episodes recorded in three separate dietary recalls. The reasons given for these were inability to avoid caffeinated drinks and fresh produce tasting for work purposes.

Examination of the temporal eating pattern during the control period (Fig. 4), shows that eating occasions occurred least frequently between 1000 h and 1559 h, likely coinciding with sleep episodes after night shifts. No distinct fast periods were observed, with eight eating occasions between 1100 h and 1159 h being the lowest frequency recorded during the 24-h period. During the intervention period, frequencies of eating occasions remained low between 1000 h and 1559 h, except for a spike at noon (Fig. 4). To enhance adherence to the period of fasting, participants seemed to redistribute their eating occasions to 0000 h–0059 h and/or 0600 h–0659 h.

Table 3 Sample size estimations using data obtained, for future definitive randomised controlled trials.

Outcome	Observed SD of difference for values from same participant[a]	Observed MD for values from the same participant[b]	MD between intervention and control periods used for sample size estimation	Sample size estimated
Postprandial TAG iAUC (mmol/l.6 h)	87	24.8	25	97
Postprandial glucose iAUC (mmol/l.3 h)	79	−11.0	25	81
Peak postprandial TAG concentration (mmol/l)	0.8	0.2	0.5	23
Glucose concentration at 2 h postprandially (mmol/l)	1.1	0.2	0.5	40
Body weight (kg)	1.3	−0.9	1	16

Estimations assume $\alpha = 0.05$, $\beta = 0.2$ (or power = 80%).
Abbreviations: iAUC, incremental area under the curve; MD, mean difference; SD, standard deviation; TAG, triglyceride.
[a] Standard deviation based on data obtained in this pilot study.
[b] MD based on data obtained in this pilot study, presented as treatment minus control.

Sample size estimation for future definitive RCT

Using the results obtained, sample sizes required for fully-powered definitive RCTs have been estimated (Table 3).

Discussion

'Shifting the Risk' is the first study to examine the effects of a meal timing intervention on CVD risk markers in night shift workers. We showed that it is feasible for night shift workers to maintain a small 5-h overnight fast. Adherence to the 1am to 6am fast was high during the 4-week intervention period, with only three episodes of non-adherence reported, and only one participant withdrawing due to inability to maintain the fasting period. Critical to a pilot, the practicality of outcome assessment methods was examined, all of which were tolerated and successfully completed by participants. Our estimation of a 30% attrition rate was confirmed; with 19 of 28 randomised participants completing the study. Whilst this pilot was not powered to detect significant changes in the primary outcome measures (postprandial blood TAG and glucose iAUC), findings indicate a small change in body weight at the end of the intervention period, warranting further investigation on the effects of meal timing on weight management in this population. Using the data generated in this pilot, we are able to provide sample size estimations based on blood TAG, glucose and body weight, which serves as a useful starting point for future meal timing interventions conducted in this population.

Examination of participants' baseline temporal eating pattern collectively, shows that while on night shifts, eating occasions occurred at all hours of the 24-h day with no distinct fast period [29]. This closely resembles the temporal eating pattern of night shifts observed during the control period, with 112 eating occasions recorded between 0100 h and 0600 h. In contrast, participants were able to adhere to the designated period of fast (0100 h–0600 h) during the 4-week intervention period. This reduced the number of eating occasions during the night, creating a temporal eating pattern that is more aligned with the regulation of our circadian clock system [14]. Metabolic disruptions associated with eating into the night have been reported in epidemiological studies. Using large population data sets that adjusted for traditional risk factors such as age and physical activity level, 'eating at night' has been associated with increased odds of being obese, in both Swedish (OR: 1.62) and Japanese cohorts (OR: 1.28) [30,31]. In the Japanese cohort of 61,364 healthy participants, 'eating at night' was also associated with increased odds of hyperglycaemia (OR: 1.13) [31]. By applying statistical modelling on cross-sectional data, Chen et al. showed that displacing 100 kcal of intake in the morning or noon to the night (2030 h–0459 h), was associated with increased LDL-C levels in a healthy Taiwanese cohort [32]. Furthermore, night shifts are typically characterised by frequent snacking of mainly discretionary food items and increased intake of sugar sweetened beverages [10,12,33], a finding we observed at baseline [29]. The introduction of the overnight fast inadvertently reduced the intake of these discretionary items, at a time when lipid and glucose metabolism are least efficient [15,16].

Exploratory analysis showed that body weight was slightly lower at the end of the intervention period, compared to the end of control, in the absence of changes in physical activity EE. This finding suggests that a short overnight fast may promote changes in energy expenditure. A number of diurnal studies have indicated lowered TEF after eating during the night, compared to during the day [17]. It could therefore be speculated that participants in our study may have experienced a small reduction in body weight after the intervention period, by avoiding energy intake during the night, a time at which our body is less efficient at "burning" energy. Although the difference was small, it could be clinically relevant, if it is maintained in the long term by shift workers. For better understanding of this speculation, it would be valuable to incorporate measures of energy expenditure in future long-term meal timing interventions. In the context of the 'Shifting the Risk' protocol, the incorporation of TEF assessment during the acute meal challenge session may be a feasible addition, assessing one component of energy expenditure. This should be supported by a comprehensive assessment of usual dietary intake during the test periods, to distinguish the effect of energy intake on body weight changes, from the effect of energy expenditure. Considering the night shift worker population, a 7-day food diary may be

required, to capture the potential differences in daily energy intake between shift types [11,34,35], as to provide an accurate proxy of usual daily energy intake. Lastly, future weight management studies in shift workers should consider harnessing the potential time of day difference in TEF, by incorporating a nightly fast window into their energy restriction regime. The combination of "how much" and "when to" eat is more likely to produce metabolic benefits that are of clinical significance.

Critical to a pilot study, is to assess the appropriateness of selected outcome measures and outcome assessment methods. Postprandial TAG and glucose responses were selected as primary outcome measures because they are stronger predictors of CVD in healthy individuals, compared to fasting TAG and glucose levels [36–38]. A prospective cohort study of 26,509 healthy American women found that those in the highest postprandial TAG tertile at baseline (1.93 mmol/l) have twice the risk of developing CVD (HR: 1.98, median follow up: 11.4 years), compared to those in the reference postprandial TAG category of 1.18 mmol/l [36]. This association was not observed with fasting TAG levels after adjustment with BMI and HDL-C levels. Moreover, a meta-analysis of 13 prospective cohort studies with non-diabetic participants reported that, postprandial glucose response has a positive linear relationship with CVD risk, from the ranges of 3.1–11.1 mmol/l. In contrast, the predictive effect of fasting glucose was capped at a threshold of 5.6 mmol/l [38]. Postprandial TAG and glucose responses were therefore chosen as primary outcome measures in the current study, as participants were relatively healthy, with only one known risk factor of CVD (abdominal obesity), whilst fasting TAG and glucose levels were within healthy ranges. However, we acknowledge that due to the small sample size, the subtle difference in postprandial TAG and glucose responses between intervention and control might have been missed, leading to type II error. As such, future studies should consider including participants presenting with multiple risk factors of CVD, such as those with diagnosed metabolic syndrome [24]. This population may be more likely to respond to the intervention, thereby maximising the magnitude of difference observed between intervention and control.

In this pilot, acute meal challenge sessions were conducted to assess postprandial outcomes. They were tolerated by participants and the test meal used successfully stimulated a metabolic response. Whilst we were able to capture the full postprandial glucose response (returned to fasting levels by two hours), this was not observed with postprandial TAG response (remained elevated at six hours). Therefore, the effect of the intervention on postprandial TAG clearance could not be properly assessed. Some studies have shown that postprandial TAG response can extend up to eight hours [39,40]. Extending challenge sessions beyond six hours is challenging, as it will increase participant burden. Peak postprandial TAG concentration, which occurred at three to four hours postprandially, may therefore be a more practical outcome to assess compared to postprandial TAG iAUC. This approach is supported by a meta-analysis of 113 studies, which showed that the plasma TAG concentration measured at 4-h after an oral fat load was highest, compared to 2, 6 and 8-h postprandially. The authors of this meta-analysis concluded that plasma TAG concentration measured at 4-h postprandially is therefore most representative of the peak postprandial TAG concentration [41]. Moreover, the aforementioned large prospective cohort study of American women reported that postprandial TAG concentration measured at two to four hours postprandially had the strongest association with CVD risk (HR: 4.48), compared to other postprandial time periods [36].

As part of the protocol, participants were asked to attend all three challenge sessions two to three days after their last night shift. This was based on findings from Lund et al., who showed that elevated postprandial TAG response induced by a block of night shifts remained two days after the last night shift [42]. Choosing this timeframe also reduced the independent effect that sleep deprivation has on glucose tolerance [43], which participants are likely to experience on their first day-off. However, approximately a quarter of participants were unable to attend the challenge sessions two to three days after their last night shift as per protocol; effects of the intervention may have diminished due to delayed outcome assessment. Acute diurnal studies have consistently shown that postprandial lipid and glucose metabolism are less efficient during the night compared to the day [15,16]. Considering this, perhaps outcome assessments should be conducted closer to the end of consecutive night shifts, so that the immediate effects of the intervention can be captured. In this case, peak postprandial TAG concentration may be better suited as an outcome measure compared to postprandial TAG iAUC, as participants are unlikely to attend extended assessment sessions immediately after a night shift. As such, we have provided sample size estimations using peak postprandial TAG concentration and postprandial glucose concentration at two hours, another postprandial risk marker of CVD commonly referred to in the literature [36,38,44–46].

The study design of 'Shifting the Risk' has numerous strengths. Prior to the intervention period, participants were provided with tailored advice on how to redistribute their meal times around the designated fast window, based on 4-day food diaries collected during the run-in period. The outcome assessment method was tightly controlled, with a standardised meal and fast prior to the challenge session, and a standardised test meal provided to all participants. During the intervention period, SMS messages reminding participants to fast were sent prior to each night shift, or at least on the first of a series of night shifts. Dietary recalls were collected once weekly as a method to assess adherence to the intervention, although the possibility of non-adherence on days when dietary recalls were not conducted should be acknowledged. The high SD and wide 95% CI is a limitation of our data, possibly due to high inter-participant variation. This may be attributed to participants' varied age, body weight and employment status,

such as years in shift work, number of night shifts worked and shift schedule (i.e.: permanent night/ rotating). In order to account for this heterogeneity, larger sample sizes will be required, to allow for covariate adjustments in statistical analyses or additional subgroup analyses.

In this pilot study, we showed that for shift workers who usually eat throughout the night whilst on night shift, it is possible to maintain a 5-h overnight fast. This created a defined fast period during the 24-h day, which was previously absent [29]. Our pilot data suggest that reducing the number of eating occasions during the night may promote a small shift in body weight, which may be related to lowered TEF observed during the night. This warrants further investigation into the role of meal timing, in mitigating the metabolic consequences of night shift work. Sample size estimations have been provided using data generated, allowing future definitive RCTs to test whether an overnight fast is able to improve postprandial TAG and glucose metabolism in night shift workers. For future meal timing interventions examining CVD risk, postprandial TAG and glucose response remain appropriate outcome measures to target. The assessment of energy expenditure would also be favourable, allowing for better explanations in weight changes that may occur. Any dietary interventions targeting the shift work population should consider “when” to eat in conjunction with “what” and “how much” to eat. Due to shift workers’ atypical temporal eating pattern, meal timing is likely to play a significant role in the prevention of metabolic diseases in shift workers.

Funding

This work was supported by the National Heart Foundation (Australia) Vanguard Grant [Grant number 101381]. G.K.W.L and R.D were supported by Australian Government RTP Scholarship. G.K.W.L was supported by the King and Amy O’Malley Trust Scholarship.

Author contributions

Leung: methodology, investigation, data curation, writing – original draft, visualisation, project administration. Davis: methodology, investigation, data curation, writing – review & editing, project administration. Ware: formal analysis, writing – review & editing. Huggins: conceptualisation, methodology, writing – review & editing, supervision, funding acquisition. Bonham: conceptualisation, methodology, resources, writing – review & editing, supervision, funding acquisition.

Declaration of competing interest

None.

Acknowledgements

The authors would like to sincerely thank the National Heart Foundation for funding this research. We would also like to thank Dr Nina Eikelis (Swinburne University of Technology, Australia) for the biochemical analysis of insulin samples. We would like to express appreciation to University of Ulster (Northern Ireland) placement student Jenny Robinson, for her assistance with day-to-day running of the study. Lastly, we would like to thank all the participants who devoted time and effort to complete the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.numecd.2021.03.008.

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Appendix C: Supplementary to Chapter 2 – meta-analysis.

Full search strategy from Ovid MEDLINE

	Searches	Results
1	exp "diet, food, and nutrition"/ or exp food/	1421719
2	food*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	437386
3	exp Meals/	2300
4	meal*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	52887
5	eat*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	106985
6	nutrient*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	85741
7	energy*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	401547
8	exp energy intake/ or exp portion size/	38109
9	(energy adj3 intake*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	44517
10	energyintake*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	2
11	intake*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	208866
12	feed*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary	366091

	concept word, rare disease supplementary concept word, unique identifier]	
13	snack*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	4556
14	nutrition*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	276445
15	beverage*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	29055
16	drink*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	142301
17	glucose.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	439405
18	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17	2561209
19	night*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	60048
20	(night adj3 time).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	4607
21	nighttime.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	6340
22	nocturnal.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	28346
23	diurnal.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	19858
24	shift.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	163034
25	evening*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary	14236

	concept word, rare disease supplementary concept word, unique identifier]	
26	(night adj3 work).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	1032
27	nightwork.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	35
28	exp Work Schedule Tolerance/	5669
29	(work adj3 tolerance).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	5967
30	exp biological clocks/ or exp circadian rhythm/	71939
31	(shift adj3 work*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	4419
32	shiftwork*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	578
33	(circadian adj3 misalignment*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	161
34	(circadian adj3 disorder*).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	2032
35	(circadian adj3 disturbance).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	218
36	(circadian adj3 d?sregulation&).mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	90
37	exp Sleep Disorders, Circadian Rhythm/	1762
38	irregular work hour*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	11
39	19 or 20 or 21 or 22 or 23 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35 or 36 or 37 or 38	157432
40	exp Postprandial Period/	7552

41	postprandial.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	19818
42	post prandial.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	2083
43	post meal.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	347
44	postmeal.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	420
45	postcibal.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	119
46	exp Glucose Tolerance Test/	30728
47	tolerance test*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	41033
48	tolerancetest*.mp. [mp=title, abstract, original title, name of substance word, subject heading word, keyword heading word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier]	2
49	40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48	61049
50	**18 and 39 and 49**	**1515**

Appendix D: Supplementary to Chapter 2 – meta-analysis.
Quality assessment of studies that met eligibility criteria of the systematic literature review

Table a. Quality assessment of studies included in meta-analyses of the systematic literature review.

ADA Quality Checklist Criteria	Al-Naimi 2004	Biston 1996	Bo 2017	Gibbs 2014	Gil-Lozano 2015	Holmback 2002	Holmback 2003	Leung 2017	Owens 1996	Van Cauter 1992
1. Was the research question clearly stated?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2. Was the selection of study subjects/patients free from bias?	x	✓	✓	✓	✓	✓	✓	✓	x	✓
3. Were the study groups comparable?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
4. Was the method of handling withdrawals described?	x	x	x	✓	x	✓	✓	✓	x	x
5. Was blinding used to prevent introduction to bias?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
6. Were intervention/therapeutic regimens/exposure factor or procedure and any comparisons described in detail? Were intervening factors described?	✓	✓	✓	✓	✓	✓	✓	✓	✓	x
7. Were outcomes clearly defined and the measurements valid and reliable?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?	x	✓	✓	✓	✓	✓	✓	✓	✓	✓
9. Were conclusions supported by results with biases and limitations also taken into consideration?	x	✓	✓	✓	✓	✓	x	✓	✓	✓
10. Is bias due to the study's findings or sponsorship unlikely?	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Quality Rating	**Neutral**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Positive**	**Neutral**	**Neutral**

Grey indicates validity items that must be satisfied for a positive quality rating. Quality rating based on Academy of Nutrition and Dietetics (formerly American Dietetic Association) guidelines (2003a).

Table b. Quality assessment of studies included in qualitative synthesis and excluded in the systematic literature review.

ADA Quality Checklist Criteria	Aparicio 1974	Lund 2001	Morris 2015	Morris 2016	King 1993	Van Gent 1979	Terpstra 1979
1. Was the research question clearly stated?	✓	✓	✓	✓	✓	✓	✓
2. Was the selection of study subjects/patients free from bias?	x	x	x	x	x	x	x
3. Were the study groups comparable?	✓	✓	✓	✓	✓	✓	✓
4. Was the method of handling withdrawals described?	x	x	x	x	x	x	x
5. Was blinding used to prevent introduction to bias?	✓	✓	✓	✓	✓	✓	✓
6. Were intervention/therapeutic regimens/exposure factor or procedure and any comparisons described in detail? Were intervening factors described?	✓	✓	✓	✓	✓	x	x
7. Were outcomes clearly defined and the measurements valid and reliable?	✓	✓	x	x	✓	x	x
8. Was the statistical analysis appropriate for the study design and type of outcome indicators?	x	✓	✓	✓	✓	x	x
9. Were conclusions supported by results with biases and limitations also taken into consideration?	x	x	✓	✓	x	x	x
10. Is bias due to the study's findings or sponsorship unlikely?	✓	✓	✓	✓	✓	✓	✓
Quality Rating	**Neutral**	**Neutral**	**Neutral**	**Neutral**	**Neutral**	**Negative**	**Negative**

Grey indicates validity items that must be satisfied for a positive quality rating.
Quality rating based on Academy of Nutrition and Dietetics (formerly American Dietetic Association) guidelines (2003a).

Appendix E: Supplementary to Chapter 2 – meta-analysis.

Glucose data used in meta-analysis

Reference	Postprandial concentration – reported units	Comparison (day time, night time)	Day (mean ± SD*)	Night (mean ± SD*)
Al-Naimi et al, 2004	AUC (mmol/l.h)	1300, 0100h	15.3 ± 1.3	17.2 ± 1.3
Biston et al, 1996	iAUC (mmol/l.min)	0830, 2000h	193 ± 76.7	540 ± 116.3
Bo et al, 2017	AUC (mg/dl x h)	0800, 2000h	15383 ± 1585.5	17183.3 ± 1775.5
Gibbs et al, 2015 – *Low GI*	iAUC (mmol/L.h)	0800, 2000h	142.0 ± 63.7	309.0 ± 63.7
Gibbs et al, 2015 – *High GI*	iAUC (mmol/L.h)	0800, 2000h	218.0 ± 80.9	351.0 ± 107.9
Gil-Lozano et al, 2015	iAUC (g/dl. 180 min)	1100, 2300h	16.7 ± 1.3	19.0 ± 1.2
Holmback et al, 2002 [a] – *High carbohydrate*	iAUC (mmol/L.3h)	1200, 0000h	165.1 ± 67.5	209.6 ± 38.6
Holmback et al, 2002 – *High fat*	iAUC (mmol/L.3h)	1200, 0000h	123.3 ± 38.3	190.6 ± 29.8
Leung et al, 2017 – *OGTT*	iAUC (mmol/l.2h)	0800, 2000h	180.4 ± 88.1	357.9 ± 109.2
Leung et al, 2017 – *low GI meal*	iAUC (mmol/l.3h)	0800, 2000h	66.9 ± 94.2	264.9 ± 135.0
Owen et al, 1996 [b]	iAUC (mmol/l.3h)	0800, 2000h	143.2 ± 52.1	285.5 ± 143.3
Van Cauter et al, 1992 - *12 hour fast*	2hr iAUC (mmol/l.min)	0800, 2000h	111.3 ± 43.2	203.5 ± 34.9
Van Cauter et al, 1992 [b] - *6 hour fast*	2hr iAUC (mmol/l.min)	0800, 2000h	83.9 ± 28.3	195.0 ± 50.0

* SD (standard deviation) was adjusted with correlation factor (0.4). [a] Plasma glucose levels supplied by Holmback et al, iAUC calculated by the review's authors.
[b] In the original article, authors stated that they were reporting AUC, but from their description of method, it was understood that they reported iAUC.
AUC: area under the curve, iAUC: incremental area under the curve.

Appendix F: Supplementary to Chapter 2 – meta-analysis.

Insulin data used in meta-analysis

Reference	Postprandial concentration – reported units	Comparison (day time, night time)	Day (mean ± SD*)	Night (mean ± SD*)
Al-Naimi et al, 2004	AUC (pmol/l.h)	1300, 0100h	699 ± 153.2	550 ± 150.9
Biston et al, 1996	iAUC (nmol/l.min)	0830, 2000h	35.4 ± 8.3	44.6 ± 14.0
Bo et al, 2017	AUC (µU/ml x h)	0800, 2000h	6968.9 ± 2323.9	8597.8 ± 3525.9
Gibbs et al, 2015 - *Low GI*	iAUC (pmol/L.h)	0800, 2000h	1356 ± 1080.4	1077 ± 1623.3
Gibbs et al, 2015 - *High GI*	iAUC (pmol/L.h)	0800, 2000h	1159 ± 1723.4	1236 ± 917.1
Gil-Lozano et al, 2015	iAUC (ng/ml.120min)	1100, 2300h	52.7 ± 21.2	64.3 ± 18.8
Holmback et al, 2003 [a] – *High carbohydrate*	iAUC (mU/L.3h)	1200, 0000h	2955.6 ± 1102.0	2900.5 ± 878.8
Holmback et al, 2003 – *High fat*	iAUC (mU/L.3h)	1200, 0000h	2516.6 ± 782.5	2792.8 ± 734.3
Leung et al, 2017 – *low GI*	iAUC (mU/L.3h)	0800, 2000h	1930.5 ± 818.2	3051.7 ± 793.1
Van Cauter et al, 1992 - *12 hour fast*	2hr iAUC (nmol/L.min)	0800, 2000h	15.0 ± 3.6	17.7 ± 4.5
Van Cauter et al, 1992 [b] - *6 hour fast*	2hr iAUC (nmol/L.min)	0800, 2000h	13.3 ± 2.9	15.6 ± 5.0

* SD (standard deviation) was adjusted with correlation factor (0.3). [a] Plasma insulin levels supplied by Holmback et al, iAUC calculated by the review's authors.
[b] In the original article, authors stated that they were reporting AUC, but from their description of method, it was understood that they reported iAUC.
AUC: area under the curve, iAUC: incremental area under the curve.

Appendix G: Supplementary to Chapter 2 – meta-analysis.

Funnel plots for meta-analyses

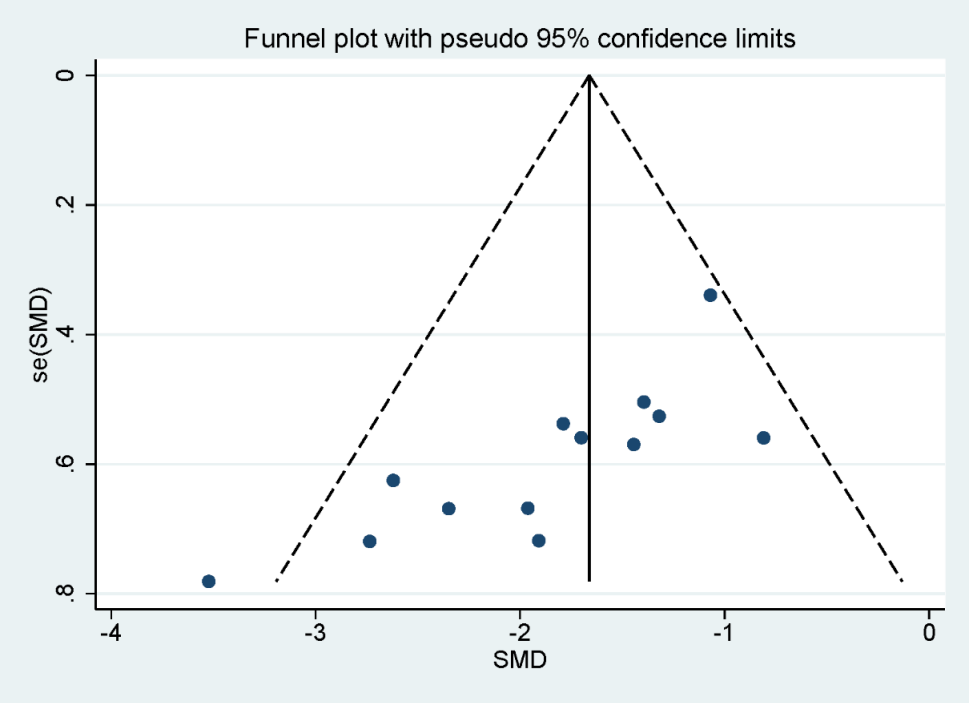

Figure 1. Funnel plot for glucose meta-analysis.

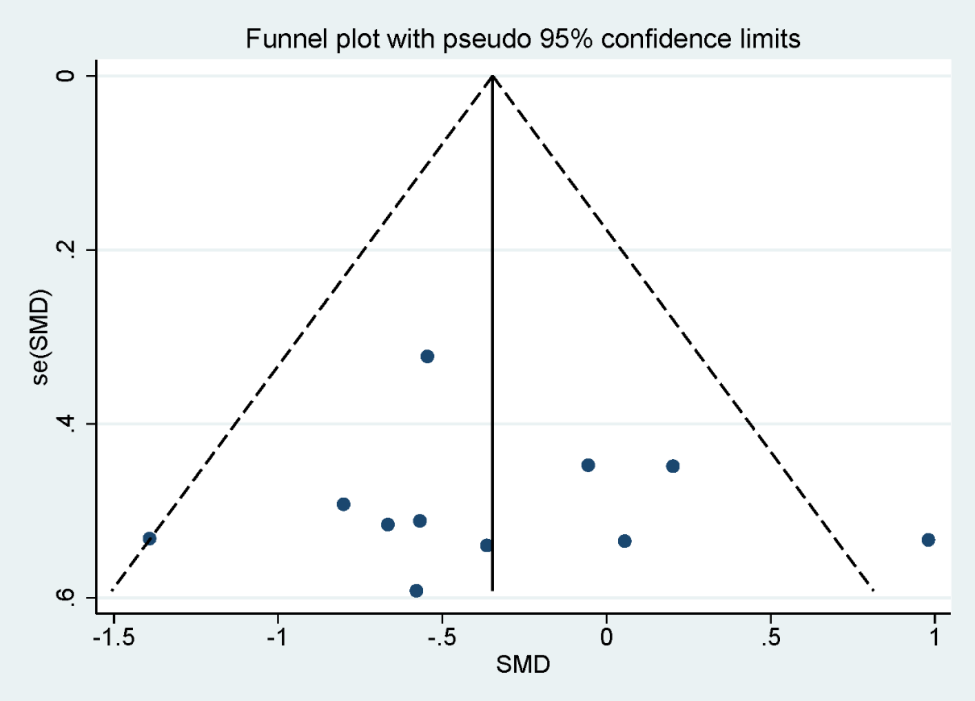

Figure 2. Funnel plot for insulin meta-analysis.

Appendix H: Supplementary to Chapter 2 – meta-analysis.

Take-one-out sensitivity analyses

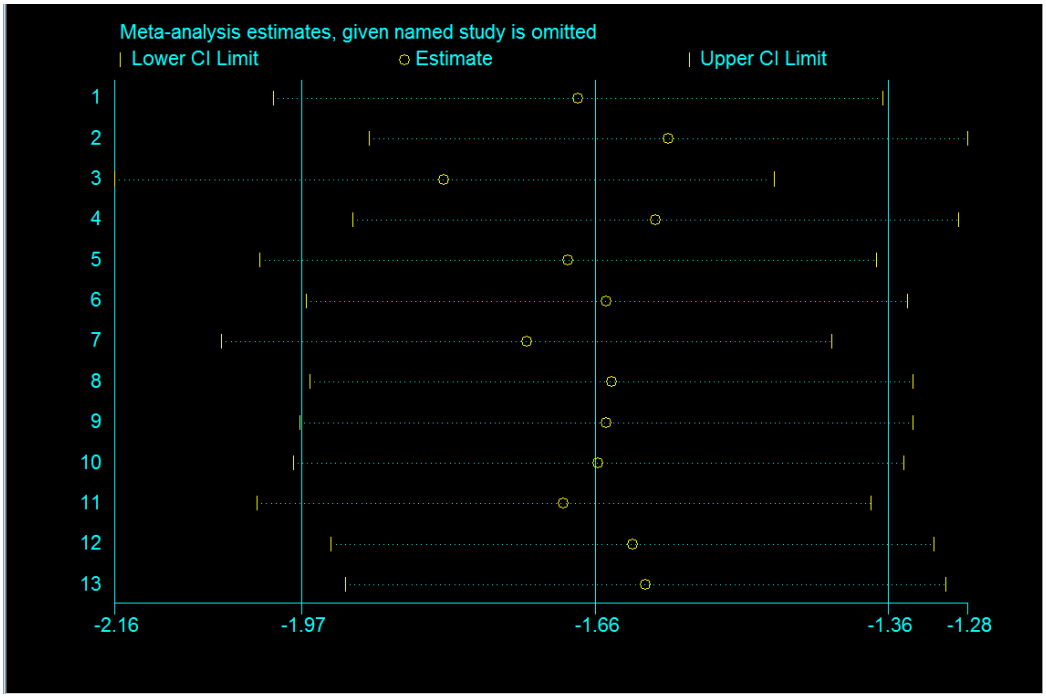

Figure 1. Take-one-out sensitivity analysis for glucose.

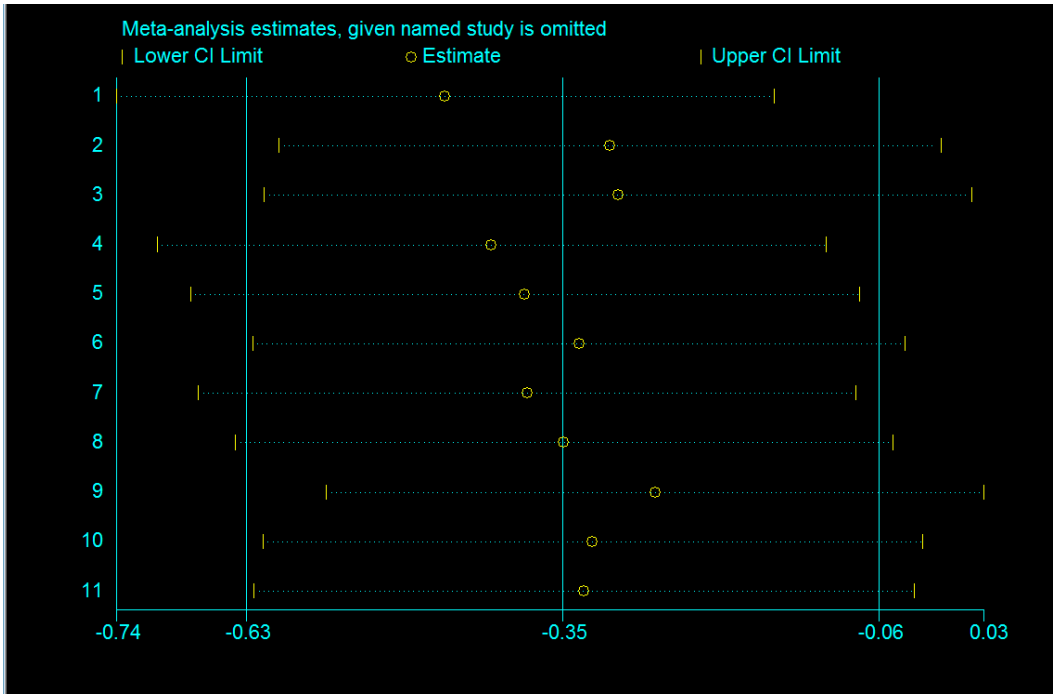

Figure 2. Take-one-out sensitivity analysis for insulin.

Appendix I: Supplementary to Chapter 2 – meta-analysis.

Sensitivity analysis for glucose response, substituting morning time points with afternoon time points where available.

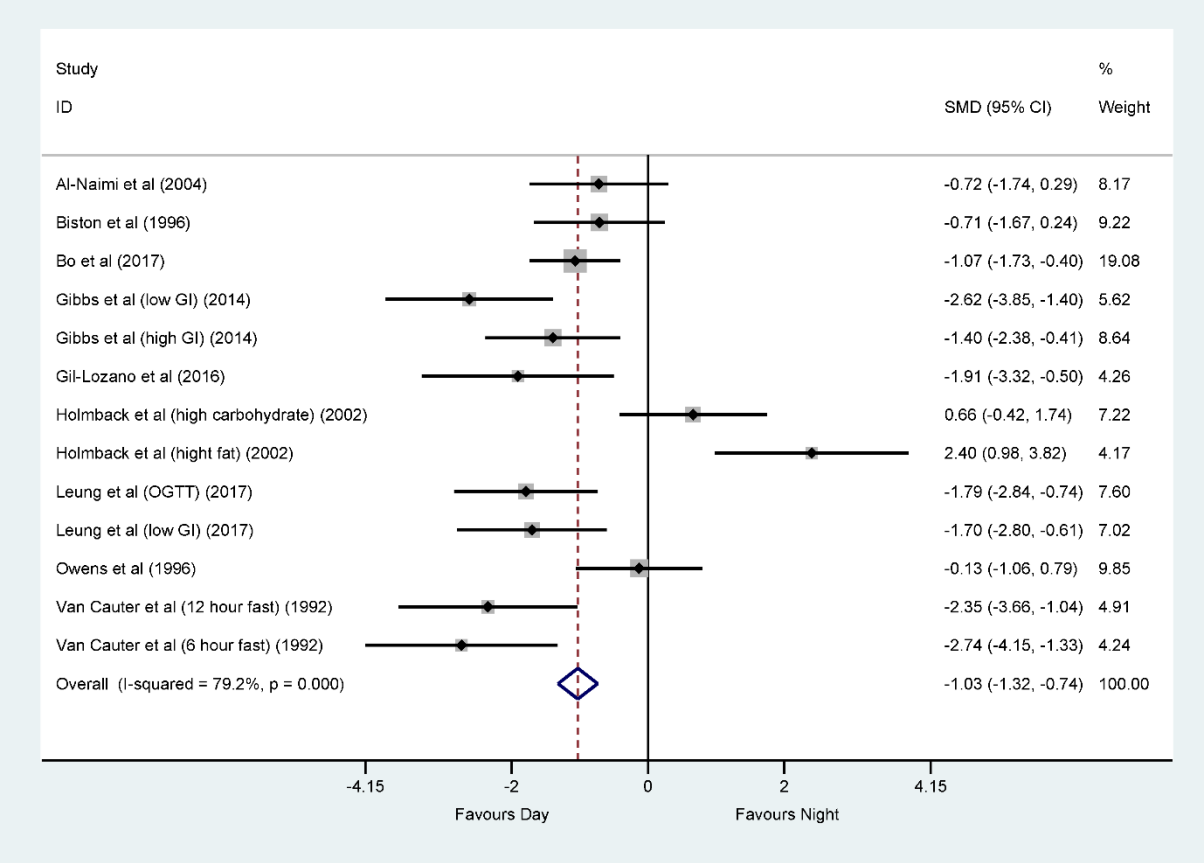

Figure 1. Forest plot of the glucose sensitivity analysis.

Table 1. Glucose data used in the sensitivity analysis. The comparisons shaded in grey were replaced by later day time points provided by the study.

Reference	Postprandial concentration – reported units	Comparison (day time, night time)	Day (mean ± SD*)	Night (mean ± SD*)
Al-Naimi et al, 2004	AUC (mmol/l.h)	1300, 0100h	15.3 ± 1.3	17.2 ± 1.3
		1600, 0400h	15.5 ± 0.9	16.5 ± 1.8
Biston et al, 1996	iAUC (mmol/l.min)	0830, 2000h	193 ± 76.7	540 ± 116.3
		1400, 2000h	457 ± 116.3	540 ± 116.3
Bo et al, 2017	AUC (mg/dl x h)	0800, 2000h	15383 ± 1585.5	17183.3 ± 1775.5
Gibbs et al, 2015 - *Low GI*	iAUC (mmol/L.h)	0800, 2000h	142.0 ± 63.7	309.0 ± 63.7
Gibbs et al, 2015 - *High GI*	iAUC (mmol/L.h)	0800, 2000h	218.0 ± 80.9	351.0 ± 107.9
Gil-Lozano et al, 2015	iAUC (g/dl. 180 min)	1100, 2300h	16.7 ± 1.3	19.0 ± 1.2
Holmback et al, 2002 [a]	iAUC (mmol/L.3h)	1200, 0000h	165.1 ± 67.5	209.6 ± 38.6
High carbohydrate		1600, 0400h	225.1 ± 49.1	190.0 ± 56.6
Holmback et al, 2002	iAUC (mmol/L.3h)	1200, 0000h	123.3 ± 38.3	190.6 ± 29.8
High fat		1600, 0400h	229.4 ± 32.9	149.8 ± 33.4
Leung et al, 2017 – *OGTT*	iAUC (mmol/l.2h)	0800, 2000h	180.4 ± 88.1	357.9 ± 109.2
Leung et al, 2017 – *low GI meal*	iAUC (mmol/l.3h)	0800, 2000h	66.9 ± 94.2	264.9 ± 135.0
Owen et al, 1996 [b]	iAUC (mmol/l.3h)	0800, 2000h	143.2 ± 52.1	285.5 ± 143.3
		1400, 0200h	226.1 ± 85.1	238.4 ± 100.7
Van Cauter et al, 1992 - *12 hour fast*	2hr iAUC (mmol/l.min)	0800, 2000h	111.3 ± 43.2	203.5 ± 34.9
Van Cauter et al, 1992 [b] - *6 hour fast*	2hr iAUC (mmol/l.min)	0800, 2000h	83.9 ± 28.3	195.0 ± 50.0

* SD (standard deviation) was adjusted with correlation factor (0.4). [a] Plasma glucose levels supplied by Holmback et al, iAUC calculated by the review's authors.
[b] In the original article, authors stated that they were reporting AUC, but from their description of method, it was understood that they reported iAUC.
AUC: area under the curve, iAUC: incremental area under the curve

Appendix J: Supplementary to Chapter 2 – meta-analysis.

Sensitivity analysis for insulin responses, substituting morning time points with afternoon time points where available.

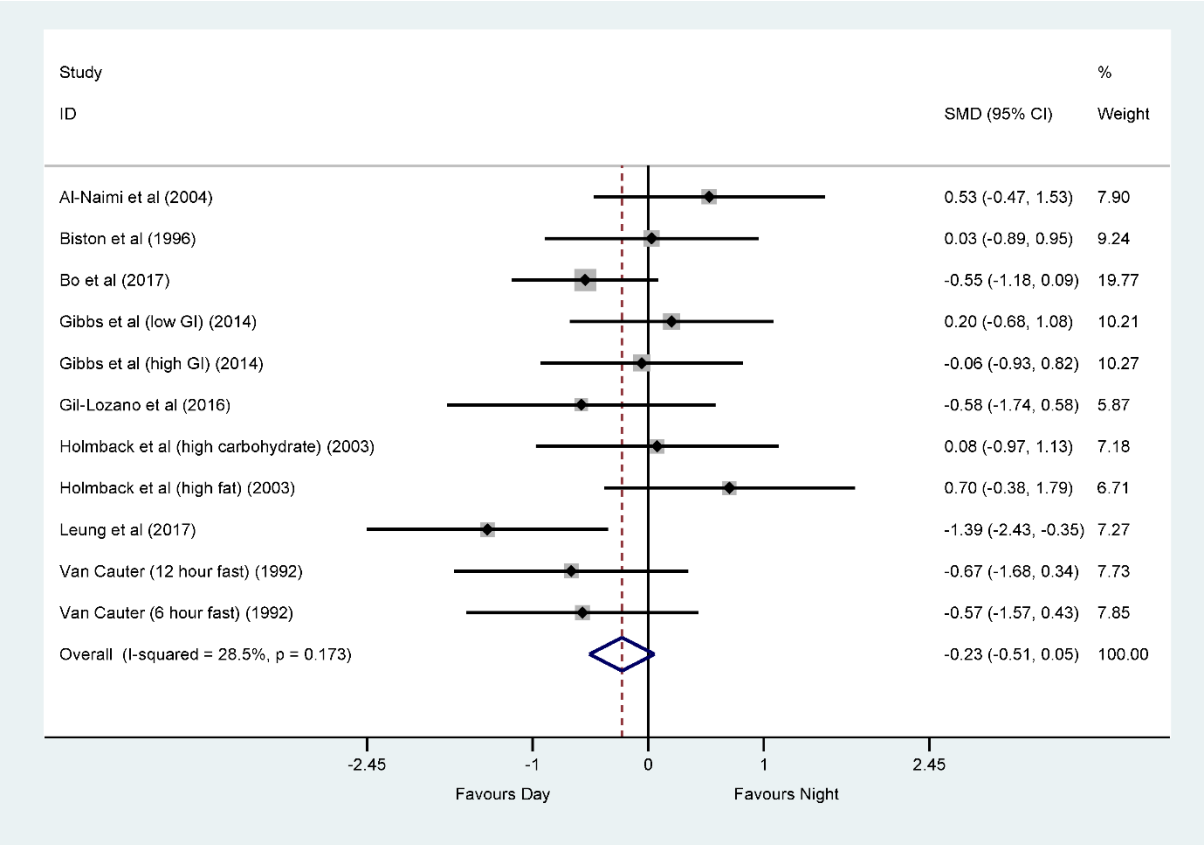

Figure 1. Forest plot of the insulin sensitivity analysis.

Table 1. Insulin data used in the sensitivity analysis. The comparisons shaded in grey were replaced by later day time points provided by the study.

Reference	Postprandial concentration – reported units	Comparison (day time, night time)	Day (mean ± SD*)	Night (mean ± SD*)
Al-Naimi et al, 2004	AUC (pmol/l.h)	1300, 0100h	699 ± 153.2	550 ± 150.9
		1600, 0400h	452 ± 148.5	384 ± 106.1
Biston et al, 1996	iAUC (nmol/l.min)	0830, 2000h	35.4 ± 8.3	44.6 ± 14.0
		1400, 2000h	45.0 ± 13.5	44.6 ± 14.0
Bo et al, 2017	AUC (µU/ml x h)	0800, 2000h	6968.9 ± 2323.9	8597.8 ± 3525.9
Gibbs et al, 2015 - *Low GI*	iAUC (pmol/L.h)	0800, 2000h	1356 ± 1080.4	1077 ± 1623.3
Gibbs et al, 2015 - *High GI*	iAUC (pmol/L.h)	0800, 2000h	1159 ± 1723.4	1236 ± 917.1
Gil-Lozano et al, 2015	iAUC (ng/ml.120min)	1100, 2300h	52.7 ± 21.2	64.3 ± 18.8
Holmback et al, 2003 [a]	iAUC (mU/L.3h)	1200, 0000h	2955.6 ± 1102.0	2900.5 ± 878.8
High carbohydrate		1600, 0400h	3603.6 ± 1379.5	3495.0 ± 1417.1
Holmback et al, 2003	iAUC (mU/L.3h)	1200, 0000h	2516.6 ± 782.5	2792.8 ± 734.3
High fat		1600, 0400h	3332.4 ± 876.7	2739.4 ± 809.1
Leung et al, 2017 – *low GI*	iAUC (mU/L.3h)	0800, 2000h	1930.5 ± 818.2	3051.7 ± 793.1
Van Cauter et al, 1992 - *12 hour fast*	2hr iAUC (nmol/L.min)	0800, 2000h	15.0 ± 3.6	17.7 ± 4.5
Van Cauter et al, 1992 [b] - *6 hour fast*	2hr iAUC (nmol/L.min)	0800, 2000h	13.3 ± 2.9	15.6 ± 5.0

* SD (standard deviation) was adjusted with correlation factor (0.3). [a] Plasma insulin levels supplied by Holmback et al, iAUC calculated by the review's authors.
AUC: area under the curve, iAUC: incremental area under the curve.

Appendix K: Supplementary to Chapter 3a – 'Shifting the Risk' (CVD risk factors).

Baseline characteristics of completers and drop-outs.

Measure	Completers (n= 19)	Drop-outs (n= 9)
Gender	Frequency (percentage)	
Female	13 (68%)	5 (55%)
Male	6 (32%)	4 (44%)
	Mean ± standard deviation	
Age (years)	41 ± 10	36 ± 11
Body weight (kg)	86.2 ± 17.2	98.1 ± 18.4
BMI (kg/m^2)	30.7 ± 5.7	33.8 ± 6.4
Waist circumference (cm)	97.3 ± 11.2	106.3 ± 14.3

Measures for completers taken at baseline acute meal challenge session (n= 19); measures for drop-outs taken at screening session (n= 9).

Appendix L: Supplementary to Chapter 3b – 'Shifting the Risk' (Feasibility).

Number of expressions of interest received from each recruitment strategy utilised in 'Shifting the Risk', including start and end date of strategy implementation.

Recruitment Strategy	Start date	End date	EOIs received (n) [a]	Online screening questionnaires completed (n)	Night shift workers identified (n)	Proportion of night shift workers within EOIs (%)	Confirmed eligible participants (n)	Proportion of eligible participants within EOIs (%)	Recruitment cost ($)
Direct ESTA engagement	2017-07-24	n/a	23	22	19	83	5	22	0.00
Cold contact to shift worker employing companies	2017-10-25	n/a	1	1	1	100	0	0	0.00
Gumtree advertisement	2017-11-14	2017-12-14	9	5	1	11	1	11	0.00
Facebook paid advertisement (traffic generation, Dept. account)	2017-11-28	2017-12-06	30	25	16	53	1	3	149.90
Facebook paid advertisement (traffic generation, Dept. account)	2017-12-06	2017-12-20	13	10	10	77	3	23	97.00
Gumtree advertisement (with boost)	2018-01-10	2018-02-09	5	4	3	60	0	0	39.00
Facebook paid advertisement (traffic generation, Dept. account)	2018-01-11	2018-01-26	6	5	5	83	1	17	105.45
Blog post on Monash Nutrition Blog & Facebook paid boost on Post	2018-01-15	2018-01-22	85	82	76	89	1	1	22.61
Letterbox drops of flyers	2018-01-27	n/a	5	5	3	60	1	20	1543.47
Facebook paid advertisement (traffic generation, Dept. and University Faculty account)	2018-02-05	2018-02-24	61	60	53	87	5	8	870.00

Recruitment Strategy	Start date	End date	EOIs received (n) [a]	Online screening questionnaires completed (n)	Night shift workers identified (n)	Proportion of night shift workers within EOIs (%)	Confirmed eligible participants (n)	Proportion of eligible participants within EOIs (%)	Recruitment cost ($)
Radio advertisement via Melbourne metropolitan radio station	2018-02-19	2018-03-02	38	34	32	84	5	13	3850.00
Facebook paid advertisement (traffic generation, Dept. account)	2018-03-06	2018-03-13	21	21	18	86	1	5	100.00
Facebook paid advertisement (lead generation, University Faculty account)	2018-03-19	2018-04-15	171	31	30	18	0	0	353.98
Facebook paid advertisement (traffic generation, University Faculty account)	2018-04-30	2018-05-27	54	53	49	91	3	6	353.98
Blog post on Monash Lens	2018-06-08	n/a	2	2	2	100	1	50	0.00
Facebook paid advertisement (traffic, University Faculty account)	2018-06-25	2018-07-08	45	45	15	33	3	7	180.16
Total			**569**	**405**	**333**	**59**	**31**	**5**	**7665.55**

[a] EOIs (expressions of interests) include completed online screening questionnaires, as well as enquiries received from SMS, email and telephone that did not proceed to completion of questionnaire. Abbreviations: Dept., Department of Nutrition, Dietetics and Food (Monash University); EOIs, expressions of interest; ESTA, Melbourne Emergency Services Telecommunications Authority.

Appendix M: Supplementary to Chapter 4 – Photovoice Study.

Participant hand-out explaining photo-taking activity

PHOTO TAKING ACTIVITY

Through your photos, share with us:
"What are shift worker's food choices and eating habits at work and outside of work?"

When should I take photos?

- Please take photos on **2 types of days**:
  1. On a day when you are working **night shift**
  2. On a day when you have a **day off**
- You can take photos over multiple days
- Remember that photos can be taken **during**, **before** and **after** your night shift
- If you work any other shift types, e.g. day or evening shifts, you can also include photos for these days

What should I take photos of?

- When do you eat?
- What do you eat?
- Where do you get food from?
- How do you prepare your food?
- Who do you eat with?
- Where do you eat?
- What is around you when you are eating?

*Photos should capture your **usual** habits

A few things to remember

- Take as many or as few photos as you like.
- The photos do not have to be beautiful, at a specific angle or with specific lighting, as long as it captures what you want it to represent, that is fine.
- Avoid taking photos of other people, especially their identifiable features. If you are going to do so, please ask for their permission. You may want to show them the expression of interest questionnaire (https://tinyurl.com/shiftworkphoto) if they ask about the study.
- **Please try to complete the activity in the next 2 – 3 weeks.**

If you can, please email your photos to Gloria at **med.shiftwork.study@monash.edu** (with your name) prior to the interview.

Otherwise, just bring your phone/camera to the interview.

Please email or call Gloria on **0490 369 973/9902 4199** if you have any questions.

Planned date, time and location for Interview: _____

Examples of photos that you may wish to take

On a day when I had to work night shift…

Before I went to work I bought…at….	 
At work I prepared my food by…	 
During my night shift I ate…	 
After my night shift I had to… So I ate…	 

On a day when I had a day-off…

On my day off I ate breakfast at…	
During my day off I ate…	 
During my day off I usually eat with…	 
On my day off I usually…	
On my day off I usually try to….	

Appendix N: Supplementary to Chapter 4 – Photovoice Study.

Participant demographics questionnaire

Please complete the following questions about yourself and your household, to help us better understand our discussion during the interview.

Details about you

1. Gender:

   ☐ Male

   ☐ Female

   ☐ I prefer not to say

2. Age (years): _____

3. Ethnicity (please select one you most identify with):

   ☐ Aboriginal Australian

   ☐ North-west European (e.g. British, Irish, Dutch, German)

   ☐ Southern and Eastern European (e.g. Italian, Spanish, Greek, Macedonian, Russian)

   ☐ North African and Middle Eastern (e.g. Arab, Jewish, People of the Sudan)

   ☐ South East Asian (e.g. Vietnamese, Indonesian, Khmer, Filipino)

   ☐ North East Asian (e.g. Chinese, Japanese, South Korean)

   ☐ Southern and Central Asian (e.g. Indian, Pakistani, Sri Lankan)

   ☐ People of the Pacific Islands (e.g. Maori, Samoan, Torres Strait Islander)

   ☐ People of the Americas (i.e. North American, South American, Central American)

   ☐ Sub-Saharan African (e.g.: Nigerian, South African)

   Other. Please specify:

   _____

4. Marital status:

   ☐ Single (never married)

   ☐ Have stable partner

   ☐ Married

   ☐ Divorced / separated

   ☐ Widowed

5. What is the highest level of education you completed?

- ☐ None
- ☐ Primary school
- ☐ Year 7 – 10 secondary school
- ☐ Year 11 – 12 secondary school
- ☐ Certificate (Trade or Business)
- ☐ Diploma or TAFE study
- ☐ Bachelor Degree (undergraduate university)
- ☐ Graduate Diploma/ Certificate
- ☐ Postgraduate Degree (Masters/ Doctorate)

About your work

6. What is your occupation and job title? _____

7. Which suburb is your workplace situated in? _____

8. Which of the following best describes your current employment?
   - ☐ Employed full-time
   - ☐ Employed part-time
   - ☐ Casual

9. What is your current shift schedule?
   - ☐ Permanent/fixed overnight shifts
     (*This means you only work overnight shifts.*
     Overnight shifts contain at least one hour between midnight and 6am.)
   - ☐ Rotating shifts
     (*This includes overnight shifts and any of the following:*
     morning, afternoon or evening shifts)
   - ☐ Split shifts
     (*This means your work day is split into 2 or more parts, separated by an extended unpaid break. E.g. you work from 11am to 2pm, then again from 8pm to 2am*)

10. Please give a description of your shift schedule cycle, include the number of days on each shift type and the number of days off. If possible, please state which day of the week this starts on. *(E.g.: 2 day shifts, 2 night shifts, 3 days off, start counting from Tuesday.)*

    _____

11. Please state the start and finish times of all your shift types.

_____

_____

_____

12. Do you work at different locations between shifts?

☐ Yes,

How many different locations do you work at? _____

☐ No

13. Have you had any previous employments?

☐ Yes.

Did any of your previous employments include night shifts? Y/N

(If yes) What was your role? _____

(If yes) How long did you work in this role for? _____

If you had more than one previous employment that included night shifts, please list them below, with the numbers of years that you were in these roles for:

_____

☐ No

Details about your household

14. How many people (including you) live in your household:

☐ I live alone

☐ Two

☐ Three

☐ Four

☐ Five (+)

15. Who do you live with? (tick all that apply)

☐ I live alone

☐ Partner

☐ Child/ children (please state their age/s)
_____

☐ Siblings

☐ Parent/s

☐ Others (please specify)
_____

16. Which suburb is your current household situated in? _____

17. Are you responsible for the food and grocery shopping in your household?
    - ☐ Yes – I do most of the food and grocery shopping
    - ☐ Yes – I am jointly responsible / share with others
    - ☐ No – someone else does it

18. Are you responsible for preparing meals / cooking in your household?
    - ☐ Yes – I do most of the cooking
    - ☐ Yes – I am jointly responsible / share with others
    - ☐ No – someone else does it
    - ☐ No – we don't often cook at home

About your lifestyle habits

19. Which of the following descriptions best applies to you?
    - ☐ Current smoker
    - ☐ Ex-smoker
    - ☐ Never smoked

20. Do you take any dietary supplements? *(This includes melatonin, vitamin/mineral/herbal supplements, fish oil, probiotics, calming and sleeping products, homeopathic medicines or meal replacement shakes.)*
    - ☐ Yes, please specify _____
    - ☐ No

END OF QUESTIONNAIRE – THANK YOU

Qualtrics link:

https://tinyurl.com/shiftworkphoto-demo

End of Thesis