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Does modifying the timing of meal intake improve cardiovascular risk factors? Protocol of an Australian pilot intervention in night shift workers with abdominal obesity

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ABSTRACT

Introduction Shift work is an independent risk factor for cardiovascular disease (CVD). Shift workers who are awake overnight and sleep during the day are misaligned with their body's endogenous circadian rhythm. Eating at night contributes to this increased risk of CVD by forcing the body to actively break down and process nutrients at night. This pilot study aims to determine whether altering meal timing overnight, in a shift working population, will impact favourably on modifiable risk factors for CVD (postprandial bplasma lipids and glucose concentration).

Methods and analysis A randomised cross-over study with two 4-week test periods, separated by a minimum of a 2-week washout will be undertaken. The effectiveness of redistributing energy intake overnight versus *ad libitum* eating patterns on CVD risk factors will be examined in night shift workers (n=20), using a standard acute test meal challenge protocol. Primary outcomes (postprandial lipids and glucose) will be compared between the two conditions: post-intervention and post-control period using analysis of variance. Potential effect size estimates to inform sample size calculations for a main trial will also be generated.

Ethics and dissemination Ethics approval has been granted by the Monash University Human Research Ethics Committee (2017-8619-10329). Outcomes from this study will determine whether eliminating food intake for a defined period at night (1–6 am) impacts favourably on metabolic risk factors for CVD in night shift workers. Collective results from this novel trial will be disseminated through peer-reviewed journals, and national and international presentations. The results are essential to inform health promotion policies and guidelines for shift workers, especially those who aim to improve their metabolic health.

Trial registration number ACTRN12617000791336; Pre-results.

Strengths and limitations of this study

- First study involving free-living night shift workers that proposes to improve risk factors for cardiovascular health by altering nocturnal energy consumption.
- Outcomes could have implications for almost 20% of the working population and drive policy change in order to improve the health and well-being of shift workers.
- Existing data are not available to make informed sample size calculations.
- Unintentional weight loss as a consequence of rearranging meal timing is a potential limitation.
- Participant compliance with the study protocol is a potential limitation.

INTRODUCTION

Circadian rhythms act to optimise many aspects of human biology.[1] The mammalian circadian system consists of the central clock located in the suprachiasmatic nuclei (SCN) of the hypothalamus and peripheral clocks which drive localised processes.[2] The SCN responds to the 24-hours light/dark cycle in the external environment and provides temporal signals to the peripheral clocks. This ensures that physiological processes, such as nutrient metabolism, are appropriately timed and occur in synchrony with each other.

The importance of the circadian system for health is demonstrated by the strong associations between circadian disruption, such as that experienced in night shift work, with morbidity[3] and disease pathology.[4 5] Shift work is defined as work outside of typical daytime working hours (6 am–6 pm)[6] and up to 20% of the population in industrialised countries are engaged in shift work.[7] Shift workers frequently experience circadian misalignment, which occurs when the timings of

lifestyle behaviours, such as sleep/wake and fast/feeding, are desynchronised with the temporal rhythm set by the central circadian clock.[8] However, it seems challenging for rotating shift workers to avoid circadian misalignment, as it is difficult for them to achieve circadian adaptation, due to constant changes in shift schedules.[9]

A systematic review and meta-analysis found that compared with day work or the general population, shift work was associated with greater risk of vascular events,[10] specifically myocardial infarction (risk ratio (RR) 1.23, 95% CI 1.15 to 1.31; I^2=0) and ischaemic stroke (RR 1.05, 95% CI 1.01 to 1.09; I^2=0). Furthermore, a meta-analysis of observational studies reported an association between type 2 diabetes and shift work compared with those only exposed to day work schedules. The overall odds of developing diabetes was 9% higher in people who have been exposed to shift work, while those in rotating shift patterns, in which employees rotate through day and night shifts on a regular basis, face a 42% increased odds compared with non-shift workers.[11] These observed associations remain statistically significant after adjusting for traditional risk factors such as adiposity, physical activity and diet.[10 11]

Eating at night time, as is common for night shift workers, adds to the metabolic disruption experienced by shift workers. Participants in studies who are kept awake and eat during their 'biological night' present with multiple metabolic responses that resemble the pre-diabetic state of elevated postprandial lipids and glucose.[12] This may be one of the causal factors underlying the increased cardiovascular disease (CVD) and diabetes risk seen in shift workers.[10 11] Night workers also commonly have irregular eating times, more snacking and fewer substantial meals. Our recent systematic review and meta-analyses confirmed that 24-hour energy intake of shift workers does not differ from that of fixed day workers. A total of 12 studies with 10 367 day workers and 4726 shift workers were used to compare energy intake, resulting in a standardised mean difference for energy intake of −0.04 (95% CI −0.11 to 0.03; I^2=54%).[13] These findings suggest that meal timing (eating at the wrong time of the 24-hour cycle) rather than energy intake per se is a key contributor to the increased risk of metabolic disturbances observed in shift workers.[14]

Emerging evidence in animal models indicate that restricting food to certain times of the day can impact favourably on metabolic risk, independent of diet.[14] Specifically, mice fed a high fat diet during a specific 'best time' weigh less than mice fed the same food during their biological night,[15] and in general, a clear metabolic advantage has been observed if food intake is confined to the active period in animals.[14] In addition, long-term time-restricted feeding without caloric reduction can attenuate the effects of diet-induced obesity in animals.[16] In humans, there is evidence to suggest that changes in the timing of meal intake can positively impact metabolic responses. A randomised crossover trial in people with type 2 diabetes showed that participants had better glycaemic control when a higher proportion of daily energy was consumed at breakfast, compared with when the same amount was consumed at dinner.[17] A laboratory protocol simulating four nights of shift work in 11 healthy men found that those who were allocated to the night eating condition exhibited an increased glucose response to an acute meal challenge post night shifts. Those who did not eat at night, instead by redistributing energy intake to the day period, showed no significant differences to baseline.[18] Currently lacking, however, is the evidence for these effects over a longer time frame in free-living shift workers.

The primary aim of the 'Shifting the Risk' crossover trial is to examine the impact of altering meal timing by redistributing energy intake overnight on cardiovascular risk factors, in a group of night shift workers with abdominal obesity. This pilot studywill also assist in determining required sample size to achieve our aims. We hypothesise that restricting energy intake overnight will impact favourably on postprandial plasma lipids and glucose. If our hypothesis is supported, this simple manipulation of energy redistribution (meal timing) would be expected to contribute to the reduction of independent risk factors for metabolic disease in this at risk population group. Given the large proportion of individuals undertaking shift work, research is warranted to identify countermeasures for the adverse effect of circadian misalignment on metabolic disease risk factors. These can be developed into evidence-based dietary guidelines specific for the shift working population.

METHODS AND ANALYSIS

Primary aim

To examine the impact of altering meal timing by redistributing energy intake overnight on cardiovascular risk factors, in a group of night shift workers with abdominal obesity.

Hypothesis

Food avoidance at night between 1 and 6 am will improve postprandial lipid and glucose metabolism in night shift workers with abdominal obesity.

Study design

A randomised cross-over trial with two 4-week test periods, separated by a minimum of a 2-week wash out.

Setting

This study will recruit from any workplaces in Melbourne, Australia, which employ night shift workers.

Participants

Eligibility criteria

Study participants are those who participate in night shifts in a rotating, permanent or split shift schedule. They would have worked a minimum of 12 months consecutively in their current shift work schedule. Participants will be aged between 18 and 60 years, presenting with abdominal obesity, as assessed by guidelines that are

specific for gender and ethnicity[19] : waist circumference (>94 cm (Non-Asian men), >90 cm (Asian men), and >80 cm (all women)).

Exclusion criteria

Exclusion criteria include those working standard day time hours only, shift workers who do not work between 1 and 6 am, work less than three to four night shifts per fortnight on average, diagnosed with diabetes or CVD, on drug therapy for diabetes, or hyperlipidaemia or taking medications known and observed to alter body composition or metabolism, for example, thyroxin, insulin sensitisers, glucocorticoids or anti-depressants. Further restrictions include being pregnant, planning a pregnancy or breastfeeding, those who have lost or gained >10% of body weight in the last 6 months, those who have obesity due to secondary causes relating to genetic or endocrinology-related disorders, those who consume four or more standard drinks on a single occasion at a 'daily or almost daily' occurrence, and those who do not routinely eat between 1 and 6 am while on night shift work.

Recruitment/screening

Night shift workers (18–60 years) at risk of CVD (abdominal obesity) will be recruited from shift work industries in the Melbourne area. The study will be advertised through professional contacts in shift working industries and also through advertisement in Monash University networks, local papers and social media.

Interested participants will complete an online screening questionnaire (Qualtrics, Provo, UT) which gathers demographic, work-related and health related information. Embedded into the online screening questionnaire is the explanatory statement and consent form. Following researcher review (GKWL or RD) of the online screening questionnaire, eligible participants will be invited to attend a screening session at the Be Active Sleep Eat (BASE) facility, Monash University, for a screening session. During this session, weight, height and waist circumference, using standardised procedures, will be assessed to ensure suitability for inclusion into the trial.

Randomisation, allocation concealment and sequence generation

Eligible participants will be randomised to begin with either the intervention or control period of the study. The randomisation procedure was performed by a researcher who is not involved in recruitment or baseline data collection (CEH). The allocation sequence was determined by computer-generated random number sequence using a permuted block design.[20] A random seed value was generated for reproducibility. Block sizes will be kept confidential to the researchers determining participant eligibility. The allocation sequence is kept in a password-protected computer file, which the researchers (GKWL and RD) involved in screening the participants do not have access to. Researchers (GKWL and RD) are provided the allocation to treatment in sealed envelopes when required.

Sample size calculations

In the absence of literature with study design and endpoints similar to that proposed in this study, a power calculation could not be conducted. The current study serves as a pilot study to determine the required sample size to achieve study aims. We aim to have 20 participants complete the study. Accounting for a 30% attrition rate, we will aim to recruit 28 participants.

Preintervention (run-in period)

Prior to starting the study, all participants will be required to complete a 4-day food diary that incorporates two to three night-shifts, 1-day shift (if any) and 1-day off. Information from this food diary will be used to assist the study dietitian in providing strategies for the participants to redistribute energy intake overnight.

Study intervention

The intervention is a 4-week (minimum) timed meal protocol, whereby participants are asked to avoid food intake for a fixed period of time (5 hours between 1 and 6 am) while maintaining their usual total energy intake (ie, over the 24-hour period). That is, just the timing of which they consume their usual food and beverages will change. Prior to starting the intervention period, participants will meet with a dietitian to discuss the most appropriate way to manage their diet during the night (figure 1). Over the course of the 4-week intervention, participants will undertake an average of eight night shifts.

Control period

During the control period, participants will be asked to maintain (or return to) their habitual dietary intake for a continuous period of 4 weeks (minimum). Refer to figure 1 for summary of study protocol.

Data collection

The following section outlines the data and biochemical samples being collected during the test periods and the acute meal challenges (see table 1 for a summary).

Demographic information and questionnaires

A general shift work questionnaire will be completed by all participants at baseline. This will gain information relating to individual shift work history, including time spent in shift work roles, current shift work schedule, sleep schedule and meal timing during a typical day shift, night shift and day off. The Morningness and Eveningness questionnaire will also be completed at baseline to identify chronotype or diurnal preference.[21]

Questionnaires collected at baseline and at the end of each test period will include the following:

- Shift Work Disorder Questionnaire, to assess risk of shift work disorder, including excessive sleepiness and insomnia.[22]

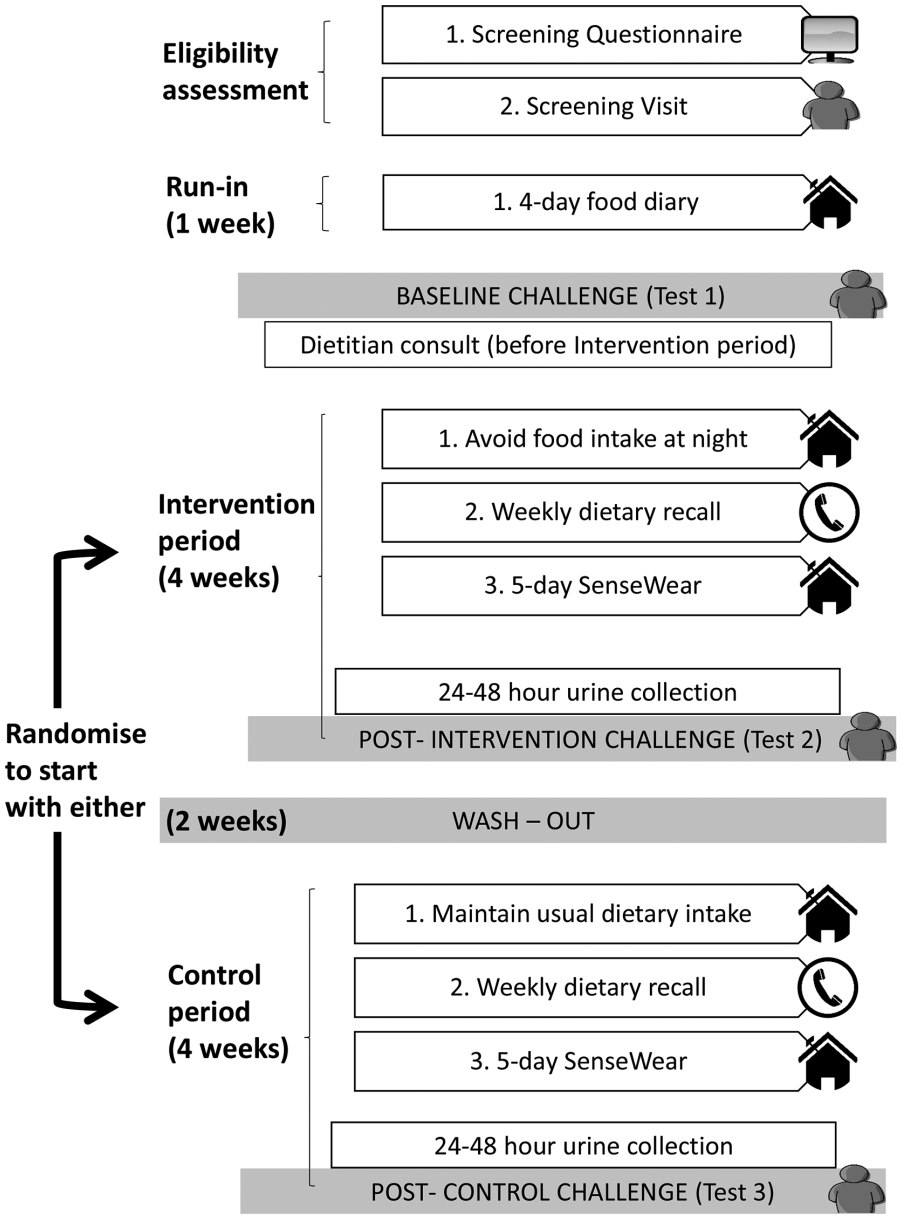

Figure 1 Summary of study protocol.

- Pittsburgh Sleep Quality Index, to evaluate self-rated sleep habits during the last month.[23]
- Depression, Anxiety and Stress Scale (DASS), to evaluate severity of symptoms of depression, anxiety and stress.[24]
- Assessment of Quality of Life questionnaire (AQoL-8D), a multiattribute utility instrument, to identify the effect of the intervention on a broad range of mental health and physical health states. Included items relate to happiness, pain, self-worth, coping and relationships.[25]

Physical activity/sleep patterns

In order to monitor physical activity levels, participants will be required to complete the long version of the International Physical Activity Questionnaire (IPAQ).[26] Participants will also wear the SenseWear activity monitor in each test period. This will be worn continuously for the preceding 5 days before they attend their next meal challenge session. The SenseWear activity monitor is a small multisensor worn on the back of the upper arm, comprising a two-axis accelerometer, heat flux sensor and galvanic skin response sensor. It estimates the wearer's physical activity duration, number of steps taken and sleep duration, as well as estimating total energy expenditure.[27]

Anthropometry

All participants will have their weight, waist circumference[28] and body composition (SECA, 515/514, SECA Group, Hamburg, Germany) measured using standardised procedures at each meal challenge session.

Acute meal challenge

At baseline (ie, pretest periods) and at the end of both test periods, participants will undergo an acute meal

Table 1 Synoptic table of outcome measures

Outcome	Run-in period	Control period	Intervention period
Diet, physical activity and sleep			
4-day food diary	X		
24-hour dietary recall		X (4 occasions)	X (4 occasions)
SenseWear monitor		X (5 days)	X (5 days)
Biochemical			
Melatonin		X (24-48 hours)	X (48 hours)
	Meal challenge session		
	Baseline (post run-in period)	**Post-control period**	**Post-intervention period**
Anthropometric			
Weight	X	X	X
BMI	X	X	X
Body composition	X	X	X
Waist circumference	X	X	X
Biochemical			
Fasting			
TAG	X	X	X
Glucose	X	X	X
Cholesterol	X	X	X
Insulin	X	X	X
TNF-α	X	X	X
Interleukin-8	X	X	X
Endothelin-1	X	X	X
sICAM-1	X	X	X
Postprandial			
TAG iAUC	X	X	X
Glucose iAUC	X	X	X
Insulin iAUC	X	X	X
TNF-α	X	X	X
Interleukin-8	X	X	X
Questionnaires			
General shift work questionnaire	X		
Morningness/Eveningness	X		
IPAQ	X	X	X
Shift work Disorder Questionnaire	X	X	X
Pittsburgh Sleep Quality Index	X	X	X
Depression, Anxiety and Stress Scale	X	X	X
AQoL-8D	X	X	X

AQoL-8D, Assessment of Quality of Life Questionnaire; BMI, body mass index; iAUC, incremental area under the curve; IPAQ, International Physical Activity Questionnaire; sICAM, soluble intercellular adhesions molecule; TAG, triacylglycerol; TNF-α, tumour necrosis factor-alpha.

challenge. The day before each meal challenge, participants will be asked to refrain from strenuous exercise and alcohol and consume a standardised dinner meal which will be provided by the researchers. The standardised meal consists of spinach and ricotta ravioli, cheese and crackers and apple juice, providing 2810 kJ as 56% carbohydrate, 24% fat and 17% protein and is to be consumed between 6 and 8 pm. After an overnight fast (12 hours), participants will attend the BASE facility at Monash University. Each meal challenge will be scheduled at the same point in participant's shift cycle, and will occur on the second or third day off after their last night shift.

Table 2 Nutrient composition of test meal

	Muffin (two)	Milkshake	Total	% Energy
Weight (g)	156	265	421	–
Energy (kJ)	2307	1472	3779	–
Fat (g)	32.8	24.8	57.6	56
Carbohydrate (g)	56.0	24.8	80.8	36
Protein (g)	8.9	8.5	17.4	8

Participants will arrive at the BASE facility between 7.30 and 8.30 am, and a cannula will be fitted in the antecubital fossa by a trained nurse or phlebotomist. A fasting baseline blood sample will be taken before participants are given a high-fat breakfast meal to be consumed within 15 min. A meal high in fat (minimum 50 g fat) is used to maximise the postprandial lipaemic response.[29] The breakfast meal consists of two muffins and a milkshake supplying 3779 kJ of energy, its nutrient composition is outlined in table 2. Blood samples (maximum total volume 120 mL) will be taken at regular intervals for a 6-hour postprandial period. During this period, participants will be permitted water (but no other food or drinks) and are to remain sedentary.

Fasting measures

During each acute meal session, fasting plasma samples will be collected for assessment of blood lipids, glucose, insulin, markers of inflammatory status (proinflammatory cytokines) and markers of endothelial function. Serum tubes will be used for collection of blood lipids (triacylglycerol (TAG), cholesterol, high-density lipoprotein and low-density lipoprotein)) and EDTA tubes will be used to collect samples for glucose, insulin and markers of inflammatory status (tumour necrosis factor-alpha (TNF-α), interleukin (IL-6)) and endothelial function (soluble intercellular adhesions molecule (sICAM-1)). All blood samples will be centrifuged and stored at −80°C until analysis. Except for markers of endothelial function, all markers will also be measured during the postprandial period. Markers of endothelial function will be analysed using ELISA technology (R&D Systems). All other markers will be analysed as per protocol outlined below under 'postprandial markers'.

Postprandial measures

Blood will be collected at the following postprandial time points: 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 min, into serum or EDTA tubes. All blood samples will be centrifuged and stored at −80°C until analysis. Serum lipids and plasma glucose concentrations will be measured on a Thermo Fisher Indiko clinical chemistry analyser (Thermo Fisher Scientific, Vantaa, Finland) by enzymatic colorimetric methods, using commercially available kits as per manufacturer's instructions (Thermo Fisher Scientific, Vantaa, Finland). Plasma insulin will be determined using the Human Insulin Specific RIA kit HI-14K (Merck Millipore, Billerica, Massachusetts, USA) according to manufacturer's instructions and read on a Gamma Counter. Inflammatory markers will be assessed on a MagPix System (Luminex Corporation, Texas, USA) using commercially available Multiplex Assays (Merck Millipore, Massachusetts, UK).

Melatonin

Urine samples will be collected on two occasions (24-48 hours in duration each) to measure the concentration of the uinary rhythm in the urinary metabolite of melatonin, 6-sulphatoxymelatonin (aMT6s). This will act as a marker of circadian phase. Participants will collect a urine sample every 4 hours (or every 8 hours during sleep) for 48 hours continuously during each test period. Participants will record the timing and volume of each collection for calculation of aMT6s excretion rate. Aliquots (5 mL) will be stored at −20°C prior to radioimmunoassay to determine the concentration of aMT6s. Urinary excretion rate for each collection episode will be subjected to cosinor analysis to determine the timing of the acrophase or peak of aMT6s. Circadian phase will be used to account for potential individual differences in metabolic outcomes between test periods.

Compliance/adherence to intervention

During the intervention period, participants will be sent automated short messages (MessageNet) on every night shift to remind them not to eat for the designated 5-hour period. Twenty-four-hour dietary recalls will be undertaken once a week during both intervention and control periods to examine compliance. This will be conducted via phone call with a researcher, on the participant's first day off after a continuous row of night shifts. They will be asked to recall their food intake from 6 am the day prior to 6 am of the current day. Weight maintenance will be measured on each meal challenge session. A variation of weight of ±2 kg will be considered usual.

Primary and secondary outcome measures

Primary

The primary outcomes for the study are postprandial TAG and glucose response, measured during each meal challenge session. This will be assessed by calculating incremental area under the curve (iAUC). This is a measure of an individual's metabolite concentrations over time, after consumption of the high fat test meal, ignoring the area beneath the fasting concentration. Postprandial lipaemia reflects the capacity of the body to process a

fat-containing meal, a factor known to be altered in shift work[30] and as a methodology, has been used successfully to show significant effects of dietary intervention in both the acute[31] and chronic setting.[32] Postprandial glucose is an important modifiable metabolic factor to target in this intervention, as evidence exists for a causal relationship between postload glucose concentration and all-cause cardiovascular mortality.[33]

Secondary

Secondary outcomes will be measured in plasma and consist of a composite of inflammatory cytokines (to include TNF-α and IL-6), markers of endothelial function (endothelin-1 and sICAM-1) and postprandial insulin. All secondary outcomes will be measured during each meal challenge. Markers of endothelial function will be measured at time 0 min only (fasting), whereas the inflammatory markers will also be assessed during the postprandial period (time 0, 60, 120, 240 and 360 min). Insulin will be assessed at eight time points between 0 and 3 hours. For a detailed synopsis of study outcomes refer to table 1.

Data management

All enrolled participants will be given a unique code, which will be used to identify their biological samples, electronic questionnaire and paper-based data. It is necessary to replace their identifiers with a code, as participants need to be tracked over multiple data collection points. A separate computer database will store participant identifiers (eg, name, address and phone number) and their associating code. This database will be password protected and only accessible by the researchers, to ensure its security and the confidentiality of any identified data. Following the completion of the study, data will be stored in a locked filing cabinet at the Monash University BASE facility, Notting Hill or in a password-protected location for a minimum of 5 years. Only the principal and associate researchers will have access to the data.

Participants who deviate from the protocol will remain in the study and the researchers will record the frequency of non-compliance events, which can be used as a covariate assessment of primary outcomes and also used to determine intervention fidelity. Outcome data cannot be collected on participants who discontinue from the study.

Protocol deviations

Protocol deviations will be communicated via an update of the Australian and New Zealand Clinical Trial Registry and also through a letter to the editor of this journal.

Statistical analysis plan

Primary outcomes (TAG and glucose iAUC) will be compared between the two test periods: post-intervention and post-control period. This will be conducted using the CROS analysis method,[34] which takes into account period effects. Briefly, CROS analysis will involve an analysis of variance (ANOVA) with TAG and glucose iAUC concentration at the end of each test period as the outcome variables and participant, period and condition group as the factor variables. Similar methods will be used to assess the difference between the two test periods for secondary outcomes. In cases where the baseline levels of an outcome are deemed important confounders, analysis of co-variance will be used. For instances where assumptions of ANOVA are not met, a non-parametric equivalent test will be used. Due to the small sample size, data will not be imputed if there are missing data.

ETHICS AND DISSEMINATION

Consent process

Prior to enrolment into the trial, participants will be able to access details of the study, specifically the consent form and explanatory statement, through an online screening questionnaire. Participants who meet the inclusion criteria will be invited to a screening session to have the study verbally explained to them by the researchers, and their waist circumference, weight and height measured. At that stage, provided they meet the waist circumference cut offs, participants can decide whether to participate. Prior to entering the study (during the screening session), written informed consent will be obtained from all participants (by GKWL or RD). All participants will be informed of their right to withdraw from the study at any time.

Confidentiality

Personal information (for example, name and contact details) will be collected by authors GKWL and RD and stored in a password-protected electronic database. Only the study's researchers will have access to this information. Only deidentified data will be presented via publications.

Data access

In accordance with the National Health and Medical Research Council Statement on Data Sharing, deidentified data should be made available for use by the other researchers unless this is prevented by ethical, privacy or confidentiality matters. The data from this study may be held on secure public repositories. Any shared data will remain anonymous and there will be no way to identify individual participants.

Dissemination

As this is a pilot study, one of the main outcomes is to generate data to inform a fully powered study. The findings of the trial will be disseminated through peer-reviewed journals, and national and international presentations. Outcomes will be reported to stakeholders and grantors including the National Heart Foundation, which has an established platform for disseminating study results to both researchers and the public. Participants will be provided with their individual body composition reports and food intake data on request. Group-level outcomes (de-identified) of the trial will also be distributed to

participants on request. Participants will be offered monetary compensation to reimburse them for their time, travel costs and inconvenience. A maximum payment of $300 is approved on completion of the trial; the payments are staggered over the three meal challenge sessions.

Trial registration and status

The protocol for this study was registered with the Australian New Zealand Clinical Trials Registry (ACTRN12617000791336) on 30 May 2017 (url: http://www.anzctr.org.au/ACTRN12617000791336.aspx).

Participant recruitment began on 24 July 2017, with the first participant entering the study on 25 August 2017.

DISCUSSION

Shift workers have been demonstrated to be at increased risk of CVD[35] and type 2 diabetes.[4] Currently, evidence-based advice with demonstrated effectiveness to target specific metabolic factors, which contribute to this increased CVD risk, is lacking. We propose that modifying meal timing may be an effective strategy to improve risk factors for cardiovascular health in night shift workers, by altering nocturnal energy consumption.

To date, the majority of interventions that examine the metabolic impact of night time eating have occurred in non-shift working populations, under conditions of simulated night shift in a laboratory setting. This study offers the first long-term intervention in free-living shift workers (4 weeks) and aims to investigate meal timing, rather than meal composition or energy intake, as a novel and modifiable risk factor to partially explain the higher rates of chronic disease observed in shift workers.

It is plausible to suggest that rearranging meal timing, without any changes in overall energy intake, could inadvertently lead to weight loss. A recent meta-analyses has linked shift work with the development of overweight and obesity, specifically abdominal obesity[36] and our previous work indicates that this is likely to do with the timing and type of foods consumed rather than overall energy intake.[13] Statistical analysis can adjust for weight loss as a covariate to examine the influence of weight changes on the primary outcomes.

It is anticipated that recruitment could be a rate-limiting step in the progression of this study, as previous intervention studies involving shift workers have reported that this population can be difficult to target and engage.[37] A study which had recruited male shift workers through close collaboration with one Australian manufacturing organisation, reported that working with organisations was a feasible strategy for recruitment and retention of study participants.[38] We have established links with a number of shift working organisations which has enhanced our capacity to successfully recruit participants.

Another challenge to this research is the potential heterogeneity introduced by differences in shift work schedules or differences between workforces, when recruiting from the general population. Recruitment will focus on organisations that have similar shift work scheduling to minimise variability in workplace practices, as it would affect outcome measures. To counteract this variability, we will also be collecting melatonin (aMT6s), which allows us to account for differences in circadian phase between participants. Furthermore, acute meal challenges are to be scheduled at the same time of day, and prestudy day conditions are controlled for to improve our likelihood of observing proposed changes in metabolic markers.[29]

Behaviours adopted during the food avoidance test period could be carried over into the control period. Compliance with the protocol, therefore, is being monitored through 24-hour dietary recalls. Individualised dietetic consultation will deliver guidance on redistributing meal timings in order to further improve compliance with the study, as simply avoiding food intake at night, without substituting the food elsewhere during the day could impact body weight. There is some evidence to support non-hungry snacking in night shift workers,[39] so unless this energy is consumed outside of the specified hours, weight loss may be observed. However, we are looking to recruit participants who work an average of four night shifts a fortnight, which should not be sufficient to significantly affect weight.

Given the large proportion of individuals undertaking shift work, this research is warranted to identify countermeasures for the adverse effect of circadian misalignment on risk factors for CVD. These can then be developed into evidence-based dietary guidelines, specific for the shift working population.

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Contributors MPB, CEH, CM and TLS: were co-applicants on the grant application and as such were involved with the original design of this pilot study. MPB: was the lead applicant, is the principal investigator for the 'Shifting the Risk' study and lead author for this paper. MPB: is also responsible for the management of the study. CEH, CM and TLS: involved with study coordination and contributed to the writing and development of the protocol paper. MJY, EAL and NE: contributed to method development. GKWL and RD: responsible for the day to day running of the trial, recruitment and sample collection. GKWL, MJY, EAL, NE and RD: also contributed to the writing and development of the protocol paper. All authors: will have responsibility for analysis, statistical interpretation of outcomes and preparation of manuscripts for publication post study completion.

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Competing interests None declared.

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REFERENCES

1. Ekmekcioglu C, Touitou Y. Chronobiological aspects of food intake and metabolism and their relevance on energy balance and weight regulation. *Obes Rev* 2011;12:14–25.
2. Johnston JD, Ordovás JM, Scheer FA, *et al*. Circadian rhythms, metabolism, and chrononutrition in rodents and humans. *Adv Nutr* 2016;7:399–406.
3. Lin X, Chen W, Wei F, *et al*. Night-shift work increases morbidity of breast cancer and all-cause mortality: a meta-analysis of 16 prospective cohort studies. *Sleep Med* 2015;16:1381–7.
4. Pan A, Schernhammer ES, Sun Q, *et al*. Rotating night shift work and risk of type 2 diabetes: two prospective cohort studies in women. *PLoS Med* 2011;8:e1001141.
5. Wang XS, Armstrong ME, Cairns BJ, *et al*. Shift work and chronic disease: the epidemiological evidence. *Occup Med* 2011;61:78–89.
6. McMenamin TM. A time to work: recent trends in shift work and flexible schedules. *Mon Labor Rev* 2007;130:3–15.
7. Stevens RG, Hansen J, Costa G, *et al*. Considerations of circadian impact for defining 'shift work' in cancer studies: IARC Working Group Report. *Occup Environ Med* 2011;68:154–62.
8. Antunes LC, Levandovski R, Dantas G, *et al*. Obesity and shift work: chronobiological aspects. *Nutr Res Rev* 2010;23:155–68.
9. Boudreau P, Dumont GA, Boivin DB. Circadian adaptation to night shift work influences sleep, performance, mood and the autonomic modulation of the heart. *PLoS One* 2013;8:e70813.
10. Vyas MV, Garg AX, Iansavichus AV, *et al*. Shift work and vascular events: systematic review and meta-analysis. *BMJ* 2012;345:e4800.
11. Gan Y, Yang C, Tong X, *et al*. Shift work and diabetes mellitus: a meta-analysis of observational studies. *Occup Environ Med* 2015;72:72–8.
12. Al-Naimi S, Hampton SM, Richard P, *et al*. Postprandial metabolic profiles following meals and snacks eaten during simulated night and day shift work. *Chronobiol Int* 2004;21:937–47.
13. Bonham MP, Bonnell EK, Huggins CE. Energy intake of shift workers compared to fixed day workers: a systematic review and meta-analysis. *Chronobiol Int* 2016;33:1086–100.
14. Moran-Ramos S, Baez-Ruiz A, Buijs RM, *et al*. When to eat? The influence of circadian rhythms on metabolic health: are animal studies providing the evidence? *Nutr Res Rev* 2016;29:180–93.
15. Arble DM, Bass J, Laposky AD, *et al*. Circadian timing of food intake contributes to weight gain. *Obesity* 2009;17:2100–2.
16. Froy O. The relationship between nutrition and circadian rhythms in mammals. *Front Neuroendocrinol* 2007;28:61–71.
17. Jakubowicz D, Wainstein J, Ahrén B, *et al*. High-energy breakfast with low-energy dinner decreases overall daily hyperglycaemia in type 2 diabetic patients: a randomised clinical trial. *Diabetologia* 2015;58:912–9.
18. Grant CL, Coates AM, Dorrian J, *et al*. Timing of food intake during simulated night shift impacts glucose metabolism: A controlled study. *Chronobiol Int* 2017;34:1003–13.
19. National Health and Medical Research Council. *Clinical practice guidelines for the management of overweight and obesity in adults, adolescents and children in Australia*. Melbourne, Australia: National Health and Medical Research Council, 2013:232.
20. Dallal G. Welcome to Randomization.com [Internet]. U.S.A. 2007. Updated Jan 2017. http://www.randomization.com/ (cited 10 Mar 2017).
21. Horne JA. Ostberg O. A self-assessment questionnaire to determine morningness-eveningness in human circadian rhythms. *Int J Chronobiol* 1976;4:97–110.
22. Barger LK, Ogeil RP, Drake CL, *et al*. Validation of a questionnaire to screen for shift work disorder. *Sleep* 2012;35:1693–703.
23. Buysse DJ, Reynolds CF, Monk TH, *et al*. The pittsburgh sleep quality index: a new instrument for psychiatric practice and research. *Psychiatry Res* 1989;28:193–213.
24. Lovibond SH, Lovibond PF. *Manual for the depression anxiety stress scales*. 2nd ed. Australia: SydneyPsychology Foundation, 1995.
25. Richardson J, Iezzi A, Khan MA, *et al*. Validity and Reliability of the Assessment of Quality of Life (AQoL)-8D Multi-Attribute Utility Instrument. *The Patient - Patient-Centered Outcomes Research* 2014;7:85–96.
26. Hagströmer M, Oja P, Sjöström M. The International Physical Activity Questionnaire (IPAQ): a study of concurrent and construct validity. *Public Health Nutr* 2006;9:755–62.
27. Johannsen DL, Calabro MA, Stewart J, *et al*. Accuracy of armband monitors for measuring daily energy expenditure in healthy adults. *Medicine & Science in Sports & Exercise* 2010;42:2134–40.
28. International Society for the Advancement of Kinanthropometry. *International standards for anthropometric assessment*. Australia: International Society for the Advancement of Kinanthropometry, 2001:139.
29. Lairon D, Lopez-Miranda J, Williams C. Methodology for studying postprandial lipid metabolism. *Eur J Clin Nutr* 2007;61:1145–61.
30. Lund J, *et al*. Postprandial hormone and metabolic responses amongst shift workers in Antarctica. *J Endocrinol* 2001;171:557–64.
31. Bonham MP, Linderborg KM, Dordevic A, *et al*. Lipidomic profiling of chylomicron triacylglycerols in response to high fat meals. *Lipids* 2013;48:39–50.
32. Piers LS, Walker KZ, Stoney RM, *et al*. The influence of the type of dietary fat on postprandial fat oxidation rates: monounsaturated (olive oil) vs saturated fat (cream). *Int J Obes* 2002;26:814–21.
33. de Vegt F, Dekker JM, Ruhé HG, *et al*. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999;42:926–31.
34. Jones B, Kenward MG. *Design and Analysis of CrossOver Trials*. 2nd ed. London: Chapman and Hall, 2003.
35. Tenkanen L, Sjoblom T, Kalimo R, *et al*. Shift work, occupation and coronary heart disease over 6 years of follow-up in the Helsinki Heart Study. *Scand J Work Environ Health* 1997;23:257–65.
36. Sun M, Feng W, Wang F, *et al*. Meta-analysis on shift work and risks of specific obesity types. *Obes Rev* 2018;19.
37. Atlantis E, *et al*. Worksite intervention effects on physical health: a randomized controlled trial. *Health Promot Int* 2006;21:191–200.
38. Morgan PJ, Collins CE, Plotnikoff RC, *et al*. Efficacy of a workplace-based weight loss program for overweight male shift workers: The Workplace POWER (Preventing Obesity Without Eating like a Rabbit) randomized controlled trial. *Prev Med* 2011;52:317–25.
39. Bonnell E, Huggins C, Huggins C, *et al*. Influences on dietary choices during day versus night shift in shift workers: a mixed methods study. *Nutrients* 2017;9:193–206.