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Randomized Control Trials

Effects of macronutrient manipulation on postprandial metabolic responses in overweight males with high fasting lipids during simulated shift work: A randomized crossover trial

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SUMMARY

Background & aims: Meals consumed out of synchronisation with normal circadian rhythms are associated with metabolic dysregulation. Changes in macronutrient composition of meals can improve metabolic responses during the day. Therefore, we aimed to investigate whether macronutrient manipulation of meals alters postprandial glucose and lipid responses and the expression of circadian genes during the night.

Methods: In a randomised crossover trial, 16 overweight males with high fasting lipids were fed isocaloric meals (2.7 MJ) at 0000 h. The meals differed primarily in total fat and total sugars content (control (8% total sugar, 5% saturated fat) vs test (16% total sugar, 26% saturated fat)). Postprandial blood samples were collected for glucose, insulin (3 h) and triglycerides (6 h) and analysed as incremental area under the curve (iAUC). RNA was extracted at 0 h, 2 h and 4 h and changes in expressions of the circadian genes *clock* and *Per 1–3* analysed.

Results: Postprandial glucose ($p = 0.04$) and insulin iAUC ($p = 0.02$) were significantly higher after consumption of the test meal compared to the control meal. Postprandial triglyceride iAUC was not statistically different between the two meal types ($p = 0.72$). No change in circadian gene expression was observed after the two meals.

Conclusions: Our results showed that macronutrient composition affects postprandial metabolic response at night. It emphasizes the need to consider the role and effects of night time eating, when developing metabolic disease prevention strategies for shift workers.

Study ID number: ACTRN12618001115224.

Website of trial registry: http://www.anzctr.org.au/. Retrospectively registered after data collection.

1. Introduction

Metabolic responses to food intake deteriorate over the 24 h cycle to coincide with the fasting and sleeping period during the night. The same meal consumed during the night, compared with during the day, is associated with increased glycaemic excursion and a reduced insulin sensitivity [1,2] - a phenotype metabolically equivalent to being pre-diabetic. Triglycerides (TG) also oscillate diurnally [3].

Circadian misalignment of behavioural and environmental cycles results in endogenous desynchronisation between central and peripheral regulators of physiological processes including energy metabolism [4,5]. Circadian misalignment has been implicated as a mechanism for increased disease risk in shift workers. Circadian responses are controlled at a transcriptional level by a group of circadian (or clock) genes including *clock*, *per1,2* and *3* and *cry* in

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response to stimuli such as sunlight, food and activity to indicate it is 'day time', while darkness, fasting and sleeping to indicate it is 'night time' [6].

Meal timing can also impact gene expression of circadian transcription factors in humans. A recent study in volunteers (n = 36) with and without diabetes observed that acute breakfast omission altered clock genes expression in RNA isolated from whole blood and resulted in an increased postprandial glycaemic response after lunch [7].

Whilst a number of studies have confirmed that eating at night produces exaggerated postprandial responses compared with during the day [8,9], the effect of varying meal composition at night to minimise these exaggerated responses has not been investigated in detail. In a recent study with healthy adults, it was hypothesised that provision of a low GI meal at night time would be effective in reducing the increased postprandial glycemia observed at night time. However, glucose response after a low GI meal was still six times greater than that observed in the morning [2]. Data reporting on the metabolic responses to high fat meals at night time are mixed, but also suggest a circadian regulation of lipid metabolism that alters the clearance of postprandial lipids at night compared with during the day [3,9,10].

This randomized crossover study was undertaken to assess the postprandial metabolic responses to two isocaloric meals (termed 'control' and 'test' meal) differing in macronutrient composition, during simulated night shift work in overweight males with high fasting lipids. We hypothesized that the 'control' meal (low in total fat, saturated fat and sugar) consumed at night will elicit a lower postprandial TG and glucose response, when compared to the 'test' meal (high in total fat, saturated fat and sugar). A secondary aim was to assess the molecular mechanism underlying the effect of eating at night and meal composition, by examining the postprandial expression of genes coding for key clock transcription factors.

2. Materials and methods

2.1. Study design

Randomized crossover trial examining the effect of macronutrient composition on postprandial metabolic responses during the night, in overweight men with high fasting blood lipids. Two separate acute meal challenges (testing sessions) were conducted (0000 hr–0600 hr), with a minimum washout period of one week. The study was carried out at the Be Active Sleep Eat (BASE) Facility at Monash University, Victoria, Australia.

2.2. Ethics

This study was approved by the Monash University Human Research Ethics Committee (Project number CF: 15/337–2015000164) and complied with the Declaration of Helsinki of 1975 as revised in 1983. All participants were provided with all relevant information regarding study procedures, and provided written informed consent prior to commencing the study.

2.3. Participants

Participants were males aged between 18 and 50 years. Recruitment was undertaken between August 2015 and April 2016 until sample size was achieved. Interested participants were recruited via social media platforms and information flyers advertised in surrounding areas, with all participants local to Melbourne, Australia. Eligible participants were, overweight or obese (Body Mass Index (BMI) $\geq$25 kg/m^2), with high fasting blood TG (>1.7 mmol/L), who maintained a regular sleep-wake cycle. Potential participants who engaged in shift work, were smokers, currently taking any lipid lowering, anti-hypertensive, anti-depressive or thyroid deficiency medications, had fasting blood glucose >6.0 mmol/L, irregular meal patterns or consumed excessive alcohol (>4 standard drinks per day) were excluded. Initial eligibility was assessed through a phone interview and confirmed via a screening session at the BASE Facility.

2.4. Screening

The screening session was to confirm a BMI of $\geq$25 kg/m^2 and to measure fasting blood TG and glucose. Upon arrival at the BASE Facility, anthropometric measures were determined without shoes and in light clothing. Height was measured using a stadiometer (Holtain Ltd, Crymych, United Kingdom), weight and body composition using a SECA Bioelectrical Impedance Analyser (515/514, SECA Group, Hamburg, Germany) and umbilical waist circumference using a stretch resistant tape (Figure Finder, Novel Products, Rockton, IL, USA). All measures were taken in duplicate following standardised procedures and recorded to the nearest 0.1 decimal place. Blood pressure was measured using a Digital Blood Pressure Monitor (Welch Allyn ProBP 3400) on two occasions, a minimum of 5 minutes apart with the participant sitting. Two finger prick blood samples were taken to measure fasting blood glucose level (Accu-Chek Performa II, Accu-Chek, Mannheim, Germany) and fasting TG levels (CardioCheck) respectively. Participants with fasting TG levels <1.7 mmol/L and/or fasting blood glucose levels >6.0 mmol/L were ineligible.

2.5. Randomization, allocation concealment and sequence generation

The sequence for allocation to order of meal type was generated using an electronic random number generator and concealed in a password protected folder. It was generated by a researcher who was not involved in eligibility assessment and data collection; MPB assigned participants to the meal order. The researcher (EK) knew in advance of each study night the allocation to meal type, however the participants were blinded to the order in which they received the meals and the macronutrient composition of the meal.

2.6. Pre-intervention procedures

Prior to the first overnight session, participants completed a seven day food diary to assess habitual diet. Dietary analysis of energy and nutrient intake was performed using Foodworks 7 (Xyris Software Pty Ltd., QLD, Australia). Sleep and activity patterns were assessed for seven days using a Sensewear armband triaxial accelerometer (SWA; Bodymedia, Pittsburgh, Pennsylvania, USA). A seven day sleep diary was also kept where participants were asked to record the times in which they went to bed at night and rose in the morning. Sleep quality over the previous month was assessed using the Pittsburg Sleep Quality Index (PSQI). A global PSQI score was generated from the survey, with lower scores correlating to better sleep quality [11] (Buysse et al.). Physical activity (previous seven days) was measured using the International Physical Activity Questionnaire (IPAQ). The IPAQ comprised of 4 items, including leisure time, domestic and gardening, work related and transport related activities [12].

One day prior to, and including the day of each testing session, participants were asked to abstain from strenuous exercise and alcohol. On the day of each session, participants were instructed to consume a standard pre-study meal, provided by the research team, at 1230 hr. The standard meal comprised of a Weight Watchers macaroni, a 150 g strawberry yogurt and 30 g of natural

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almonds (2.6 MJ, 29.4% total energy (E) protein, 49% E carbohydrate, 32% E fat). They were also instructed to consume a provided snack at 1800 h; it consisted a 250 ml chocolate dairy drink (Up and Go) and a Be Natural trail bar (32 g) (1.3 MJ, 14% protein, 66% carbohydrates, 15% fat). After consumption of the snack at 1800 hr, participants were asked to refrain from eating or drinking (except for water) until the start of the testing session at 0000 hr.

2.7. Meal challenge

On study days, participants were instructed to arrive at the BASE Facility at 2300 h. Prior to each session, participants were asked to maintain their regular sleep/wake cycle for at least three days. The participants were given one of two isocaloric meals: the control meal or the test meal (refer to Supplementary Table 1 for macronutrient breakdown of meals). The control meal was a homemade vegetarian pasta dish (2.7 MJ, 57% E from carbohydrate (8% E total sugars), 28% E fat (5% E saturated), 13% E protein). The test meal consisted of a spinach and ricotta pastry with 200 ml can of sprite (2.8 MJ, 45% E from carbohydrates (16% total sugars), 46% E from fat (26% saturated), 8% E from protein). Participants were asked to consume the meal within 15 mins. Midnight was selected for meal administration as we have previously reported increased post prandial glucose and insulin responses at midnight compared with the morning (8am) in a crossover trial [2]. Furthermore, starting the study at midnight and collecting samples for 6 hours incorporates the whole overnight period and the 0300 hr peak or nadir of circadian rhythms in melatonin, body temperature and alertness [13].

2.8. Procedures

2.8.1. Anthropometry

Participants' blood pressure, height, body composition and umbilical waist circumference were measured at each testing session, as per screening procedures.

2.8.2. Fasting and postprandial blood measures

Prior to the meal challenge an indwelling cannula (BD Insyte Auto guard 20 GA 1.1 × 25 mm) was placed in an antecubital vein by a trained nurse. Fasting baseline blood measures were taken, before consumption of the meal at 0000 hr. Eleven blood samples were drawn over the 6 hour testing period: at baseline (time 0) and at 15, 30, 45, 60, 90, 120, 180, 240, 300 and 360 mins after commencement of meal consumption. Samples were collected into two 4.0 ml BD EDTA Vacutainer collection tubes (13 × 75 mm) for glucose, insulin and TG. Samples were centrifuged (Eppendorf, 5702R) at 1,300 RCF for 15 mins. Plasma was aliquoted and stored at −80 °C degrees for future batch analysis. Blood (at 0000 hr, 0200 hr and 0400 hr) was also collected into BD CPT glass tubes, for isolation of peripheral blood mononuclear cells (PBMC), which RNA was batch extracted from.

2.8.3. Questionnaires

During the 6 hour testing period, participants completed the Karolinska sleepiness scale (KSS) at hourly intervals. This validated scale measures the subjective level of sleepiness in the last 10 min of each hour [14]. The scale was from 'one', which describes the participant as being 'extremely alert' to 'ten' which was 'very sleepy.' Self-reported appetite was also assessed hourly using visual analogue (line) scales (VAS). Each VAS scale consists of a 100 mm horizontal line rating scale, corresponding to a question related to appetite [15]. The questions were worded as "how hungry do you feel" and "how full do you feel", with anchors of 'not at all' to 'extremely' at each end of the line respectively.

2.9. Biochemical analysis

Plasma glucose (Thermo Fisher, 981779) and TG (Thermo Fisher, 981786) levels were analysed using an Indiko™ Clinical and Speciality Chemistry System (Thermo Fisher Scientific, Vantaa, Finland) as per manufacturer's instructions. The multi calibrator sCal (981831) was used to calibrate the analyser, together with the control serums Nortrol (981043) and Abtrol (981044). Intra-assay coefficient of variation was <5% for both glucose and TG. All samples were run in duplicate; any sample with a coefficient of variation >10% was rerun. Plasma insulin levels were quantified by radioimmunoassays (HI-14K Human Insulin-Specific, Merck Millipore, Missouri, USA) according to the manufacturers' instructions (RIA, Millipore). The lowest level of insulin that can be detected by this assay is 2.715 μU/mL when using a 100 μl sample size. The between assay coefficient of variation was <5%.

2.10. Peripheral blood mononuclear cell isolation and RNA extraction

Four millilitres of blood was drawn at baseline (0000 hr), 0200 hr and 0400 hr into BD CPT glass tubes (BD, Melbourne, Australia) and centrifuged (Eppendorf, 5702R) for 25 min, at 1500 RCF to separate PBMC from red blood cells. Isolated PBMC were collected into a falcon tube and PBS added to a total volume of 10 ml prior to a second centrifugation step (15 mins, at 1000 RCF). After centrifugation, the cell pellet was resuspended in 100 ul of PBS, 500 ul of cell protectant (Qiagen, Hilden Germany) was added and the sample was stored at −80 °C. Approximately 10^7 cells were obtained from the 4 ml of whole blood collected. On completion of the trial, RNA was isolated using the RNeasy Plus Mini Kit (with genomic DNA eliminator column) (Qiagen, Hilden Germany) following manufacturer's instructions. RNA was eluted in 60uL of RNAse-free water and immediately kept on ice whilst concentration and quality of RNA in the sample and ratio of absorbance at 260/280 nm ratio was determined by NanoPhotometer® Classic (Implen GmbH München Germany), and agarose electrophoresis.

2.11. Real time qPCR

A total of 500 ng RNA was reverse transcribed to cDNA with the High Capacity RNA-to-cDNA kit (ThermoFisher Scientific, Waltham, MA USA). cDNA was diluted 40 times, and real-time quantitative PCR was performed with Applied Biosystems™ TaqMan® Gene Expression Assays (ThermoFisher Scientific, Waltham, MA USA) and run on an Eppendorf Realplex real time PCR machine (Eppendorf, Eppendorf South Pacific Pty., North Ryde Australia).

MIQE guidelines were followed to identify a reference gene suitable for our experimental conditions. The software Geneinvestigator (https://genevestigator.com/gv/index.jsp) [16] was used to explore previous human microarray data and this analysis identified three genes namely TBP (TATA-box binding protein), RHOT2 (Ras Homologue Gene Family, Member T2), and KRT8 suitable for the experimental conditions [17]. TBP was selected to normalise qPCR relative expression results, as it was consistently stable in postprandial experimental tests and its expression levels were in the same range as the test genes. Relative gene expression levels were determined using the comparative ΔCt method as described in Schmittgen & Livak (2008) [18]. Fold changes were relative to the baseline (0000 hr) expression for each study visit.

Applied Biosystem Assay IDs are shown in Table 1.

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Table 1
List of circadian genes analysed by TaqMan®.

Gene symbol	TaqMan Assay ID	Amplicon length	Ref Seq
TATA-box binding protein (TBP)	Hs00427620_m1	91	NM_001172085 NM_003194.4
Period (Per) 1	Hs00242988_m1	66	NM_002616.2
Period (Per) 2	Hs00256143_m1	121	NM_022817.2
Period (Per) 3	Hs00213466_m1	64	NM_001289861.1
Circadian locomotor output cycles kaput (CLOCK)	Hs00231857_m1	88	NM_001267843.1

2.12. Statistical analysis

A power calculation was conducted *a priori* based on data from a previous study [19]. Assuming a standard deviation of the difference in means in TG of 189 mmol/L/5 hr and a minimum difference in the AUC between the two meals of 157 mmol/L/5 hr, a minimum of 15 participants were required to detect a significant difference between the meals with 80% power at an alpha value of 0.05. No data was available from comparative evening studies. The variation in glucose response is much smaller than that of TG, thus there is sufficient power with 15 participants to detect a difference in glucose [19].

2.12.1. Primary outcomes

Postprandial TG and glucose responses, estimated using iAUC were the primary outcome measures. TG iAUC was calculated over a six hour period (0–360 mins), whereas the iAUC for glucose was assessed over a three hour period (0–180 min). iAUC was calculated

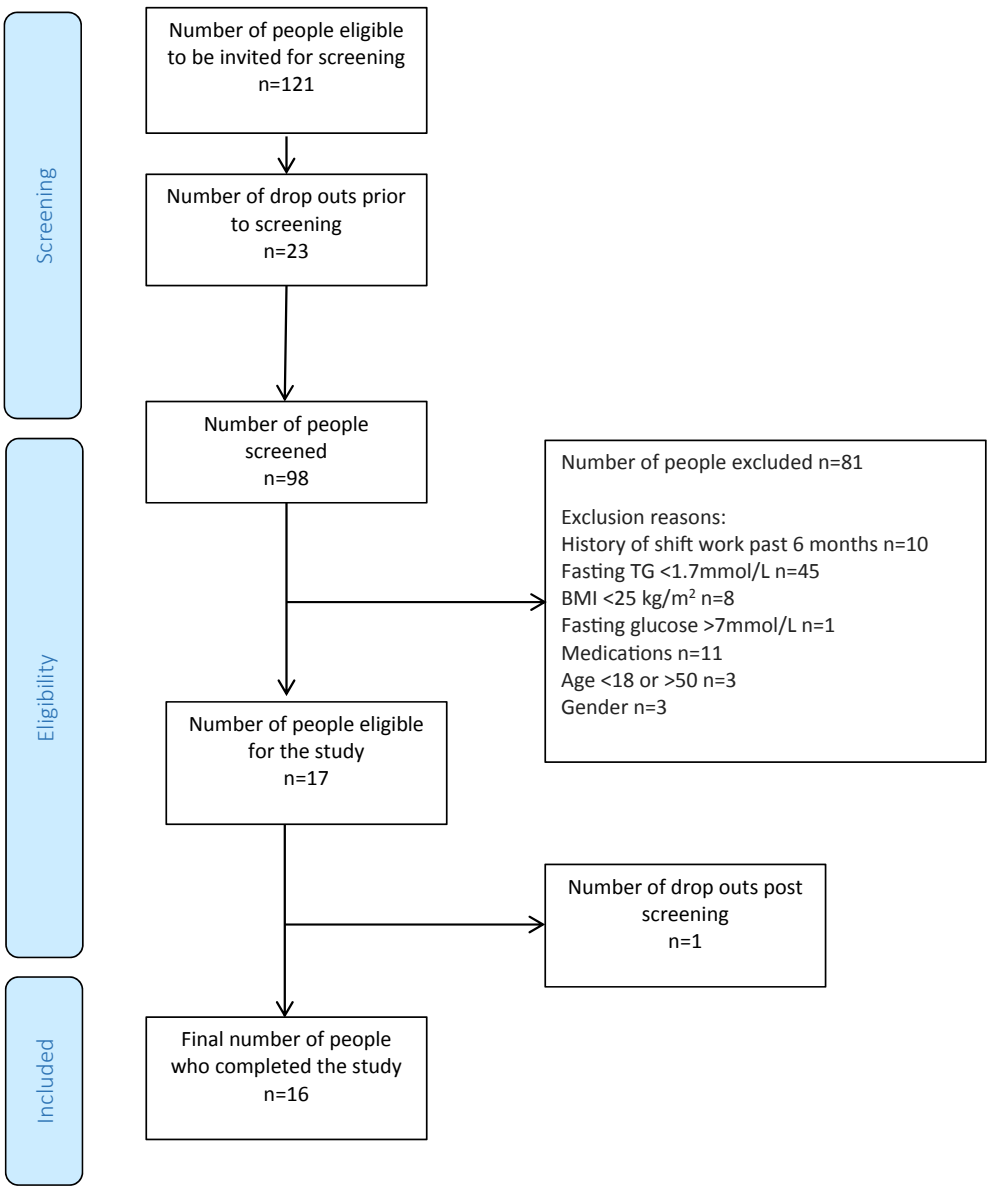

Fig. 1. Participant flow through study.

using the trapezoid rule, which ignores the area beneath the baseline concentration. The baseline concentrations were taken as the fasting values at 0000 hr. All data are reported as median (IQR) except for the plotted data (Fig. 2) which are reported as mean ± SEM. Differences in postprandial iAUC responses to the two meals challenges were analysed using the Wilcoxon signed-rank test for TG and glucose. Statistical significance was considered at $p < 0.05$.

2.12.2. Secondary outcomes

Analysis of postprandial insulin was similar to glucose and estimated using iAUC with samples were collected over a 3 hour period (0–180 min). Data are reported as median (IQR) except for the plotted data (Fig. 2) which is reported as mean ± SEM. Postprandial appetite and sleepiness as assessed by visual analogue scales (VAS). VAS scores were assessed using total area under the curve (tAUC). Statistical differences were examined using the Wilcoxon signed-rank test. Statistical analysis for primary and secondary outcomes were conducted using IBM SPSS (version 25). Statistical significance was considered at $p < 0.05$.

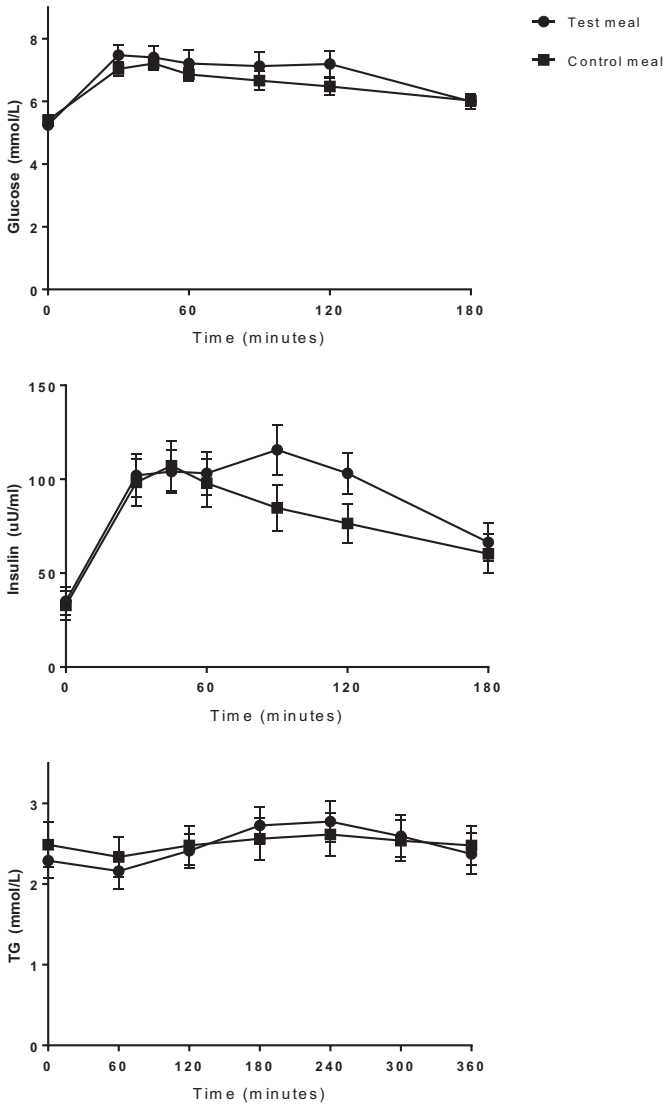

Fig. 2. Postprandial glucose, insulin and triglyceride concentrations after a control meal and a test meal at midnight. Values expressed as mean ± SEM, n = 16.

To assess changes in gene expression (secondary outcomes) the mean of the three PCR replicates for each sample was calculated, and the normalised gene expression ascertained by subtracting the Ct values of the housekeeping gene TBP from the Ct values of the target gene. Statistical tests were performed on the transformed $2^{-\Delta Ct}$ values. Analysis was completed on a sample size of n = 12 for all genes, bar *clock* where n = 8 due to sample loss. Due to non-normal distributions, and 2.65% of total data points missing at random due to low RNA yield, median differences in gene expression were compared between each meal type (control/test) at each time point (0, 120, and 240 mins) by non-parametric repeated omnibus test Skillings Mack [20], a generalisation of the Friedman test for missing observations. Where a significant difference was observed, post hoc Wilcoxon signed-ranks tests were conducted between paired groups to determine if genes were differentially expressed. Bonferroni correction was applied, p-values < 0.0083 were considered significant. All data are reported as median (IQR). Statistical analyses were conducted using R version 3.4.2 [21].

3. Results

3.1. Participant enrolment and characteristics at baseline

A flowchart for recruitment is detailed in Fig. 1. Of the 121 participants who identified for screening, 16 participants completed the trial (Fig. 1). The median age (IQR) of participants was 25 (9) years and the median BMI was 35.1 (10.6) kg/m^2. Baseline characteristics of participants are presented in Table 2. Due to missing data, body composition data (fat mass, lean mass and fat weight) and blood pressure measurements from one participant were not included.

3.2. Postprandial responses

3.2.1. Glucose

Glucose concentration from 0 to 180 min were used in the final analysis. We omitted the 15 min samples for all participants, as we

Table 2
Baseline characteristics of participants (n = 16) participating in a postprandial meal challenge at night collected at their screening visit.

Baseline characteristics	Median (IQR)
Age	25 (9)
Weight (kg)	111 (39.1)
Height (cm)	173.5 (9.4)
BMI (kg/m^2)	35.1 (10.6)
Waist circumference (cm)	113.3 (24.1)
Fat mass (%)[a]	38.6 (13.5)
Fat weight (kg)[a]	42.2 (26.9)
Fat free mass (%)[a]	63.8 (14.9)
Fat free weight (kg)[a]	66.0 (12.5)
Systolic BP (mmHg)[a]	124 (13.5)
Diastolic BP (mmHg)[a]	80 (11.0)
Fasting blood glucose (mmol/L)	5.3 (0.9)
Fasting triglycerides (mmol/L)	2.2 (0.8)
Habitual sleep duration (min)[a]	468.8 (67.0)
MET equivalents	1.4 (0.5)
MEQ score (range)[a]	43–57
Intermediate type	14/14
PSQI[a] (score from 1 to 14)	
Poor sleep quality (≥5)	N = 7
Good sleep quality (<5)	N = 8
Energy intake (MJ)	9.7 (2.9)

BMI: Body mass index, BP: blood pressure, MEQ: Morningness and eveningness questionnaire, MET: Metabolic equivalents, PSQI: Pittsburgh Sleep Quality Index.
[a] N = 15 for body composition assessment, blood pressure and PSQI, N = 14 for MEQ and habitual sleep duration.

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had no data for two participants, owing to complications with cannulation at that time point. The iAUC was therefore calculated based on eight samples rather than nine. Median (IQR) for blood glucose iAUC are shown in Table 3. Postprandial blood glucose levels are depicted in Fig. 2. Fasting glucose was no different ($P = 0.733$) between the two meal challenges (control 5.3 (0.9) vs test 5.2 (1.1)). The Wilcoxon signed rank test revealed that the median (IQR) iAUC was significantly greater after the test meal compared to the control meal (297.5 (241.4) vs. 219.0 (223.3) mmol/L.3 h, $p = 0.044$). The median glucose concentrations at 180 min postprandial was significantly raised compared to the baseline value after both the control meal (5.3 (0.9) vs. 6.0 (1.1) mmol/L, $p = 0.003$) and test meal (5.2 (1.1) vs. 5.6 (1.7) mmol/L, $p = 0.011$).

3.2.2. Insulin

Insulin concentration from 0 to 180 mins (excluding 15 min samples as per glucose) were used in the final analysis. Median (IQR) for insulin iAUC values are shown in Table 3. Baseline insulin concentrations were not different between the two meal challenges ($p = 0.569$). Postprandial blood insulin levels are depicted in Fig. 2. A significantly larger iAUC response was observed after the test meal compared with the control meal (11022 (7017) vs. 7948 (2673) mU/L.3 h, $p = 0.023$).

3.2.3. Triglycerides

Baseline TG levels were not different prior to the acute meal challenge (2.1 (1.4) vs 2.4 (1.2) mmol/L, $p = 0.215$). Hourly TG values from 0 to 360 mins (inclusive) were used in the final analysis. Median (IQR) for blood TG iAUC values are shown in Table 3. The median TG concentrations at 360 mins postprandial were similar to baseline after both the control meal (2.4 (1.2) vs. 2.3 (1.4) mmol/L, $p = 0.717$) and test meal (2.1 (1.4) vs. 2.1 (0.9) mmol/L, $p = 0.776$). Postprandial blood TG levels are depicted in Fig. 2 and whilst non-significant, a 26% greater increase in postprandial TG concentrations in the blood after the test meal compared to the control meal (105.9 (239.9) vs 62.9 (151.7)) was observed.

3.2.4. Subjective appetite (VAS scale) and sleepiness (KSS scale) measures

Subjective feelings of fullness and hunger were collected hourly over the 6 hour postprandial period. No differences in subjective fullness and satiety were observed after consumption of the control vs the test meal at night time (Table 3). When subjective sleepiness was examined, a significant effect of meal type was observed, with participants reporting increased feelings of sleepiness after the test meal compared with the control meal (1620 (840) vs 1312 (711), $p = 0.017$).

Table 3
Night time fasting and postprandial (incremental/total area under the curve) responses for blood glucose, insulin, triglycerides, sleepiness and appetite after two meal challenges varying in macronutrient composition, in men with high fasting lipids (n = 16). Data reported as median (IQR).

	Control meal	Test meal	*p* value
Glucose (mmol/L) at baseline	5.3 (0.9)	5.2 (1.1)	0.733
Glucose iAUC (mmol/L 3 h)	219.0 (223.3)	297.5 (241.4)	0.044
Insulin (mU/L) at baseline	22.0 (28.2)	23.3 (25.5)	0.569
Insulin iAUC (mU/L 3 h)	7948 (2673)	11022 (7017)	0.023
TG (mmol/L) at baseline	2.4 (1.2)	2.1 (1.4)	0.215
TG iAUC (mmol/L 6 h)	62.9 (151.7)	105.9 (239.9)	0.717
Sleepiness (tAUC)	1312 (711)	1620 (840)	0.017
Hunger (tAUC)	1110 (223.3)	870 (765)	0.969
Satiety (tAUC)	1290 (28.2)	1050 (1095)	0.172

iAUC: incremental area under the curve; tAUC: total area under the curve; TG: triglyceride. n = 14 for hunger and satiety.
Values are median (IQR). Significant differences reported using Wilcoxon signed-rank test. $P < 0.05$ deemed a significant difference.

3.2.5. Gene expression of clock genes

Meal composition at night did not influence gene expression of *Per1 and Per3* or *Clock* ($p > 0.05$). An effect of time was initially observed for *Per2*, however post hoc analyses on *Per-2* rendered the initial observed effect over time non-significant. Instead we describe a 1.4 fold increase in *Per2* expression 4 hours after consumption of the control meal and a 1.8 fold increase in *Per2* expression 4 hours after consumption of the test meal (Fig. 3).

4. Discussion

This study examined the response to two isocaloric meals differing in macronutrient composition on metabolic markers in overweight men with high fasting lipids during simulated shift work. We also examined postprandial expression of circadian genes (*Per1*, *Per2*, *Per 3* and *Clock*). The identification of metabolic responses to food intake specifically at night has increasing relevance in a society which operates on a 24/7 basis, especially as people in shift work roles reportedly consume a significant proportion of their daily caloric load at night [22].

We hypothesized that provision of a meal lower in total, saturated fats and sugars (control meal) would result in lower postprandial glycaemia and lipidemia at night (0000 hr) when compared with a test meal. Glycaemic control was significantly improved when participants were provided with the control meal compared with a meal higher in sugar (test meal) whilst no difference was observed in postprandial lipemic responses between the meal challenges, even though the control meal was lower in total and saturated fat.

Glycaemic responses at night time are typically reported as being higher than during the day [9,23]. In this study, we confirmed our hypothesis that by manipulating meal choice, we can help mediate the higher glycaemic responses observed at night. After the test meal, we observed higher glucose and insulin levels than that observed after the control meal, even though the test meal had a lower proportion of total carbohydrates (45% vs 57%). However, the proportion of sugars in the test meal was double that of the control meal, possibly contributing to the raised glycaemic response. This increase in glycaemic response at night may also be attributed to a reduction in insulin sensitivity at night [5,24], which is consistent with the increased postprandial insulin response we observed after consumption of the test meal. Avoiding foods that challenge the body's response to a meal may have beneficial implications for diabetes risk reduction, in people who have no choice but to eat at night such as shift workers.

Similar to glucose, there is also supporting evidence for a diurnal variations in postprandial TG, with higher postprandial lipids observed at night compared to during the day [10]. The reason for this relative lipid intolerance at night is unclear, but has been speculated to relate to the relative insulin resistance at night [3,25]. This may cause a reduction in the activity of lipoprotein lipase and a subsequent decrease in the hydrolysis of plasma TG, resulting in higher circulating plasma TGs at night [25].

It is useful to note therefore, that even though night time is associated with poor lipid tolerance, meals considered moderate in fat content (20–35 g) did not generate exaggerated postprandial TGs at night in the current study. These findings suggest that for those who have no choice but to eat at night, meals with a moderate amount of fat combined with a complex carbohydrate source, compared with more refined carbohydrate (specifically total sugars), may be preferential in terms of mediating adverse

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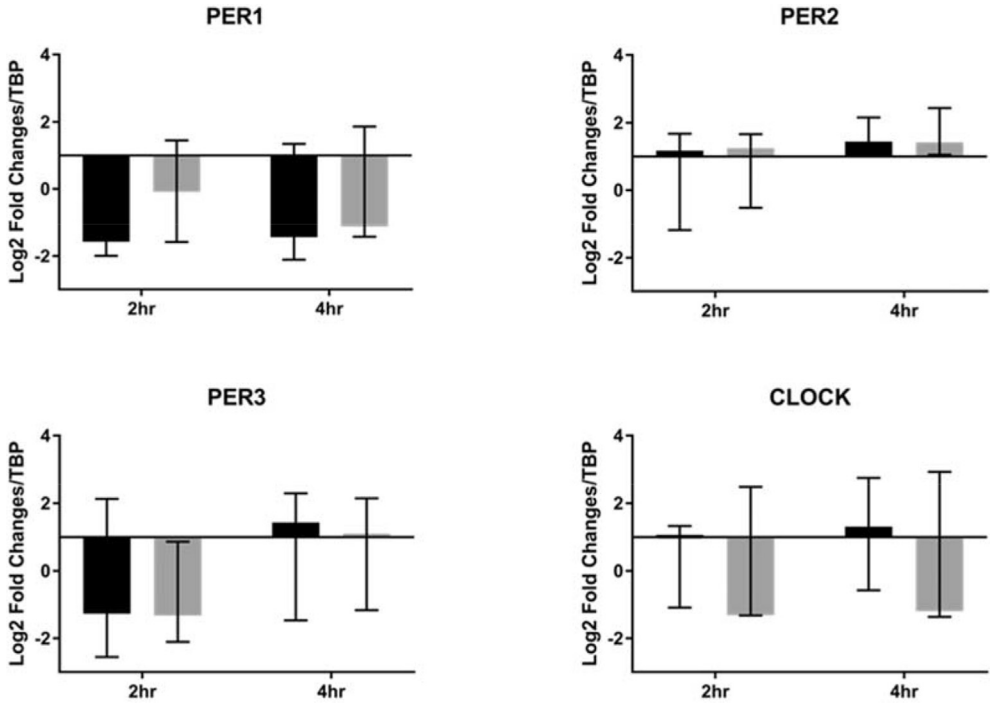

Fig. 3. Postprandial regulation of gene expression in human PBMC. Blood samples were collected before the meal (baseline), 2 hr and 4 hr after meal consumption. Results were calculated by $2^{-\Delta\Delta Ct}$ relative quantification methods and expressed as median $\log_2$ of fold change from baseline, error bars represent interquartile range (IQR). Statistical significance was calculated as $p < 0.05$. Black box: control meal; grey box: test meal. PER1, PER2, PER3 ($n = 12$), CLOCK ($n = 8$).

postprandial responses. Other modest benefits from a higher fat meal compared with a high carbohydrate meal under simulated shift work conditions is a more effective maintenance of performance and reduced sleepiness [26]. However, this study was only in seven healthy male participants.

The effect of meal composition at night on sleepiness and subjective appetite was also assessed in the current study. Whilst the meals did not impact subjective feelings of hunger and fullness, we did see an impact of meal in our analysis of subjective sleepiness. Participants who completed the KSS reported increased sleepiness after the test meal compared to the control meal. These findings are of interest, as studies in shift workers have indicated an increased tendency for sugary foods [27] whilst on night shift, possibly based on a perceived notion of improved alertness.

Both the nutrient composition of food and timing of food intake can affect peripheral circadian clocks [28]. Restricted feeding regimens can act to synchronise the oscillations of peripheral clocks and attenuations in core clock gene expression in peripheral tissues have been observed after a high fat diet in an animal model [29]. In the current study, the macronutrient composition of a meal was not shown to affect postprandial expression of core clock genes. In human PBMCs, it has been noted that negative regulators *Per1*, *2* and *3* reach their lowest level of expression in the evening and start to be upregulated overnight, reaching their peak in the morning hours [30], and is consistent with the observed upregulation of *Per2* in the 4 hour overnight postprandial period in the current study. Although this was rendered non-significant after a post-hoc correction was applied.

In a study performed by Pivovarova et al. [31] in non-obese individuals ($n = 29$) with normal glucose tolerance, the expression of core clock genes (PER1, PER2, PER3, and TEF) in monocytes were reported to be modulated by a switch from an isocaloric high carbohydrate/low fat diet to 6 weeks consumption of a low carbohydrate/high fat diet. The authors suggest that the alterations in diurnal oscillations of these circadian genes, as a result of a change in diet, actuated an uncoupling of peripheral circadian oscillators from the central pacemaker. This uncoupling of peripheral and central clocks could impact the metabolic regulation of nutrient digestion and absorption in peripheral tissues. The results of the current study have not found that acute consumption of a meal with a higher fat composition produces similar changes in circadian gene expression. Irregular meal timing (skipping of breakfast) has also been associated with changes in circadian gene expression that correlated with an increased postprandial glycaemic response after lunch [7]. These emerging findings highlight the complex interaction at play between meal timing and/or meal composition on circadian disruption and highlight food as an entraining cue for peripheral body clocks. Furthermore, the significant effect of eating on the expression of key clock genes that are upstream of the synthesis of enzymes and hormones involved in glucose and lipid metabolism [28], suggests that more sensitive biomarkers could be employed to define the metabolic effect of eating at night and the impact of food composition.

As access to healthy food choices at night may be limited [32]. it is important to understand the consequences of food choice at night in the shift working population bearing in mind their food preferences [33] and that appetite regulation may be impacted by circadian disruption [34]. This study benefitted from tightly controlled conditions with energy intake during the day of testing controlled from midday. In addition, selection of participants with high fasting lipids allowed us to investigate the impact of night time eating in a population at risk of cardiovascular disease.

The reproducibility of individual postprandial responses to the meal challenges was not examined in this study. But as the authors have previously reported data indicating metabolically adverse postprandial lipaemic responses to isocaloric meals high in non dairy fat compared with meals high in dairy fat in the morning [19] this methodology is suitable for examining such responses without the need for reproducibility testing.

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Not having a morning comparator could be considered a limitation, but there is now convincing evidence to support diurnal variations in glucose and lipid metabolism [2,35]. Whereas information relating to the effect of macronutrient composition on metabolic abnormalities at night are still limited. As our participants were overweight males, these findings are applicable for a significant proportion of the population, but may not be transferable to women or those individuals of a healthy body weight.

Currently, there are limited studies which report the metabolic, genetic and subjective appetite effects of altering meal composition under simulated night shift conditions, which demonstrates the novelty of these data. In this study, we report differential effects of isocaloric meals varying in macronutrient composition, on postprandial triglyceride, glucose and insulin response, and sleepiness. We did not find evidence that acute consumption of meals differing in macronutrient composition resulted in differential expression of genes involved in circadian regulation. Whilst overall these findings are not unexpected, in that they suggest that a meal lower in saturated fat and sugar at night time was associated with an improved glycaemic response and reduced sleepiness, they contribute to the growing literature on the impact of meal timing and food choice on metabolic health. It is critical to consider the role of night time eating, when developing treatment and prevention strategies for metabolic disease risk in shift workers.

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Statement of authorship

Bonham: Conceptualization, Formal Analysis, Methodology, Supervision, Original Draft, Reviewing and Editing. **Kaias:** Project Administration, Investigation, Review and Editing. **Huggins:** Conceptulization, Supervision, Review and Editing. **Davis:** Methodology, Formal Analysis, Review and Editing. **Leung:** Methodology, Review and Editing. **Eikelis:** Data Acquisition, Review and Editing. **Shaw:** Data Curation, Formal Analysis **Murgia:** Methodology, Supervision, Review and Editing.

Conflict of interest

No conflicts of interest.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2019.02.018.

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