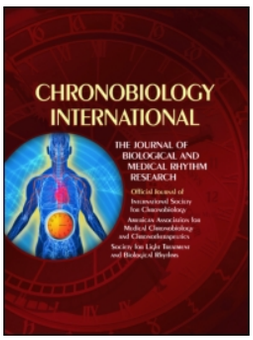

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Timing of food intake during simulated night shift impacts glucose metabolism: A controlled study

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ABSTRACT

Eating during the night may increase the risk for obesity and type 2 diabetes in shift workers. This study examined the impact of either eating or not eating a meal at night on glucose metabolism. Participants underwent four nights of simulated night work (SW1–4, 16:00–10:00 h, <50 lux) with a daytime sleep opportunity each day (10:00–16:00 h, <3 lux). Healthy males were assigned to an eating at night (NE; n = 4, meals; 07:00, 19:00 and 01:30 h) or not eating at night (NEN; n = 7, meals; 07:00 h, 09:30, 16:10 and 19:00 h) condition. Meal tolerance tests were conducted post breakfast on pre-night shift (PRE), SW4 and following return to day shift (RTDS), and glucose and insulin area under the curve (AUC) were calculated. Mixed-effects ANOVAs were used with fixed effects of condition and day, and their interactions, and a random effect of subject identifier on the intercept. Fasting glucose and insulin were not altered by day or condition. There were significant effects of day and condition × day (both $p < 0.001$) for glucose AUC, with increased glucose AUC observed solely in the NE condition from PRE to SW4 ($p = 0.05$) and PRE to RTDS ($p < 0.001$). There was also a significant effect of day ($p = 0.007$) but not condition × day ($p = 0.825$) for insulin AUC, with increased insulin from PRE to RTDS in both eating at night ($p = 0.040$) and not eating at night ($p = 0.006$) conditions. Results in this small, healthy sample suggest that not eating at night may limit the metabolic consequences of simulated night work. Further study is needed to explore whether matching food intake to the biological clock could reduce the burden of type 2 diabetes in shift workers.

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Introduction

The sleep–wake cycle plays a pivotal role in human physiology and behaviour across the 24-h day. A number of key metabolic functions follow this 24-h cycle or circadian rhythm, including digestion (Konturek et al., 2011), insulin production (Boden et al., 1996a) and sensitivity (Boden et al., 1996) and glucose tolerance (Boden et al., 1996b; Van Cauter et al., 1991). Misaligning these functions impairs glucose metabolism (Kalsbeek et al., 2014; Scheer et al., 2009) and epidemiological studies confirm long-term circadian misalignment, as associated with night work, is associated with the development of insulin resistance (Demir et al., 2016), metabolic syndrome (Karlsson et al., 2001), type 2 diabetes (Cappuccio et al., 2010) and obesity (Cappuccio et al., 2008; Di Lorenzo et al., 2003; Suwazono et al., 2008).

Workers tend to redistribute food intake to when they are on shift. Many night workers, for example, eat meals and snacks throughout the night (Lennernäs et al., 1995). At night gastric emptying is slower (Goo et al., 1987), and insulin resistance is increased (Morgan et al., 1999). These physiological changes cause a reduction in glucose tolerance to meals eaten during the night (Al-Naimi, Hampton et al., 2004; Morgan et al., 2012) and increase plasma triacylglycerol concentrations (Hampton et al., 1996; Morgan et al., 1998). Meals consumed later in the day predict higher body mass index (BMI) (Baron et al., 2011) and have been shown to reduce the effectiveness of weight loss programmes

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(Garaulet et al., 2013). Thus, inappropriately timed meals may increase the risk of developing chronic obesity-related disease outcomes.

Restricting food intake at night limits the impact of circadian misalignment on glucose metabolism in rodents, even when fed high fat diet (Arble et al., 2009). Studies in rodents have shown that many of the metabolic deficits associated with circadian misalignment are reversed if food intake is withheld when they would normally be asleep (Barclay et al., 2012; Salgado-Delgado et al., 2010).

Therefore, the aim of this study was to investigate the impact of eating a meal at night versus not eating a meal at night on glucose metabolism following four nights of circadian misalignment in healthy, young men. We hypothesised that redistributing food intake to the daytime hours would limit the metabolic consequences of shift work. To test the hypothesis, we assessed glucose and insulin response to a breakfast meal under both conditions, following simulated night work.

Materials and methods

This study was approved by the University of South Australia Human Research Ethics Committee (0000033621) and was conducted in accordance with the Declaration of Helsinki. This study is registered with the Australian New Zealand Clinical Trials Registry (ACTRN12615001107516). Participants were provided with all relevant information regarding study procedures, and provided written informed consent prior to entering the laboratory.

Participants

Healthy young males, aged 18–45 years, were enrolled via flyers and social media. Interested participants underwent a detailed screening process to determine suitability. Participants were of good physical (confirmed by a general health questionnaire and fasting blood screen) and mental health (assessed by clinical history and Beck Depression Inventory) (Beck, Steer et al.). Participants were excluded if they reported a known sleep disorder (general health questionnaire) or sleep disturbances (>6 on the Pittsburgh Sleep Quality Index) (Buysse et al., 1989)), food allergy/intolerance, were restrained eaters, BMI was greater than 30 kg/m^2 or used prescription or over-the-counter medications known to affect glucose metabolism or sleep. Participants did not engage in night shift work, trans-meridian travel, smoking or illicit drug use, excessive alcohol (>2 standard drinks/day) or caffeine consumption (>2 cups/day) in the 2 months prior to the study.

During the week prior to the study, participants reported regular sleep–wake schedules (average total sleep time 8.9 ± 1.2 h), verified using sleep diaries and wrist worn activity monitors (Actiwatch 2, Philips Respironics, Bend, OR). Participants were instructed to abstain from caffeine and alcohol intake in the week prior to the study, with compliance verified using a 3-day food diary completed on non-consecutive days.

Design

Procedure

This study was completed in the sleep laboratory at the Centre for Sleep Research at the University of South Australia, Adelaide, Australia, from January to July 2015 using a controlled, parallel study design. Since the two conditions could not be run simultaneously, allocation was done at the group level (participants were run 2–4 at a time), rather than the participant level. Participants were not aware of the separate meal times; however, it was not possible to blind the research staff due to logistical reasons.

Groups were assigned to either the eating at night (control) or not eating at night condition (intervention). Participants entered the laboratory at 12:00 h on adaption day and were familiarised with the laboratory environment. All participants were then given one sleep opportunity of 8 h time in bed (TIB) (PRE; 22:00–06:00 h) before transitioning to the night-work protocol. During the 4 days of simulated night work, participants were awake from 16:00 to 10:00 h with a daytime sleep of 6 h TIB (SW1–SW4; 10:00–16:00 h). On return to the daytime schedule (RTDS), participants had a nocturnal sleep of 8 h TIB (22:00–06:00 h). Light intensity was maintained at <50 lux during wake periods and <0.03 lux (complete darkness) during sleep periods. A detailed study protocol is shown in Figure 1.

Participants remained in the sleep laboratory for the duration of the protocol. Participants

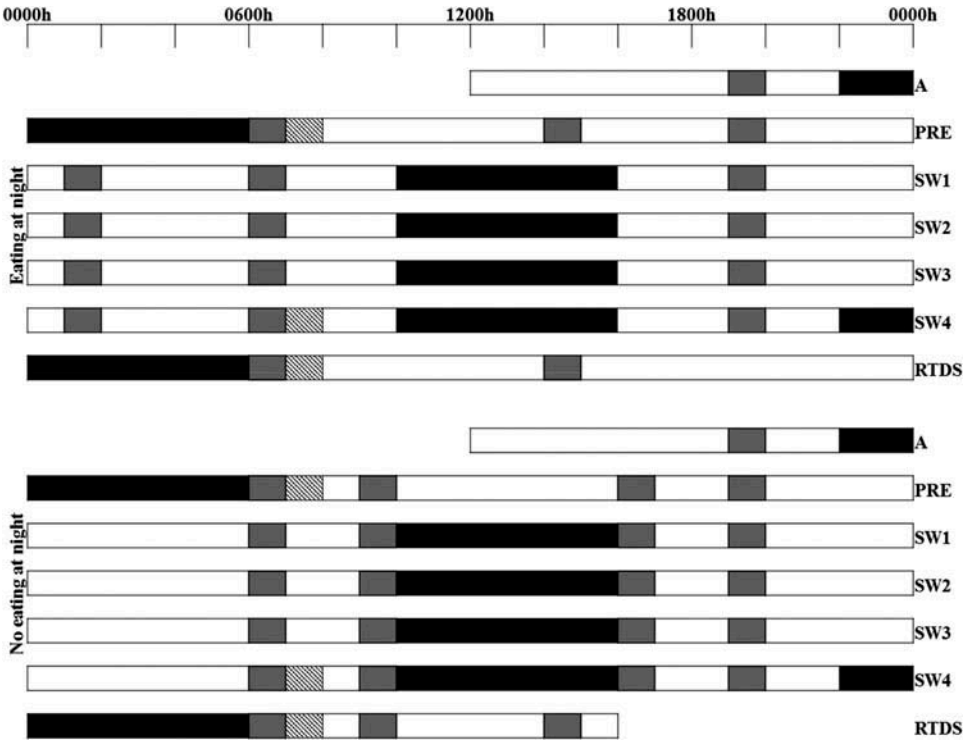

Figure 1. Protocol schematic. Black bars, scheduled sleep opportunities (<3 lux); white bars, periods of wake (<50 lux); patterned box, blood testing in response to breakfast meal; grey box, meal times. A, Adaption day; PRE, pre night work; SW1–4, night shift work days; RTDS, return to daytime schedule.

completed a number of neurobehavioural test batteries (questionnaires and cognitive performance) and a driving simulation task, reported elsewhere (Grant et al., 2016; Gupta et al., 2016). In the sleep laboratory all participants were allocated an individual bedroom, in which all tests were conducted. Study personnel monitored participants throughout the protocol to ensure compliance. When not completing scheduled tasks, participants were free to read, watch DVDs, play board games, talk or listen to music. Participants were advised that vigorous exercise was prohibited.

The laboratory was kept at an ambient temperature 23°C ± 1°C. Participants were not permitted access to clocks or social time cues (i.e. mobile phones, Internet and live television).

Food intake

Food intake was strictly controlled throughout the study and participants were not allowed to bring any food or beverages into the laboratory.

Participants in the eating at night condition consumed a meal at 19:00 and 01:30 h each day. These meals were ≈30% and ≈40% of the daily energy requirement, respectively. In the not eating at night condition, the 01:30 h meal was redistributed to the daytime hours in the form of two "snacks" provided at 09:30 h (≈20% daily energy) and 14:10 h (≈10% daily energy). In the not eating at night condition, a meal was also provided at 19:00 h (≈40% daily energy). The energy content of the meal was based on individual daily dietary energy requirements (kilojoules), calculated using the validated Harris Benedict equation (Roza & Shizgal, 1984) with a light/sedentary activity level (laboratory condition). Once calculated the individual energy requirement was further reduced by 15% to allow for extreme sedentary laboratory conditions. The energy content was increased by 30% on adaption day to allow for the extended time spent awake. The macronutrient content of meals was based on the average Australian diet (Australian Bureau of Statistics, 1997).

Participants were not permitted to use the bathroom during allocated meal times. Between meal

Table 1. Breakfast meal macronutrient composition.

Foods (quantity)	Energy (kJ)	Total fat (g)	Protein (g)	Carbohydrate (g)	Fibre (g)
White bread toasted (35 g)	305.0	0.6	3.1	13.1	1.0
Margarine (5 g)	140.2	3.8	0.0	0.0	0.0
Strawberry jam (5 g)	74.1	0.0	0.0	4.5	0.1
Reduced fat milk (200 mL)	428.0	2.5	7.7	12.1	0.0
Orange juice (200 mL)	340.2	2.0	1.2	18.0	0.4
Cornflakes (57 g)	884.9	0.3	4.9	45.7	1.8
Total	2171.9	9.2	16.9	93.5	3.2

g, grams; mL, millilitres; kJ, kilojoules.

times participants were allowed water *ad libitum*, access to other foods or beverages was restricted.

Meal tolerance test

A meal tolerance test was conducted to simulate a naturalistic metabolic challenge. All participants consumed a high carbohydrate "breakfast", which included foods with a high glycaemic index (GI) (cornflakes GI ≈ 77 and white bread GI ≈ 73) between 06:30 and 07:00 h that was identical for each participant, each day. The high GI foods were included to challenge the participants' ability to handle a rapid increase in blood glucose. The content and macronutrient composition of this meal are shown in Table 1. Meal tolerance tests were conducted pre- and post breakfast on PRE, SW4 and RTDS. Blood samples were collected at −15 and 0 min prior to and 15, 30, 60, 90 and 120 min post breakfast on each of these days. Blood was collected via an in-dwelling cannula in the median cubital vein into EDTA (insulin) and sodium fluoride (glucose) tubes.

Blood analysis

Blood samples were centrifuged at 4000 rpm for 10 min at 4°C and plasma stored at −80°C for later analyses. Plasma glucose concentrations were analysed using a commercial assay kit with a Konelab 20XT clinical chemistry analyser (Thermo Fisher Scientific, Waltham, MA). Plasma insulin concentrations were determined by ELISA (Mercodia, Uppsala, Sweden). The intra- and inter-assay coefficients of variation were <4.0%.

Sleep data

Sleep EEG was recorded on PRE: 22:00–06:00 h, SW2: 10:00–16:00 h and RTDS: 22:00–06:00 h, using Compumedics GRAEL recorders (Melbourne, Australia). EEG data were collected from sites F3, F4, C3, C4, O1 and O2 and were referenced to a contralateral mastoid (M1, M2). Participants were monitored via an infrared camera overnight by an experienced human sleep technician. Studies were scored according to Rechtschaffen and Kales sleep staging criteria (Rechtschaffen & Kales, 1968). Sleep variables analysed included total sleep time (TST), wake after sleep onset (WASO), sleep efficiency (SE), sleep onset latency (SOL) and time in minutes of rapid eye movement (REM), Stage 1, Stage 2, Stage 3 and Stage 4.

Statistical analyses

Analyses were completed using SPSS 21.0 software (IBM Corp, Armonk, NY). Independent sample *t*-tests were used to examine differences between conditions for mean age, BMI and habitual sleep prior to entry into the sleep laboratory.

Area under the curve (AUC) was calculated (0–120 min post breakfast) using the trapezoidal estimation method (Le Floch et al., 1990) to indicate overall glucose and insulin response to the breakfast meal. If missing values occurred, the values from the sample directly before and after the missing data point were averaged. Mixed-effects ANOVAs were then used to analyse glucose and insulin AUC, peak and 120 min post breakfast with mixed effects of condition (eating at night, not eating at night) and day (PRE, SW4, RTDS), and their interaction (condition × day), with a random effect of the subject identifier on the intercept. *Post hoc* pairwise comparisons were conducted when significant interaction effects were found. A study by Scheer et al. (2009) found a mean difference of 1.78 mmol/L (SD ~ 2.04 mmol/L) in average 2-h postprandial glucose attributable to circadian misalignment. Based on this large effect size estimate, we

required a total study sample of $N = 10$ participants for our primary outcome measure, glucose, to be sufficiently powered (for the condition (2 levels) × day (3 levels) interaction effect, $\alpha = 0.05$, $1 - \beta = 0.80$; $r_{repeated\_measurements} = 0.5$).

Fasting glucose and insulin were calculated by averaging values obtained at −15 and 0 min. Mixed-effects ANOVAs were used to analyse the fasting variables (glucose and insulin) with mixed effects of condition (eating at night, not eating at night) and day (PRE and RTDS), and their interaction (condition × day), with a random effect of the subject identifier on the intercept.

Mixed-effects ANOVAs were conducted for the sleep data with fixed effects of condition (eating at night, not eating at night) and day (PRE, SW4, RTDS), and their interaction (condition × day), and a random effect of the subject identifier on the intercept (to appropriately model different starting values for each participant).

A within-subjects focus was used throughout analyses. The primary effect of interest was the condition × day interaction (examining whether within-subject changes were different between the two groups) and these effects were further examined using within-subjects contrasts. Denominator degrees of freedom were corrected with the Satterthwaite approximation and are reported to the nearest whole number. In all analyses, results were considered statistically significant if $p \leq 0.05$ (two tailed).

Results

Participants

Fifteen healthy male participants were enrolled in the study. Two participants withdrew prior to entering the laboratory (Figure 2). After commencement of the study one participant

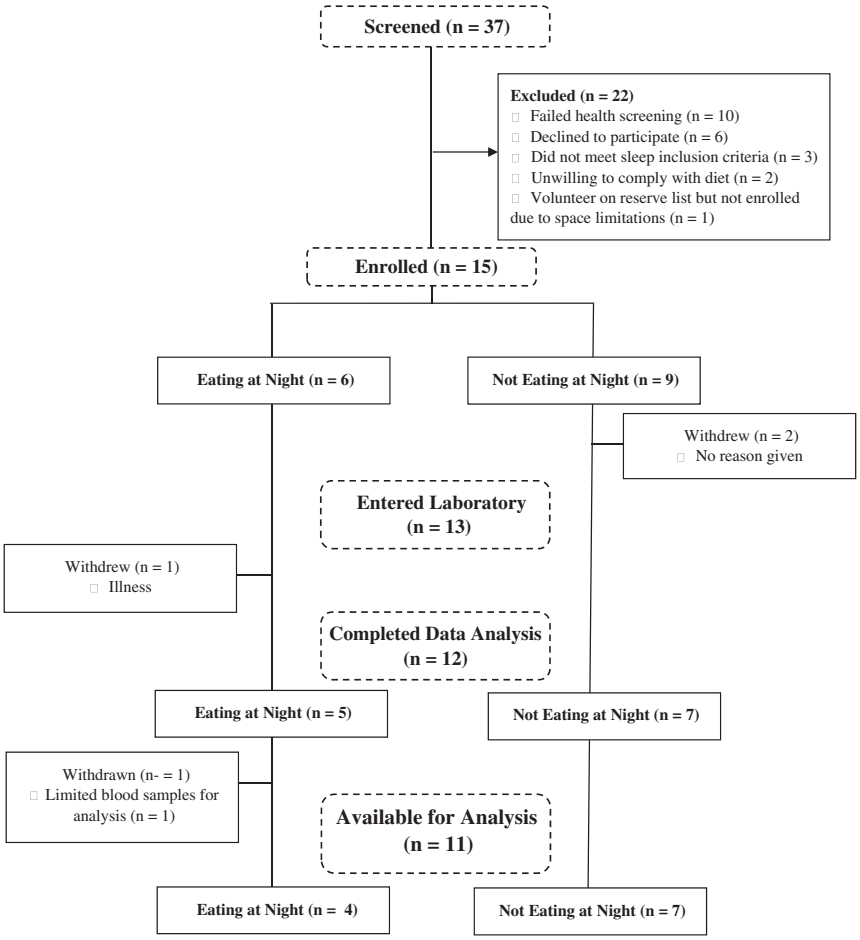

Figure 2. Consort diagram of participants who were screened, allocated a condition, completed the laboratory protocol and data analysed. Fifteen healthy male participants were enrolled and 11 completed the study. Two participants withdrew prior to entering the laboratory. After commencement of the study, one participant withdrew due to illness. One person was excluded from analysis as he was unable to provide adequate blood samples.

withdrew due to illness, while data from a further participant were excluded from analysis as we were unable to obtain adequate blood samples. A total of 11 participants completed the study, with 4 in the eating at night condition (age 24.5 ± 5.4 years; BMI 22.1 ± 1.4 kg/m^2) and 7 in the not eating at night condition (age 25.6 ± 5.4 years; BMI 22.4 ± 1.9 kg/m^2). There was no significant difference in age (p = 0.770), BMI (p = 0.755) or total sleep time (p = 0.533) between conditions prior to the commencement of the study. The following ethnic backgrounds were included in this study: Asian (45%), Hispanic (18%), white (27%) and aboriginal (9%).

Plasma glucose

There were significant effects of day (AUC; $p < 0.001$, peak; p = 0.044) and condition × day (AUC; $p < 0.001$ peak; p = 0.020, 120 min; p = 0.013) (Table 2), such that glucose increased from PRE to SW4 (p = 0.05) and PRE to RTDS ($p < 0.001$) in participants eating at night (Figure 3). By contrast, participants not eating at night did not show a change in glucose between PRE and SW4 (p = 0.127) or from PRE to RTDS (p = 0.787).

In those eating at night, the average glucose AUC response to breakfast was 27% higher on SW4, and 69% higher on RTDS compared to PRE. In participants not eating at night, there was a 12% increase in the glucose AUC response to breakfast on SW4, and a 2% increase on RTDS, relative to PRE.

Plasma insulin

There was an effect of day for insulin AUC (p = 0.007), peak (p = 0.011) and 120 min response to breakfast (p = 0.003), but not for condition × day interaction (AUC; p = 0.825, peak; p = 0.531, 120 min; p = 0.134), such that there was no significant change in insulin AUC from PRE to SW4 (p = 0.550, p = 0.091), but insulin AUC did increase from PRE to RTDS (p = 0.040, p = 0.006) in both eating at night and not eating at night conditions, respectively (Figure 3).

In the eating at night condition, insulin AUC in response to breakfast increased by 11% following SW4 and 35% following RTDS, relative to PRE. In participants not eating at night, insulin AUC increased by 18% following SW4 and 16% following RTDS, relative to PRE.

Table 2. Metabolic response to breakfast meal.

			Eating at night	Not eating at night	Condition		Day		Condition × day	
			Mean ± SD	Mean ± SD	F_{df}	p_{value}	F_{df}	p_{value}	F_{df}	p_{value}
Glucose (mmol/L)	AUC	PRE	596.2 ± 134.7	801.4 ± 178.7	$0.42_{(1,9)}$	0.533	$10.40_{(2,18)}$	<0.001	$10.29_{(2,18)}$	<0.001
		SW4	734.1 ± 210.6	883.6 ± 160.5						
		RTDS	969.0 ± 173.5	815.5 ± 215.7						
	Peak	PRE	7.2 ± 1.1	8.7 ± 1.9	$0.00_{(1,9)}$	0.989	$3.73_{(2,18)}$	0.044	$4.92_{(2,18)}$	0.020
		SW4	8.7 ± 2.8	8.8 ± 2.4						
		RTDS	10.1 ± 2.3	8.5 ± 2.5						
	120 min	PRE	3.8 ± 0.9	5.6 ± 1.9	$2.88_{(1,9)}$	0.124	$3.18_{(2,18)}$	0.066	$5.62_{(2,18)}$	0.013
		SW4	4.4 ± 1.0	6.5 ± 0.8						
		RTDS	7.1 ± 2.5	5.6 ± 1.6						
Insulin (pmol/L)	AUC	PRE	49.5 ± 10.4	54.7 ± 11.0	$0.42_{(1,9)}$	0.531	$6.73_{(2,18)}$	0.007	$0.19_{(2,18)}$	0.825
		SW4	54.4 ± 23.6	65.6 ± 22.0						
		RTDS	67.4 ± 28.9	73.9 ± 24.8						
	Peak	PRE	635.9 ± 153.8	651.0 ± 119.7	$0.42_{(1,9)}$	0.535	$5.85_{(2,18)}$	0.011	$0.66_{(2,18)}$	0.531
		SW4	710.8 ± 348.3	807.8 ± 261.0						
		RTDS	884.6 ± 339.6	844.8 ± 273.4						
	120 min	PRE	324.5 ± 150.8	361.9 ± 87.6	$0.86_{(1,9)}$	0.379	$8.15_{(2,18)}$	0.003	$2.25_{(2,18)}$	0.134
		SW4	304.9 ± 109.2	466.6 ± 182.5						
		RTDS	699.3 ± 340.0	617.2 ± 328.0						

SD, standard deviation; AUC, Area under the curve; min, minute. AUC was calculated (0–120 min post breakfast) using the trapezoidal estimation method. Results shown are from linear mixed model analyses with main effects of meal timing; eating at night/not eating at night and day; pre night work (PRE), following four consecutive days of night shift (SW4), and following a return to day shift (RTDS) and their interactions (condition × day). Denominator df corrected with Satterthwaite approximation and reported to the nearest whole number (F value with degrees of freedom ($_{df}$) displayed as subscript), and significance (p_{value}) values were presented.

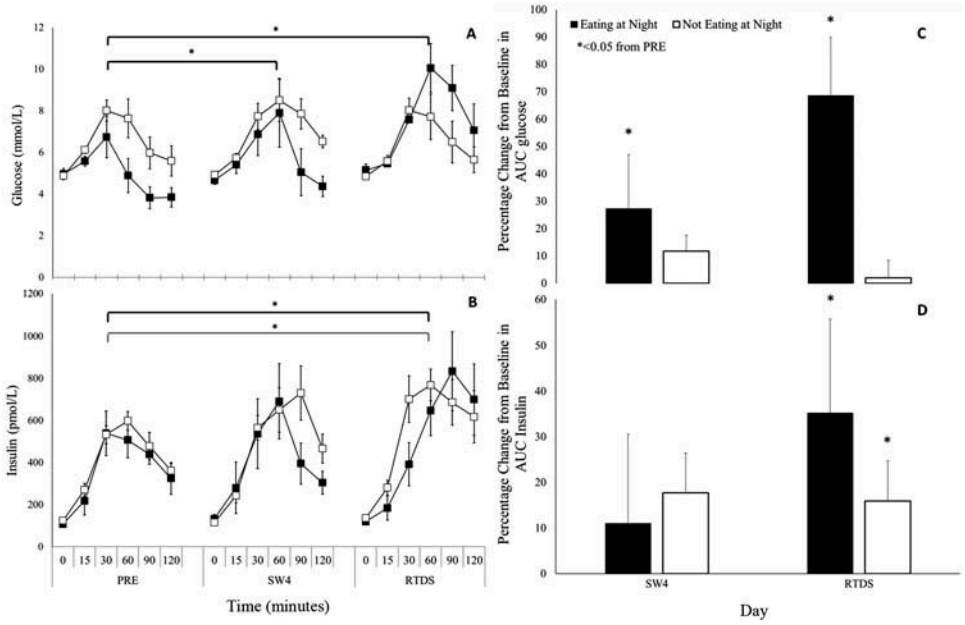

Figure 3. Metabolic outcomes following a breakfast meal. Graphs A and B show mean response to breakfast at −15 and 0 min pre-breakfast (averaged for baseline) and 15, 30, 60, 90, and 120 min post-breakfast: A – glucose and B – insulin. Eating at night condition, solid square marker; not eating at night condition, open circle marker. Bars represent standard error. Mean metabolic response to breakfast was collected at baseline, following the fourth night of shift work, and following a return to regular day work schedule. Graphs C and D show percentage change from baseline AUC in response to the breakfast: C – glucose and D – insulin. Eating at night condition: black solid fill; not eating at night condition: white solid fill. Bars represent standard error. *$p < 0.05$, significance from pre night work (PRE).

Table 3. Fasting baseline versus return to day shift (single time point 0).

		Eating at night	Not eating at night	Condition		Day		Condition × day	
		Mean ± SD	Mean ± SD	F_{df}	p_{value}	F_{df}	p_{value}	F_{df}	p_{value}
Glucose (mmol/L)	PRE	5.0 ± 0.5	4.9 ± 0.4	$0.45_{(1,9)}$	0.522	$0.41_{(1,9)}$	0.539	$1.68_{(1,9)}$	0.228
	RTDS	5.1 ± 0.6	4.9 ± 0.3						
Insulin (pmol/L)	PRE	107.9 ± 7.4	124.0 ± 31.0	$0.85_{(1,9)}$	0.380	$4.79_{(1,9)}$	0.056	$0.00_{(1,9)}$	0.958
	RTDS	119.7 ± 29.2	136.3 ± 34.9						

SD, standard deviation. Results shown are from linear mixed model analyses with main effects of meal timing; eating at night/not eating at night and day; pre night work (PRE), and following a return to day shift (RTDS) and their interactions (condition × day). Denominator df corrected with Satterthwaite approximation and reported to the nearest whole number (F value with degrees of freedom ($_{df}$) displayed as subscript), and significance (p_{value}) values were presented.

Fasting plasma glucose and insulin

There were no significant effects of condition ($p = 0.522$, $p = 0.380$), day ($p = 0.539$, $p = 0.056$) or condition × day ($p = 0.228$, $p = 0.958$) for fasting glucose and insulin, respectively (Table 3).

Sleep data

There was no significant effect of condition for any of the sleep variables (Table 4). There was a main effect of day for TST, SOL, Stage 1, Stage 2 and Stage 4 ($p < .05$), such that participants had significantly less sleep during the day than at night. There was no significant condition × day interaction effect shown for any of the sleep variables.

Discussion

Our results from a small, healthy, all male sample studied in a controlled laboratory environment suggested that eating a meal during simulated night work impaired glucose metabolism in response to breakfast with ~30% increase in

Table 4. Sleep length and architecture.

		Eating at night	No eating	Condition		Day		Condition × day	
Variable	Day	Mean ± SD	Mean ± SD	$F_{(df)}$	p_{value}	$F_{(df)}$	p_{value}	$F_{(df)}$	p_{value}
TST (min)	PRE	424.6 ± 18.5	389.7 ± 87.3	$0.15_{(1,11)}$	0.707	$6.31_{(2,21)}$	0.007	$0.73_{(2,21)}$	0.494
	SW4	324.1 ± 19.8	325.6 ± 33.5						
	RTDS	419.0 ± 24.5	353.7 ± 121.6						
WASO (min)	PRE	36.4 ± 13.6	68.8 ± 92.0	$0.17_{(1,11)}$	0.687	$1.23_{(2,21)}$	0.312	$0.48_{(2,21)}$	0.625
	SW4	29.1 ± 17.7	29.6 ± 34.3						
	RTDS	40.6 ± 15.8	96.8 ± 131.0						
SE (%)	PRE	88.9 ± 3.5	81.5 ± 18.3	$0.15_{(1,11)}$	0.707	$1.70_{(2,21)}$	0.202	$0.67_{(2,21)}$	0.524
	SW4	91.2 ± 5.4	90.6 ± 9.3						
	RTDS	87.4 ± 5.1	73.7 ± 25.3						
SOL (min)	PRE	16.9 ± 3.6	20.0 ± 13.7	$0.01_{(1,11)}$	0.944	$5.19_{(2,21)}$	0.010	$0.36_{(2,21)}$	0.700
	SW4	2.4 ± 1.5	4.2 ± 2.5						
	RTDS	20.1 ± 14.0	29.6 ± 32.9						
REM (min)	PRE	74.5 ± 8.4	66.8 ± 29.2	$0.00_{(1,11)}$	0.951	$0.86_{(2,22)}$	0.436	$0.55_{(2,22)}$	0.583
	SW4	66.4 ± 7.3	72.8 ± 19.8						
	RTDS	83.8 ± 10.9	73.8 ± 31.4						
Stage 1 (min)	PRE	32.8 ± 9.5	44.9 ± 13.7	$0.70_{(1,11)}$	0.421	$5.40_{(2,22)}$	0.012	$1.96_{(2,22)}$	0.166
	SW4	25.5 ± 12.5	28.0 ± 9.6						
	RTDS	37.5 ± 3.8	34.0 ± 14.3						
Stage 2 (min)	PRE	233.1 ± 8.6	196.1 ± 58.3	$1.03_{(1,11)}$	0.332	$26.10_{(2,21)}$	<0.001	$0.65_{(2,21)}$	0.534
	SW4	134.1 ± 29.1	119.9 ± 25.2						
	RTDS	236.8 ± 19.0	183.7 ± 75.4						
Stage 3 (min)	PRE	28.0 ± 6.4	33.4 ± 16.6	$0.33_{(1,11)}$	0.579	$2.31_{(2,22)}$	0.123	$0.03_{(2,22)}$	0.969
	SW4	21.1 ± 9.7	20.9 ± 7.1						
	RTDS	20.0 ± 4.7	22.6 ± 19.6						
Stage 4 (min)	PRE	56.3 ± 27.0	48.4 ± 25.5	$0.10_{(1,11)}$	0.770	$24.70_{(2,22)}$	<.001	$0.30_{(2,22)}$	0.746
	SW4	77.0 ± 27.4	84.1 ± 16.3						
	RTDS	41.0 ± 5.5	39.6 ± 10.5						

Data presented (n = 11). TST, total sleep time; min, minute; WASO, wake after sleep onset; SE, sleep efficiency; SOL, sleep onset latency; REM, rapid eye movement. Results shown are from linear mixed model analyses with main effects of meal timing; eating at night/not eating at night and day; pre night work (PRE), following four consecutive days of night shift (SW4) and following a return to day shift (RTDS) and their interactions (condition × day). Denominator df corrected with Satterthwaite approximation and reported to the nearest whole number (F value with degrees of freedom ($_{df}$) displayed as subscript), and significance (p_{value}) values were presented.

glucose AUC, relative to baseline, after four nights and ~70% increase after returning to the daytime schedule. This impairment was limited when food intake was withheld during the night and redistributed to the daytime hours.

Findings of the current study extend previous carefully controlled, laboratory studies using either circadian misalignment or sleep restriction protocols, which demonstrated that eating meals during the biological night impairs immediate postprandial glucose metabolism or glucose tolerance to test meals (Hampton et al., 1996; Ribeiro et al., 1998; Scheer et al., 2009; Schmid et al., 2011). These results have also been verified in field studies of shift workers (Knutsson et al., 2002; Lund et al., 2001).

To our knowledge, this is the first study in humans to demonstrate that withholding food intake at night, during simulated night shifts, may limit impairment in glucose tolerance. While our findings are necessarily preliminary due to the small sample, results are consistent with studies in animal models. Although the concept of withholding food intake across the night shift is novel in humans, our findings are consistent with a number of studies in rodents examining the effect of withholding food intake across the biological night (Barclay et al., 2012; Sherman et al., 2012). Together these findings suggest that the inappropriate timing of meals may impact on glucose metabolism, and that withholding food during the night may prevent these deleterious metabolic effects of working night shifts.

There were no differences in measures of fasting glucose or insulin pre and post the simulated night-work protocol. This has also been found in sleep restriction and sleep deprivation studies (Donga et al., 2010; Reynolds et al., 2012; Schmid et al., 2011). Morris et al. (2015) reported no

changes in fasting glucose or insulin following 5 days of circadian misalignment with an 8-h daytime sleep (11:00–19:00 h). The impairments in metabolic regulation in the current study were only evident after a meal tolerance test challenge. The stable preprandial levels suggest the increased glucose response to a meal, despite increased insulin secretion observed in the eating at night condition could be associated with decreased insulin sensitivity (Scheer et al., 2009).

Changes in insulin metabolism were apparent in both groups after the return to day shift (following an 8-h night sleep and an 11-h fast); however, impairments in glucose tolerance were noted only in the eating at night group. This suggests the benefits of withholding food intake during the night may continue even after sleep is returned to the night-time hours. However, the increased metabolic disturbance seen after return to day shift may have been due to an "unmasking" effect caused by the 11-h fast the night before. That is, on SW4 in the eating at night condition, a second meal effect may have blunted the glucose response to breakfast (Robertson et al., 2003; Wolever et al., 1988). The "second meal" effect describes the impact of an initial meal on a second meal and is well described in a literature review examining the ability of high fibre/low glycaemic load meals to reduce the glucose AUC in a subsequent meal (Higgins, 2012). During simulated night work, there was a minimum 4.5-h gap between the night-time meal (01:30 h) and the test breakfast meal (consumed between 06:30 and 07:00 h). While it is possible that this meal may have reduced the glucose response to breakfast in the eating at night condition on SW4, impaired glucose tolerance was still observed in this group, while impairment was not seen in the group in which food was withheld. Thus, in the not eating at night condition, whilst there was insulin resistance, metabolic impairment was limited following return to day shift, suggesting glucose homeostasis was maintained when food intake was withheld across the simulated night shifts.

The results of this study should be viewed in the light of the fact that it was a laboratory-controlled study in healthy, young, lean men. Populations that are older, or with obesity, may respond differently. Furthermore, whilst the participants in this study were restricted to 6 h TIB during night work, they slept well during the day due to the ideal sleeping environment in the laboratory. Whilst at home, daytime sleep quality and quantity could be reduced due to light, sound, temperature, caffeine and alcohol consumption (Akerstedt, 2003; Novak & Auvil-Novak, 1996). The results of this study are also limited by the sample size. Relatively small samples are common in this field due to the resource-intensive nature of the research (Reynolds et al., 2012; Scheer et al., 2009; Spiegel et al., 1999). A vulnerability of small samples in experimental designs is that they are open to baseline differences between conditions resulting from spurious differences simply by chance. However, the glucose response to the test meal, seen in the current study, in both conditions at baseline is within the normal range for healthy participants (Caumo et al., 2000). Whilst we cannot completely rule out the potential impact of baseline differences, the glucose values seen at baseline are not sufficiently large so as to blunt the response to the effects of the protocol. The practicalities of the no eating at night protocol also need to be tested in a real-world shift working population, given that some studies suggest that night workers redistribute their food consumption to the night (De Assis et al., 2003; Morikawa et al., 2008), whilst some report a higher proportion of their total daily intake during the day (Lennernäs et al., 1995; Ohtsuka, 2001). Therefore, not eating at night may be challenging for some night workers. However, recent data suggest that not eating at night actually improves cognitive and driving performance (Grant et al., 2016; Gupta et al.).

In conclusion, this study is the first to examine the impact of eating versus not eating at night on metabolic outcomes. As found in rodent studies, these early results suggested that not eating at night limited the impaired metabolic response seen in the eating at night condition. Altering meal timing and restricting food intake during the night could be an intervention to reduce the burden of metabolic disease in night workers.

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