A bedtime milk snack does not impact resting metabolic rate, substrate utilisation, and appetite the following morning in mildly overweight males

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Abstract

Nighttime eating is often associated with a negative impact on weight management and cardiometabolic health. However, data from recent acute metabolic studies have implicated a benefit of ingesting a bedtime snack for weight management. The present study compared the impact of ingesting a milk snack containing either 10 (BS10) or 30 g (BS30) of protein with a non-energetic placebo (BS0) 30 min before bedtime on next morning metabolism, appetite and energy intake in mildly overweight males (age: 24.3 (SEM 0.8) years; BMI: 27.4 (SEM 1.1) kg/m^2). Next morning measurements of resting metabolic rate (RMR), appetite and energy intake were measured using indirect calorimetry, visual analogue scales and an *ad libitum* breakfast, respectively. Bedtime milk ingestion did not alter next morning RMR (BS0: 7822 (SEM 276) kJ/day, BS10: 7482 (SEM 262) kJ/day, BS30: 7851 (SEM 261) kJ/day, $P = 0.19$) or substrate utilisation as measured by respiratory exchange ratio ($P = 0.64$). Bedtime milk ingestion reduced hunger ($P = 0.01$) and increased fullness ($P = 0.04$) during the evening immediately after snack ingestion, but elicited no effect the next morning. Next morning breakfast (BS0: 2187 (SEM 365) kJ, BS10: 2070 (SEM 336) kJ, BS30: 2582 (SEM 384) kJ, $P = 0.21$) and 24 h post-trial ($P = 0.95$) energy intake was similar between conditions. To conclude, in mildly overweight adults, compared to a non-energetic placebo, a bedtime milk snack containing 10 or 30 g of protein does not confer changes in next morning whole-body metabolism and appetite that may favour weight management.

Introduction

Several observational studies reveal that eating late in the day, e.g. immediately before bedtime, is counter-productive for body weight management and cardiometabolic health [1–3]. Consistent with this notion, physiological data from acute metabolic studies exist to demonstrate that energy intake in the hours immediately leading up to bedtime results in a lower acute diet-induced thermogenesis[4] and a reduced feeling of satiation compared to energy intake in the morning or afternoon[1]. Thus, it is intuitive that over a chronic time period, a dietary pattern in which energy intake is prioritised close to bedtime may promote a positive energy balance and weight gain.

Conversely, there are emerging data from acute studies indicating that consumption of lower energy and single macronutrient snacks 30 min before bedtime may confer favourable outcomes with regards to whole-body metabolism and appetite[5]. These bedtime snack studies have focused primarily on comparing the impact of acute ingestion of the individual macronutrient constituents of milk (whey protein, casein protein, and carbohydrate) on next morning RMR, substrate utilisation, and appetite[6–10]. For instance, the consumption of 30 g of whey protein, 30 g of casein protein or 33 g of carbohydrate (equivalent to an energy intake of 586-627 kJ) 30 min before bedtime was reported to increase next morning RMR in active young men[7]. In addition, next morning fat oxidation rates were increased with bedtime casein ingestion compared to whey and carbohydrate ingestion[7]. Furthermore, a subsequent study in overweight and obese females reported an increased next morning satiety and decreased desire to eat with bedtime whey, casein, or carbohydrate ingestion compared to the omission of a bedtime snack[6]. Hence, a growing body of scientific evidence from acute metabolic studies supports the notion that a low energy snack (~586-627 kJ) prior to bedtime may be beneficial for weight management.

Casein protein is commonly perceived to be an ideal bedtime snack given its slower digestion properties that allows for a sustained elevation in plasma amino acid concentrations for the duration of sleep[5,11]. Nonetheless, based on findings from acute studies, whey protein and carbohydrate also appear to be an important component of a bedtime snack since an increase in next morning RMR has been shown to be comparable to casein protein in active young men[7]. Given that milk is a protein-dense foodstuff, consisting of 80% casein and 20% whey protein, and contains carbohydrate[12], in theory milk may be considered an ideal bedtime snack for increasing next morning RMR because of its macronutrient composition. Readily available in both fluid and powder form, milk provides a more practical and economically viable bedtime snack compared with an isolated (or hydrolysed) whey or casein protein supplement[13]. Moreover, within an acute study setting, the provision of milk as a mid-

day snack, or as part of a standardised breakfast, has been shown to be effective in decreasing perceived appetite when compared to ingestion of water and beverages comprised primarily of carbohydrate[14,15]. However, to our knowledge, only a single study to date in female athletes has examined the impact of bedtime milk ingestion, administered in chocolate milk form, compared to a non-energetic placebo and observed an increase in RMR and reduction in appetite the following morning[10]. A logical follow-up study is to investigate the impact of bedtime consumption of a mixed macronutrient food source such as milk on next morning RMR, substrate utilisation, and appetite in healthy, overweight adults.

Evidence regarding the optimal bedtime protein dose required to effectively modulate RMR and appetite the following morning also remains unknown. Interestingly, the intake of ~30 g of protein during the day has been shown to induce greater diet-induced thermogenesis, modulate appetite, and enhance satiety[16,17]. However, to date, no acute study has examined whether a protein dose less than 30 g confers a similar increase in RMR and modulatory effect on appetite the following morning.

Accordingly, the primary aim of this acute metabolic study was to compare the impact of bedtime skimmed milk ingestion to a non-energetic placebo on next morning RMR, substrate utilisation, subjective appetite ratings, and subsequent energy intake in healthy, mildly overweight young men. The secondary aim was to determine the dose-response relationship between bedtime milk ingestion and next morning RMR, substrate utilisation, appetite and energy intake. We hypothesised that ingestion of the bedtime milk beverage containing 30 g of protein will increase next morning RMR, reduce appetite, and increase fat oxidation rates to a greater extent than a milk beverage containing 10 g of protein or a non-energetic control.

Methods

Participants and ethics approval

Twelve healthy, mildly overweight males participated in the present study. *A priori*, we conducted a power calculation (GPower v3 software) of appropriate sample size based on previous published data[10] that measured, on average, a 5% higher RMR the following morning after bedtime ingestion of chocolate milk *v.* placebo using the same indirect calorimetry technique conducted in the present study. By setting statistical power (1-ß err prob) at 0.8, α error probability at 0.05 and effect size (Cohen's *d*) at 1.4 (based on previous data[10]), our power calculation revealed a minimum sample size of 10 participants (using a crossover research design) would be necessary to detect a statistical difference in RMR between milk and placebo treatment conditions. Exclusion criteria included any

known diagnosis of cardiovascular disease, stroke, diabetes mellitus, and thyroid or kidney dysfunction. Participants taking medications that may affect appetite, taste and smell were excluded. Smokers and those with lactose intolerance or a dislike of dairy products also were excluded. Baseline anthropometric parameters including age, height, weight, BMI, waist and hip circumferences, and sum of five skinfolds (triceps, biceps, subscapular, iliac crest, calf) were measured prior to the start of experimental trials (Table 1). The present study was conducted according to guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the University of Stirling, Faculty of Health Sciences and Sport Research Ethics Committee. Written informed consent and health questionnaires were obtained from all participants prior to participation.

Protocol overview

Each experimental trial was conducted over two days (see Fig. 1 for protocol overview). On day one, participants consumed a standardised evening meal at 19.30 h and then a bedtime snack at 22.30 h on the night before the morning laboratory visit. Leading up to the standardised evening meal, participants were instructed to continue with their habitual diet during the day in terms of meal timing and content. Subjective appetite and thirst were assessed before and after the bedtime snack and before the standardised bedtime at 23.00 h. Overnight, participants wore actigraphy devices on their wrists for the assessment of sleep quality.

The next morning, participants woke up at 06.30 h and immediately completed a questionnaire to assess sleep quality prior to attending the laboratory. Sleep quality (including sleep duration) was also assessed objectively using actigraphy (see *measurements of sleep quality*). Participants arrived at the laboratory fasted at 08.00 h, having abstained from moderate-to-high intensity exercise, alcohol intake, and caffeine consumption for 24 h, and rested supine on a bed for 10 min. Subjective appetite and thirst was assessed at the end of the 10 min rest period. Metabolic measurements were then completed using indirect calorimetry for 30 min. Subsequently, subjective appetite and thirst was assessed again followed by collection of the first blood sample and the *ad libitum* breakfast. Subjective appetite and thirst also were assessed before and after breakfast and again 30 min after breakfast. Blood samples were collected immediately after the 15 min breakfast period and 30 min after the end of breakfast.

Bedtime beverage treatments

The study was randomised and cross-over in design. Treatments were double-blind except for the non-energetic placebo (BS0), which was water. A third party, not involved in other aspects of the

study, prepared the beverages in advance and randomised the treatments in a counterbalanced order, with at least 4 days separating trials. Treatments were given to participants as pre-weighed Tesco© Instant Dried Skimmed Milk powder in opaque plastic beverage bottles instead of fluid milk to ensure treatments were isovolumetric. Participants were instructed to add 400 ml of water to dissolve the skimmed milk powder thoroughly by shaking the bottle prior to ingestion at home. Macronutrient breakdown and energy content of treatments are described in Table 2. The treatment condition containing 10 g of protein (BS10) was chosen to mimic the approximate amount of protein in a typical glass of milk. The treatment with the highest amount of protein (BS30) was chosen to meet the 30 g protein threshold postulated to be required to suppress appetite[17] and to match the protein dose administered in previous bedtime snack studies[6–9]. Participants were given an empty bottle for BS0 and filled it with 400 ml of tap water to be consumed at the time of the bedtime beverage.

Diet control

Participants completed a weighed food diary for three separate evening meals prior to beginning the study. Energy and macronutrient intakes were calculated using dietary analysis software (Nutritics Academic Edition v4.267, Nutritics). The average energy intake of the three evening meals was used to determine the total energy content of the standardised evening meal. The standardised evening meal was designed to provide the same macronutrient breakdown of diets of UK adults according to the National Diet and Nutrition Survey 2008/09 – 2011/12 (carbohydrate: 50%; fat: 32%, protein 18%)[18]. The standardised evening meal consisted of Tesco© Fusilli Pasta Twists, Tesco© Bolognese Pasta Sauce, Tesco© Beef Lean Steak Mince 5% Fat, and olive oil. The ingredients were supplied to the participants and instructions were provided to prepare the meal at home. Compliance was verified verbally and by return of empty food containers.

Participants also kept a 2 d food and activity diary 48 h prior to the first experimental trial and were asked to replicate the same food intake and activity in the 48 h prior to the subsequent trials. No other food or drink was permitted after consumption of the bedtime beverage the night before the morning trials. Participants were asked to consume 300 ml of water in the morning prior to visiting the laboratory.

Metabolic measurements

Oxygen consumption and carbon dioxide production was measured via indirect calorimetry (Oxycon Pro, Cardinal Health) using a ventilated metabolic hood placed over the participant's head. Prior to starting the measurements, a calibration program within the software application accompanying the metabolic cart (LabManager, V5 30.0) was used to determine ambient conditions

(temperature, relative humidity, and barometer pressure). Volume calibration was completed manually using a 3 litre calibration pump and gas analyzer calibration was completed using verified gases of known concentrations (16% O_2 and 5% CO_2). Measurements were completed with participants resting supine on a bed in a quiet and temperature-controlled room (20-24 °C). Gas exchange was measured continuously for 30 min and data were captured every 30 s. The software application determined the RER and calculated the RMR using the formula derived by Weir[19]. Only the final 20 min of the data collection period was used for analysis to ensure participants were at a physiological steady state.

Subjective assessment of hunger, fullness, desire to eat, and thirst

Hunger, fullness, desire to eat, and thirst were assessed subjectively using a validated visual analogue scale (VAS)[20]. The questions accompanying the VAS were "How hungry do you feel?", "How full do you feel?", "How much do you think you could eat now?", and "How thirsty are you?". The horizontal lines were anchored by the statements "Not at all hungry/full/thirsty" and "As hungry/full/thirsty as I have ever felt" at each end. For desire to eat, the statements "Nothing at all" and "A large amount" were used at each end of the horizontal line. Participants placed a vertical mark on a 100 mm horizontal scale to rate how they felt regarding each sensation. Participants were instructed not to refer to previous scales when completing each new set of scales.

Ad libitum breakfast and 24 h post-trial energy intake

Participants were given 15 min to consume an *ad libitum* breakfast at a dining table in an isolated area of the research kitchen to minimize external distractions. Participants were provided a packet of Kellogg's Corn Flakes®, a 500 ml jar of semi-skimmed milk, and instructed to eat until they were comfortably full. If participants finished eating before the allotted 15 min, they remained seated at the table. The packet of Kellogg's Corn Flakes® (1582 kJ per 100 g) was weighed before and after the *ad libitum* breakfast to determine the amount the participant consumed. The volume of semi-skimmed milk (Tesco© British Semi Skimmed milk, 50 kcal per 100 ml) remaining in the jar was measured in a graduated cylinder to determine volume consumed. All participants answered 'yes' to whether they would like corn flakes and milk for breakfast in the pre-study questionnaire. Participants were not informed that the energy intake of the cereal was being measured.

At the end of each trial, participants were instructed to keep a detailed food record of all food and beverages consumed in the 24 h post-trial period. The food records were analyzed using dietary analysis software. Ten participants were included in the analysis of energy intake in the 24 h post-trial period as two participants were unable to provide complete food records.

Measurements of sleep quality

Given that sleep restriction has been associated with reduced next morning RMR[21], objective and subjective measurements of sleep were assessed to investigate the acute effect of bedtime milk ingestion on sleep. The MotionWatch 8© (CamNtech Ltd.) tri-axial wrist-worn actigraphy device was used to obtain three objective measurements of sleep quality – actual sleep time, sleep latency, and fragmentation index. Actual sleep time was defined as total minutes categorized as sleep by the actigraphy device and the accompanying software (MotionWare, 1.125, CamNtech Ltd.). Sleep latency was defined as the time between 'lights out' and 'fell asleep' time points. Fragmentation index, expressed as the sum of total mobile time and immobile bouts not exceeding 1 min in duration, is a measure of disruption to sleep periods used as a marker of sleep quality, with a higher value indicating lower quality sleep.

Participants completed the Leeds Sleep Evaluation Questionnaire (LSEQ) immediately upon waking on the morning of the experimental trials for subjective measurements of sleep quality. The LSEQ was validated in individuals aged 18-49 years and consists of ten VAS questions that evaluate four domains of sleep: the ease of getting to sleep, the perceived quality of sleep, the ease of awakening from sleep, and behaviour following wakefulness[22]. Participants were asked to place a mark on the 100 mm line based on how they felt between two extremes, e.g. "less sleepy than usual" and "more sleepy than usual". The scores were averaged to give a score for each domain.

Blood sampling and analyses

A cannula (Becton, Dickinson & Company) was inserted into a forearm vein for blood sampling. At each timepoint, 10 ml of blood was dispensed evenly between lithium heparin or clot activator vacutainer tubes. Within 120 min, lithium heparin vacutainers were centrifuged at 3500 rpm at 4°C and plasma aliquots were dispensed into Eppendorf tubes. Clot activator vacutainers were allowed to clot for 60 min at room temperature before centrifugation and dispensing serum aliquots into Eppendorf tubes. Plasma and serum samples were stored at -80°C for future analysis of glucose and insulin concentrations, respectively. Serum glucose concentrations was analyzed with use of an automated analyzer (ILab Aries, Instrumentation Laboratory) and plasma insulin concentrations was analyzed with use of a commercially available ELISA kit (Demeditec Diagnostics GmbH) according to manufacturer's instructions. The HOMA2 Calculator V2.2.3[23] was used to determine the homeostatic model assessment of insulin resistance (HOMA-IR) value. The averages of duplicate samples were for data analysis used. The intra-assay CV and inter-assay CV for insulin concentrations were 8.5% and 10.8%, respectively. Two participants were unable to provide blood samples for all 3 trials; therefore 10 participants were included in the final analysis of blood samples.

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194 *Data presentation and statistical analysis*

195 Statistical analyses were conducted using IBM® SPSS® Statistics software package version 23
196 (IBM Corporation). AUC was calculated using the trapezoidal method with the baseline set as the value
197 measured immediately after bedtime snack ingestion for the evening period and at 0 min for the next
198 morning period (see Fig. 1). One-way repeated measures ANOVA was conducted to examine
199 differences in RMR, RER, estimated carbohydrate oxidation and fat oxidation rates, energy intake at *ad*
200 *libitum* breakfast, 24 h post-trial energy intake, HOMA-IR, the AUC of subjective appetite and thirst
201 assessments, actual sleep time, sleep latency, fragmentation index, and the 4 domains of sleep in the
202 LSEQ. Two-way repeated measures ANOVA was conducted to test for treatment, time, and treatment-
203 by-time interaction effects on subjective assessment of hunger, fullness, desire to eat, and thirst and
204 also glucose and insulin concentrations. Where a significant treatment and/or interaction effect was
205 detected, Bonferroni *post hoc* test was used to determine specific differences for both one-way and
206 two-way repeated measures ANOVA. Statistical significance was determined at an alpha level of $P <$
207 0.05, and data were reported as mean with standard errors unless specified otherwise.

208

209 **Results**

210 *Pre-trial dietary intake*

211 Analysis of the pre-trial 2 d food diary revealed a daily mean energy intake of 26.3 (SEM 3.4)
212 kJ/kg/d and a macronutrient breakdown of 45.5 (SEM 2.5)% carbohydrate, 19.2 (SEM 1.2)% protein,
213 and 35.3 (SEM 1.7)% fat.

214 *Metabolic measurements*

215 There was no significant effect of bedtime snack treatment on next morning RMR ($P = 0.19$)
216 (Fig. 2a) or RER ($P = 0.64$) (Fig. 2b). Likewise, there was no significant effect of bedtime snack
217 treatment on estimated carbohydrate ($P = 0.51$) or fat ($P = 0.17$) oxidation rates (Fig. 2c).

218 *Subjective assessment of hunger, fullness, desire to eat, and thirst*

219 Subjective assessments of hunger, fullness, and desire to eat are represented in Fig. 3. A
220 significant main effect of bedtime snack treatment was observed on subjective measurements of hunger
221 ($P = 0.01$) and fullness ($P = 0.04$) during the evening period after bedtime milk ingestion. Hunger
222 ratings for BS30 were significantly lower than BS0 during the evening at 5 ($P = 0.04$) and 30 min ($P =$
223 0.001) after bedtime milk ingestion, but was only significantly lower at 30 min for BS10 *v.* BS0 ($P =$
224 0.01) (Fig. 3a). Evening fullness ratings for BS30 were significantly higher than BS0 at 30 min ($P =$

0.007) after bedtime milk ingestion, while BS10 fullness ratings were higher than BS0 at 5 min (P = 0.02) (Fig. 3b). There were no differences between BS30 and BS10 in subjective hunger or fullness during the evening after bedtime milk ingestion ($P > 0.05$).

There was a trend for a significant effect of bedtime snack on the next morning rating of fullness ($P = 0.07$), but not next morning hunger ($P = 0.60$). No significant effect of bedtime snack was observed on desire to eat or thirst both during the evening after ingestion (desire to eat: $P = 0.21$; thirst: $P = 0.71$) or the following morning (desire to eat: $P = 0.42$; thirst: $P = 0.91$).

Subjective appetite and thirst measurements also were expressed as AUC calculated over periods between bedtime snack ingestion and sleep, and from 0 to 95 min on the morning of the trials (Fig. 4). There was a significant effect of bedtime snack treatment on the AUC for hunger ($P = 0.006$) and fullness ($P = 0.02$) during the evening period. The bedtime snack treatment had no effect on AUC for hunger measured the following morning ($P = 0.62$), but there was a trend for a significant effect on the AUC of fullness the following morning ($P = 0.05$). No effect of bedtime snack treatment was observed for AUC of desire to eat and thirst calculated over the evening period (desire to eat: $P = 0.21$; thirst: $P = 0.23$) or the following morning (desire to eat: $P = 0.39$; thirst: $P = 0.91$).

Ad libitum breakfast and 24 h post-trial energy intake

There was no significant effect of bedtime snack treatment on energy intake at the *ad libitum* breakfast (BS0: 2187 (SEM 356) kJ, BS10: 2070 (SEM 336) kJ, BS30: 2582 (SEM 384) kJ, $P = 0.21$). Likewise, bedtime snack did not have a significant effect on 24 h post-trial energy intake when expressed per kg body weight (BS0: 105 (SEM 16) kJ/kg, BS10: 108 (SEM 11) kJ/kg, BS30: 108 (SEM 16) kJ/kg, $P = 0.95$).

Blood glucose and insulin concentrations

There was no significant bedtime snack and time interaction on next morning serum glucose (P = 0.60) or plasma insulin ($P = 0.57$) concentrations. Bedtime snack did not have a significant effect on next morning serum glucose ($P = 0.61$), plasma insulin ($P = 0.56$), or HOMA-IR ($P = 0.85$) (Table 3). A main effect of time on serum glucose and plasma insulin concentrations ($P < 0.01$) was observed.

Sleep measurements

As measured by the actigraphy devices, there was no significant effect of bedtime snack treatment on actual sleep time (BS0: 351 (SEM 9) min, BS10: 366 (SEM 12) min, BS30: 333 (SEM 20) min, $P = 0.18$). Likewise, no significant effect of bedtime snack treatment on sleep latency was observed (BS0: 20.3 (SEM 7.0) min, BS10: 23.7 (SEM 8.8) min, BS30: 30.3 (SEM 11.6) min, P = 0.76). There also was no significant effect of bedtime snack treatment on fragmentation index (BS0:

28.8 (SEM 2.4), BS10: 29.2 (SEM 4.9), and BS30: 35.9 (SEM 5.5), $P = 0.41$). Similarly, bedtime snack treatment had no significant effect on any of the 4 domains of subjective sleep in the LSEQ (data not shown): "getting to sleep" ($P = 0.95$), "quality of sleep" ($P = 0.66$), "awake following sleep" ($P = 0.77$), and "behaviour following awakening" ($P = 0.86$).

Discussion

The primary aim of the present study was to investigate the influence of bedtime skimmed milk ingestion on acute changes in whole-body metabolism and appetite the following morning in mildly overweight males. The main finding was that bedtime ingestion of a milk snack containing either 10 g or 30 g of protein did not increase next morning RMR compared to a non-energetic placebo. In addition, next morning RER, as well as carbohydrate oxidation and fat oxidation rates, were similar between milk and non-energetic placebo conditions. Whereas the bedtime milk conditions tended ($P = 0.074$) to increase subjective fullness the next morning, no differences in hunger and desire to eat were observed between milk and non-energetic placebo conditions. Accordingly, energy intake at an *ad libitum* breakfast the next morning and 24 h post-trial was similar between conditions. Hence, refuting our original hypothesis, bedtime milk ingestion failed to increase RMR and fat oxidation or reduce appetite the next morning compared to a non-energetic placebo in mildly overweight males.

In the present study, we anticipated a dose-dependent increase in next morning RMR with bedtime milk intake due, at least in part, to differences in protein and energy content of test drinks. The two primary factors known to influence diet-induced thermogenesis are protein and energy content, with protein estimated to contribute up to 30% of diet-induced thermogenesis[24]. Hence, previous bedtime snack studies have proposed an energy-induced increase in thermogenesis to be a key mechanism behind the increase in next morning RMR following bedtime snack ingestion[6,7,10]. In the present study, the BS10 condition was chosen to mimic the 7-10 g of protein contained in a typical glass of milk and was similar to the 12 g of protein in the bedtime chocolate milk intervention administered previously by Ormsbee et al. (2016)[10]. In addition to being higher in protein and energy content than BS10 and the previously described chocolate milk intervention[10], the BS30 condition in the present study was protein matched to a similar bedtime snack study that found that 30 g of whey or casein increased next morning RMR[7]. Ormsbee et al. (2016)[10] reported a higher RMR with the bedtime ingestion of 355 ml of skimmed chocolate milk (12 g protein, 30 g carbohydrate, 0 g fat, 753 kJ) compared to a non-energetic placebo in young, trained, lean females. By contrast, in the present study of mildly overweight males, next morning RMR was similar between milk and non-energetic

control conditions, irrespective of the dose of protein and energy content in the bedtime milk snack. Multiple factors may explain these discrepant findings, including differences in time elapsed between bedtime snack ingestion and metabolic measurements and differences in participant characteristics between studies. Sleep quality can be excluded because bedtime milk ingestion had no impact on sleep duration and quality in the present study.

One plausible explanation for the inconsistent findings regarding RMR between bedtime snack ingestion studies concerns time elapsed between bedtime snack ingestion and metabolic measurements the next morning. Utilising a respiratory chamber, previous studies have demonstrated that when an evening meal was consumed at 17.30 h and then an evening snack at 19.30 h, the increase in energy expenditure due to diet-induced thermogenesis returned to basal levels ~6 h after ingestion of the evening snack[24,25]. Conversely, data also exist demonstrating that diet-induced thermogenesis persists for longer than 6 h[26]. In the present study, we standardised the time between consumption of a bedtime milk snack (22.30 h) and next morning measurements of indirect calorimetry (08.10 h) at 9 h and 40 min and observed no increase in RMR with milk ingestion. Similarly, in a study of obese men, Kinsey *et al* (2016)[8] reported no increase in next morning RMR measured ~8 h after bedtime ingestion of 30 g of casein protein compared to a non-energetic placebo. In contrast, the same authors demonstrated next morning RMR to be increased by approximately 5% compared with a non-energetic placebo in lean, trained females when bedtime chocolate milk was consumed as little as 7 h before the measurement of RMR the following morning[10]. As such, in the present study, we potentially missed the impact of diet-induced thermogenesis of bedtime milk ingestion on next morning RMR because we collected metabolic measurements 3 h and 40 min beyond the proposed ~6 h cut off point[24,25]. Taken together, these data suggest the time elapsed between bedtime snack ingestion and the next morning measurement of energy expenditure impacts, at least in part, the ability to detect an increase in next morning RMR through diet-induced thermogenesis.

In theory, the discrepant findings between past[6–9] and present investigations of bedtime snack ingestion and next morning metabolism also may relate to the characteristics of recruited participants. Diet-induced thermogenesis has been reported to be greater in lean *v.* obese males[27], which implies that bedtime snack ingestion confers a greater potential to increase next morning RMR in lean compared with obese males. Accordingly, a previous study in physically-active men demonstrated an increase in RMR the following morning after bedtime ingestion of whey protein, casein protein, and carbohydrate[7]. In contrast, a study in obese men with a BMI of 36.1 kg/m^2 observed no difference in next morning RMR following bedtime ingestion of casein protein compared to a non-energetic

placebo[8]. Consistent with this finding, we observed no increase in RMR the following morning after bedtime skimmed milk ingestion in overweight men with a BMI of 27.4 kg/m^2. Interestingly, although a previous study reported no difference in diet-induced thermogenesis between lean and obese females fed during the day[28], other studies have reported a higher next morning RMR after bedtime snack ingestion in lean, trained females[10], but not in obese females[6,9] when compared to no bedtime snack ingestion at baseline. Hence, future studies should compare sex-differences in next morning RMR following bedtime snack ingestion between lean and obese individuals.

The timing of next morning metabolic measurements and blood sampling also may explain why we failed to observe any modulation of substrate utilisation with bedtime milk ingestion. Milk consists of all macronutrients, of which protein composition constitutes 80% casein and 20% whey. The bedtime ingestion of casein protein has been shown to increase fat oxidation rates the next morning compared to whey protein and carbohydrate[7]. It was speculated that the lower insulin response to ingested casein compared to whey protein and carbohydrate resulted in a reduced inhibition of fat oxidation the following morning[7]. Therefore, we anticipated that bedtime milk ingestion, which is rich in casein protein, would elicit an increase in fat oxidation the following morning. However, in the present study, morning fasting glucose and insulin concentrations in both milk conditions were similar to the non-energetic placebo condition, suggesting that, as perhaps could be expected, the glucose and insulin concentrations had returned to basal levels the next morning following bedtime milk ingestion. Accordingly, we observed no differences in substrate utilisation the following morning as estimated by RER between milk and placebo conditions. We also acknowledge that, in the present study, carbohydrate and fat oxidation rates may have been overestimated given that our calculations of substrate utilisation assumed negligible protein oxidation. Previous bedtime snack studies have made the same assumption with the bedtime provision of 30 g of protein[6–9]. Future studies are warranted that collect overnight gas exchange measurement using a respiratory chamber to determine the timecourse of change in overnight energy expenditure and substrate utilisation following bedtime snack ingestion.

Given that bedtime chocolate milk ingestion elicited a reduction in appetite the following morning compared to a non-energetic placebo in lean, trained females[10], we anticipated that bedtime skimmed milk ingestion also would promote the suppression of appetite the following morning in mildly overweight males. However, in the present study, whereas evening hunger was suppressed and fullness increased immediately after bedtime consumption of milk compared to a non-energetic placebo, this effect was not maintained the following morning, even in the BS30 condition.

Interestingly, other bedtime snack studies examining whey, casein, and carbohydrate ingestion reported inconsistent results relating to next morning appetite[6–10]. For example, the bedtime ingestion of 30 g of casein has been reported to be more satiating the next morning compared to whey or carbohydrate ingestion, but conversely, was found to increase desire to eat the next morning compared to a non-energetic placebo at bedtime[8]. Future bedtime snack studies are required to clarify the differences in next morning appetite after intake of various mixed macronutrient food sources, e.g. milk, compared to single macronutrient snacks, both administered in solid and liquid form. Such studies should include measurements of candidate appetite regulating hormones (e.g. ghrelin) to provide mechanistic insight into the potential role of a bedtime snack in modulating next morning appetite.

The practical implications of modulating next morning RMR, substrate utilisation and appetite with bedtime snack ingestion relates to weight management. In theory, increasing next morning RMR and decreasing appetite may contribute to an overall negative energy balance. In addition to obtaining subjective measurements of appetite, we also assessed subsequent energy intake the following morning using an *ad libitum* breakfast of cornflakes, as well as energy intake during the following 24 h. Given that subjective hunger and desire to eat were similar between conditions, and that there was only a trend ($P = 0.07$) for an effect of bedtime snack on fullness the following morning, it follows that bedtime milk ingestion failed to modulate energy intake during the *ad libitum* breakfast. Interestingly, although not statistically significant ($P = 0.21$), energy intake at breakfast for the BS30 condition was 18% and 25% higher than BS0 and BS10 conditions, respectively. Although not favourable from a weight management perspective, it is plausible that those with sarcopenia and aiming to retain lean mass, e.g. older adults[29], may benefit from the increased energy intake over time. Furthermore, in the present study, the bedtime milk snack failed to impact energy intake during the 24 h post-trial period. We acknowledge that participant preference for the breakfast option, i.e. cornflakes, may have affected their overall energy intake since no alternative food choice to cornflakes was offered at breakfast. In addition, we cannot discount the possibility that participants may have under-reported or made changes to their usual food intake[30] since food records were the only method employed to assess 24 h post-trial energy intake. Nevertheless, based on our findings, it appears that bedtime milk ingestion does not impact energy intake the following day in mildly overweight men.

Although the bedtime milk snack did not impact appetite and subsequent energy at breakfast the following morning, perhaps unsurprisingly, appetite was reduced during the evening period immediately following milk ingestion compared with placebo. Hence, it may be argued that bedtime

385 milk ingestion could play a role in reducing energy intake prior to bedtime. Evidence exists to suggest
386 that individuals with weight management issues may benefit most from controlling appetite over the
387 evening period[1]. Night eating, defined as waking at night at least once a week to consume food and/or
388 consuming 25% or more of total daily energy intake after the last meal of the day, has been
389 demonstrated to be 2.5 times more prevalent in obese compared to normal weight individuals[2].
390 Furthermore, total daily energy intake appears to increase as energy intake increases at night between
391 18.00 h and 02.00 h[1,31]. In the present study, whilst milk ingestion suppressed appetite prior to
392 bedtime, no differences in appetite were observed between BS10 and BS30 conditions. Therefore,
393 ingesting a low energy and nutrient-rich snack such as a typical 200 ml glass of milk containing 7-10 g
394 of protein (as in the BS10 condition in the present study) ~30 min before bedtime appears adequate to
395 modulate appetite in the evening and may serve to displace intake of potentially energy dense foods
396 that can contribute to higher total daily energy intake. This notion is supported by a study in which
397 overweight or obese participants with self-reported night snacking behaviours were instructed to
398 consume a fixed ready-to-eat cereal with milk daily 90 min after the evening meal[32]. After 4 weeks of
399 the intervention, participants who complied with the daily evening snack protocol significantly reduced
400 their post-evening meal energy intake, resulting in a trend towards greater body weight reduction
401 compared to participants who continued on their normal diet[32]. Participants in the present study
402 consumed each bedtime snack treatment on one occasion only, hence future studies are warranted to
403 investigate if the chronic ingestion of a low energy and nutrient-dense bedtime snack can contribute to
404 weight management, without long-term implications on cardiometabolic health.
405 To conclude, in our hands, the bedtime ingestion of milk containing 10 or 30 g of protein does
406 not modify RMR, substrate utilisation, and appetite the following morning (>9 h post-prandial)
407 compared with a non-energetic placebo snack in mildly overweight males. Consequently, energy intake
408 in the subsequent breakfast and 24 h post-trial period was similar between conditions. To date, findings
409 from bedtime snack studies have been inconsistent, rendering the role of bedtime energy intake as a
410 potential weight management strategy inconclusive. Future studies that include chronic bedtime energy
411 intake of foods with different macronutrient composition and texture are warranted to characterise the
412 long-term implications of a structured bedtime snack *v.* free living bedtime eating habits.

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Tables

Table 1. Participant Characteristics

(Mean values with their standard errors; *n* 12)

	Mean	SEM
Age (years)	24.3	0.8
Height (cm)	182.0	2.0
Weight (kg)	91.0	4.4
BMI (kg/m^2)	27.4	1.1
Waist Circumference (cm)	90.7	3.2
Hip Circumference (cm)	106.9	2.7
Skinfolds (mm)*	92.6	13.2

*Sum of skinfolds included triceps, biceps, subscapular, iliac crest, and calf

Table 2. Energy and macronutrient content of bedtime snack treatments

	BS0	BS10	BS30
Skimmed milk powder (g)	0	28	84
Energy (kJ)	0	410	1234
Protein (g)	0	10	30
Casein (g)	0	8	24
Whey (g)	0	2	6
Carbohydrate (g)	0	14	42
Fat (g)	0	0.2	0.5

BS0, placebo; BS10, 10 g protein; BS30, 30 g protein.

Table 3. Serum glucose and plasma insulin concentrations
(Mean values with their standard errors; *n* 10)

	Before *ad libitum* Breakfast		After *ad libitum* Breakfast		30 min After *ad libitum* Breakfast	
Serum glucose (mmol/l)	Mean	SEM	Mean	SEM	Mean	SEM
BS0	4.7[a]	0.3	6.0[ab]	0.7	6.9[b]	0.5
BS10	4.4[a]	0.2	5.6[a]	0.6	6.6[a]	0.8
BS30	4.5[a]	0.3	5.5[ab]	0.5	7.0[c]	0.7
Plasma insulin (pmol/l)						
BS0	63.6[a]	5.8	311.5[ab]	90.7	504.6[b]	68.0
BS10	69.5[a]	8.3	254.5[b]	64.4	423.2[b]	50.7
BS30	63.8[a]	4.7	244.7[b]	56.2	506.9[c]	79.4

BS0, placebo; BS10, 10 g protein; BS30, 30 g protein.
[a,b,c] Mean values across a row with different superscript letters were significantly different from each other ($P<0.05$, repeated measures two-way ANOVA, Bonferroni *post hoc* test).

Figure Captions

Fig. 1. Schematic diagram of study protocol on (a) day one and day two prior to arriving at the laboratory and (b) during the trial on day two. A standardised dinner was consumed at 19.30, followed by the bedtime snack at 22.30. Participants went to sleep at 23.00 and woke up at 06.30 the next day. Participants arrived at the laboratory at 08.00 and rested in supine position for 10 min. Metabolic measurements were completed via indirect calorimetry for 20 mins, which was proceeded by the 15 min *ad libitum* breakfast. The appetite and thirst questionnaire was completed before and after both the metabolic measurements and breakfast. The first and second blood sample was taken before breakfast and after breakfast. The final questionnaire and blood sample was taken 30 min after breakfast. , *ad libitum* breakfast of cornflakes and semi-skimmed milk; , appetite and thirst questionnaire; , bedtime snack; , Leeds Sleep Evaluation Questionnaire; , arrival at laboratory; , blood sample; , indirect calorimetry.

Fig. 2. Values are means with their standard errors of next morning (a) resting metabolic rate, (b) respiratory exchange ratio, and (c) carbohydrate and fat oxidation following bedtime milk ingestion. No significant main effect of bedtime snack was observed for all measurements ($P > 0.05$, one-way repeated measures ANOVA). BS0, 0 g protein; BS10, 10 g protein; BS30, 30 g protein.

Fig. 3. Values are means with their standard errors of next morning subjective (a) hunger, (b) fullness, and (c) desire to eat following bedtime milk ingestion. Dashed line denotes time when bedtime milk was ingested. Dotted line denotes time when *ad libitum* breakfast was ingested. Data were analyzed using a two-way (bedtime snack x time) repeated measures ANOVA. Measurements from the night before and morning of trial were analyzed separately. At night, there was a significant main effect of bedtime snack on hunger and fullness ($P < 0.05$). The following morning, there was a trend towards a significant effect of bedtime snack on fullness ($P = 0.07$), but no significant effect was observed for hunger and desire to eat ($P > 0.05$). Bonferroni's post hoc test was conducted to determine differences between means. * Mean value was significantly different between BS0 and BS30. # Mean value was significantly different between BS0 and BS10.

Fig. 4. Values are means with their standard errors of the area under the curve (AUC) of subjective (a) hunger, (b) fullness, and (c) desire to eat. Data were analyzed using a one-way repeated measures ANOVA. Data from the night before and morning of trial were analyzed separately. There was a significant main effect of bedtime snack on hunger and fullness AUC at night ($P < 0.05$), but not the next morning ($P > 0.05$). No significant main effect of bedtime snack was found for desire to eat AUC. Bonferroni's post hoc test was conducted to determine differences between means. [a,b] Mean values with different letters were significantly different for the night before.

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