

**This article has been accepted for publication in *British Journal of Nutrition*. This version is free to view and download for private research and study only. Not for re-distribution, re-sale or use in derivative works. © The Authors 2018**

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

Arthur H. H. Lay<sup>1</sup>, Daniel R. Crabtree<sup>2</sup>, Tom G. Campbell<sup>3</sup>, Gillian M. Dreczkowski<sup>1</sup>, Stuart D. R. Galloway<sup>1</sup>, Kevin D. Tipton<sup>1</sup>, Oliver C. Witard<sup>1\*</sup>

<sup>1</sup> *Physiology, Exercise and Nutrition Research Group, Faculty of Health Sciences and Sport, University of Stirling, Stirling, FK9 4LA, Scotland, UK*

<sup>2</sup> *Centre for Health Science, University of the Highlands and Islands, Inverness, IV2 3JH, Scotland, UK*

<sup>3</sup> *School of Applied Sciences, Edinburgh Napier University, Edinburgh, EH11 4BN, Scotland, UK*

**\*Corresponding author:** Oliver C. Witard, University of Stirling, Stirling, FK9 4LA, Scotland, UK, +44 (0) 1786 466298, oliver.witard@stir.ac.uk

**Shortened title:** Bedtime snack and next morning metabolism

**Keywords:** milk, bedtime snack, resting metabolic rate, appetite

## 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<sup>2</sup>). 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.

## 1 **Introduction**

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

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

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

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

41 Evidence regarding the optimal bedtime protein dose required to effectively modulate RMR and  
42 appetite the following morning also remains unknown. Interestingly, the intake of ~30 g of protein  
43 during the day has been shown to induce greater diet-induced thermogenesis, modulate appetite, and  
44 enhance satiety<sup>(16,17)</sup>. However, to date, no acute study has examined whether a protein dose less than  
45 30 g confers a similar increase in RMR and modulatory effect on appetite the following morning.

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

54

## 55 **Methods**

### 56 *Participants and ethics approval*

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

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

74

#### 75 *Protocol overview*

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

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

#### 94 *Bedtime beverage treatments*

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

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

#### 108 *Diet control*

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

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

#### 124 *Metabolic measurements*

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

129 (temperature, relative humidity, and barometer pressure). Volume calibration was completed manually  
130 using a 3 litre calibration pump and gas analyzer calibration was completed using verified gases of  
131 known concentrations (16% O<sub>2</sub> and 5% CO<sub>2</sub>). Measurements were completed with participants resting  
132 supine on a bed in a quiet and temperature-controlled room (20-24 °C). Gas exchange was measured  
133 continuously for 30 min and data were captured every 30 s. The software application determined the  
134 RER and calculated the RMR using the formula derived by Weir<sup>(19)</sup>. Only the final 20 min of the data  
135 collection period was used for analysis to ensure participants were at a physiological steady state.

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

137 Hunger, fullness, desire to eat, and thirst were assessed subjectively using a validated visual  
138 analogue scale (VAS)<sup>(20)</sup>. The questions accompanying the VAS were “How hungry do you feel?”,  
139 “How full do you feel?”, “How much do you think you could eat now?”, and “How thirsty are you?”.  
140 The horizontal lines were anchored by the statements “Not at all hungry/full/thirsty” and “As  
141 hungry/full/thirsty as I have ever felt” at each end. For desire to eat, the statements “Nothing at all” and  
142 “A large amount” were used at each end of the horizontal line. Participants placed a vertical mark on a  
143 100 mm horizontal scale to rate how they felt regarding each sensation. Participants were instructed not  
144 to refer to previous scales when completing each new set of scales.

#### 145 *Ad libitum breakfast and 24 h post-trial energy intake*

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

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

#### 160 *Measurements of sleep quality*

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

171 Participants completed the Leeds Sleep Evaluation Questionnaire (LSEQ) immediately upon  
172 waking on the morning of the experimental trials for subjective measurements of sleep quality. The  
173 LSEQ was validated in individuals aged 18-49 years and consists of ten VAS questions that evaluate  
174 four domains of sleep: the ease of getting to sleep, the perceived quality of sleep, the ease of awakening  
175 from sleep, and behaviour following wakefulness<sup>(22)</sup>. Participants were asked to place a mark on the  
176 100 mm line based on how they felt between two extremes, e.g. “less sleepy than usual” and “more  
177 sleepy than usual”. The scores were averaged to give a score for each domain.

#### 178 *Blood sampling and analyses*

179 A cannula (Becton, Dickinson & Company) was inserted into a forearm vein for blood  
180 sampling. At each timepoint, 10 ml of blood was dispensed evenly between lithium heparin or clot  
181 activator vacutainer tubes. Within 120 min, lithium heparin vacutainers were centrifuged at 3500 rpm  
182 at 4°C and plasma aliquots were dispensed into Eppendorf tubes. Clot activator vacutainers were  
183 allowed to clot for 60 min at room temperature before centrifugation and dispensing serum aliquots  
184 into Eppendorf tubes. Plasma and serum samples were stored at -80°C for future analysis of glucose  
185 and insulin concentrations, respectively. Serum glucose concentrations was analyzed with use of an  
186 automated analyzer (ILab Aries, Instrumentation Laboratory) and plasma insulin concentrations was  
187 analyzed with use of a commercially available ELISA kit (Demeditec Diagnostics GmbH) according to  
188 manufacturer’s instructions. The HOMA2 Calculator V2.2.3<sup>(23)</sup> was used to determine the homeostatic  
189 model assessment of insulin resistance (HOMA-IR) value. The averages of duplicate samples were for  
190 data analysis used. The intra-assay CV and inter-assay CV for insulin concentrations were 8.5% and  
191 10.8%, respectively. Two participants were unable to provide blood samples for all 3 trials; therefore  
192 10 participants were included in the final analysis of blood samples.

193

#### 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 ( $P =$

225 0.007) after bedtime milk ingestion, while BS10 fullness ratings were higher than BS0 at 5 min ( $P =$   
226 0.02) (Fig. 3b). There were no differences between BS30 and BS10 in subjective hunger or fullness  
227 during the evening after bedtime milk ingestion ( $P > 0.05$ ).

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

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

#### 240 *Ad libitum breakfast and 24 h post-trial energy intake*

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

#### 246 *Blood glucose and insulin concentrations*

247 There was no significant bedtime snack and time interaction on next morning serum glucose ( $P$   
248  $= 0.60$ ) or plasma insulin ( $P = 0.57$ ) concentrations. Bedtime snack did not have a significant effect on  
249 next morning serum glucose ( $P = 0.61$ ), plasma insulin ( $P = 0.56$ ), or HOMA-IR ( $P = 0.85$ ) (Table 3).  
250 A main effect of time on serum glucose and plasma insulin concentrations ( $P < 0.01$ ) was observed.

#### 251 *Sleep measurements*

252 As measured by the actigraphy devices, there was no significant effect of bedtime snack  
253 treatment on actual sleep time (BS0: 351 (SEM 9) min, BS10: 366 (SEM 12) min, BS30: 333 (SEM  
254 20) min,  $P = 0.18$ ). Likewise, no significant effect of bedtime snack treatment on sleep latency was  
255 observed (BS0: 20.3 (SEM 7.0) min, BS10: 23.7 (SEM 8.8) min, BS30: 30.3 (SEM 11.6) min,  $P =$   
256 0.76). There also was no significant effect of bedtime snack treatment on fragmentation index (BS0:

257 28.8 (SEM 2.4), BS10: 29.2 (SEM 4.9), and BS30: 35.9 (SEM 5.5),  $P = 0.41$ ). Similarly, bedtime  
258 snack treatment had no significant effect on any of the 4 domains of subjective sleep in the LSEQ (data  
259 not shown): “getting to sleep” ( $P = 0.95$ ), “quality of sleep” ( $P = 0.66$ ), “awake following sleep” ( $P =$   
260 0.77), and “behaviour following awakening” ( $P = 0.86$ ).

261

## 262 **Discussion**

263 The primary aim of the present study was to investigate the influence of bedtime skimmed milk  
264 ingestion on acute changes in whole-body metabolism and appetite the following morning in mildly  
265 overweight males. The main finding was that bedtime ingestion of a milk snack containing either 10 g  
266 or 30 g of protein did not increase next morning RMR compared to a non-energetic placebo. In  
267 addition, next morning RER, as well as carbohydrate oxidation and fat oxidation rates, were similar  
268 between milk and non-energetic placebo conditions. Whereas the bedtime milk conditions tended ( $P =$   
269 0.074) to increase subjective fullness the next morning, no differences in hunger and desire to eat were  
270 observed between milk and non-energetic placebo conditions. Accordingly, energy intake at an *ad*  
271 *libitum* breakfast the next morning and 24 h post-trial was similar between conditions. Hence, refuting  
272 our original hypothesis, bedtime milk ingestion failed to increase RMR and fat oxidation or reduce  
273 appetite the next morning compared to a non-energetic placebo in mildly overweight males.

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

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

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

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

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

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

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

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

362  
363 The practical implications of modulating next morning RMR, substrate utilisation and appetite  
364 with bedtime snack ingestion relates to weight management. In theory, increasing next morning RMR  
365 and decreasing appetite may contribute to an overall negative energy balance. In addition to obtaining  
366 subjective measurements of appetite, we also assessed subsequent energy intake the following morning  
367 using an *ad libitum* breakfast of cornflakes, as well as energy intake during the following 24 h. Given  
368 that subjective hunger and desire to eat were similar between conditions, and that there was only a  
369 trend ( $P = 0.07$ ) for an effect of bedtime snack on fullness the following morning, it follows that  
370 bedtime milk ingestion failed to modulate energy intake during the *ad libitum* breakfast. Interestingly,  
371 although not statistically significant ( $P = 0.21$ ), energy intake at breakfast for the BS30 condition was  
372 18% and 25% higher than BS0 and BS10 conditions, respectively. Although not favourable from a  
373 weight management perspective, it is plausible that those with sarcopenia and aiming to retain lean  
374 mass, e.g. older adults<sup>(29)</sup>, may benefit from the increased energy intake over time. Furthermore, in the  
375 present study, the bedtime milk snack failed to impact energy intake during the 24 h post-trial period.  
376 We acknowledge that participant preference for the breakfast option, i.e. cornflakes, may have affected  
377 their overall energy intake since no alternative food choice to cornflakes was offered at breakfast. In  
378 addition, we cannot discount the possibility that participants may have under-reported or made changes  
379 to their usual food intake<sup>(30)</sup> since food records were the only method employed to assess 24 h post-trial  
380 energy intake. Nevertheless, based on our findings, it appears that bedtime milk ingestion does not  
381 impact energy intake the following day in mildly overweight men.

382 Although the bedtime milk snack did not impact appetite and subsequent energy at breakfast the  
383 following morning, perhaps unsurprisingly, appetite was reduced during the evening period  
384 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<sup>(1)</sup>. 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<sup>(2)</sup>.  
390 Furthermore, total daily energy intake appears to increase as energy intake increases at night between  
391 18.00 h and 02.00 h<sup>(1,31)</sup>. 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<sup>(32)</sup>. 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<sup>(32)</sup>. 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.

## References

1. de Castro JM (2004) The time of day of food intake influences overall intake in humans. *J Nutr* **134**, 104–111.
2. Tholin S, Lindroos A, Tynelius P, et al. (2009) Prevalence of night eating in obese and nonobese twins. *Obesity (Silver Spring)* **17**, 1050–1055.
3. Lennernäs M, Akerstedt T & Hambræus L (1994) Nocturnal eating and serum cholesterol of three-shift workers. *Scand J Work Environ Health* **20**, 401–406.
4. Romon M, Edme JL, Boulenguez C, et al. (1993) Circadian variation of diet-induced thermogenesis. *Am J Clin Nutr* **57**, 476–480.
5. Kinsey AW & Ormsbee MJ (2015) The health impact of nighttime eating: old and new perspectives. *Nutrients* **7**, 2648–2662.
6. Kinsey AW, Eddy WR, Madzima TA, et al. (2014) Influence of night-time protein and carbohydrate intake on appetite and cardiometabolic risk in sedentary overweight and obese women. *Br J Nutr* **112**, 320–327.
7. Madzima TA, Panton LB, Fretti SK, et al. (2014) Night-time consumption of protein or carbohydrate results in increased morning resting energy expenditure in active college-aged men. *Br J Nutr* **111**, 71–77.
8. Kinsey AW, Cappadona SR, Panton LB, et al. (2016) The Effect of Casein Protein Prior to Sleep on Fat Metabolism in Obese Men. *Nutrients* **8**, E452.
9. Ormsbee MJ, Kinsey AW, Eddy WR, et al. (2015) The influence of nighttime feeding of carbohydrate or protein combined with exercise training on appetite and cardiometabolic risk in young obese women. *Appl Physiol Nutr Metab* **40**, 37–45.
10. Ormsbee MJ, Gorman KA, Miller EA, et al. (2016) Nighttime feeding likely alters morning metabolism but not exercise performance in female athletes. *Appl Physiol Nutr Metab* **41**, 719–727.
11. Trommelen J & van Loon LJC (2016) Pre-Sleep Protein Ingestion to Improve the Skeletal Muscle Adaptive Response to Exercise Training. *Nutrients* **8**, E763.
12. Pereira PC (2014) Milk nutritional composition and its role in human health. *Nutrition* **30**, 619–627.
13. Sports Dietitians Australia (2011). Fact sheet protein and amino acid supplementation. [https://www.sportsdietitians.com.au/wp-content/uploads/2015/04/110701-Protein-Supplementation\\_General.pdf](https://www.sportsdietitians.com.au/wp-content/uploads/2015/04/110701-Protein-Supplementation_General.pdf) (accessed June 2017).
14. Dougkas A, Minihane AM, Givens DI, et al. (2012) Differential effects of dairy snacks on appetite, but not overall energy intake. *Br J Nutr* **108**, 2274–2285.
15. Dove ER, Hodgson JM, Puddey IB, et al. (2009) Skim milk compared with a fruit drink acutely reduces appetite and energy intake in overweight men and women. *Am J Clin Nutr* **90**, 70–75.

16. Halton TL & Hu FB (2004) The effects of high protein diets on thermogenesis, satiety and weight loss: a critical review. *J Am Coll Nutr* **23**, 373–385.
17. Phillips SM, Chevalier S & Leidy HJ (2016) Protein ‘requirements’ beyond the RDA: implications for optimizing health. *Appl Physiol Nutr Metab* **41**, 565–572.
18. NDNS: results from Years 1 to 4 (combined) - Publications - GOV.UK. <https://www.gov.uk/government/statistics/national-diet-and-nutrition-survey-results-from-years-1-to-4-combined-of-the-rolling-programme-for-2008-and-2009-to-2011-and-2012> (accessed February 2017).
19. Weir JBDB (1949) New methods for calculating metabolic rate with special reference to protein metabolism. *J Physiol* **109**, 1–9.
20. Flint A, Raben A, Blundell JE, et al. (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *Int J Obes Relat Metab Disord* **24**, 38–48.
21. Spaeth AM, Dinges DF & Goel N (2015) Resting metabolic rate varies by race and by sleep duration. *Obesity (Silver Spring)* **23**, 2349–2356.
22. Parrott AC & Hindmarch I (1978) Factor analysis of a sleep evaluation questionnaire. *Psychol Med* **8**, 325–329.
23. HOMA Calculator : Overview. <https://www.dtu.ox.ac.uk/homacalculator/> (accessed July 2017).
24. Westerterp KR (2004) Diet induced thermogenesis. *Nutr Metab* **1**, 5.
25. Verboeket-van de Venne WP, Westerterp KR, Hermans-Limpens TJ, et al. (1996) Long-term effects of consumption of full-fat or reduced-fat products in healthy non-obese volunteers: assessment of energy expenditure and substrate oxidation. *Metabolism* **45**, 1004–1010.
26. Reed GW & Hill JO (1996) Measuring the thermic effect of food. *Am J Clin Nutr* **63**, 164–169.
27. Segal KR, Edaño A & Tomas MB (1990) Thermic effect of a meal over 3 and 6 hours in lean and obese men. *Metabolism* **39**, 985–992.
28. Tentolouris N, Pavlatos S, Kokkinos A, et al. (2008) Diet-induced thermogenesis and substrate oxidation are not different between lean and obese women after two different isocaloric meals, one rich in protein and one rich in fat. *Metabolism* **57**, 313–320.
29. Keller K & Engelhardt M (2014) Strength and muscle mass loss with aging process. Age and strength loss. *Muscles Ligaments Tendons J* **3**, 346–350.
30. Ortega RM, Pérez-Rodrigo C & López-Sobaler AM (2015) Dietary assessment methods: dietary records. *Nutr Hosp* **31 Suppl 3**, 38–45.
31. de Castro JM (2007) The time of day and the proportions of macronutrients eaten are related to total daily food intake. *Br J Nutr* **98**, 1077–1083.

32. Waller SM, Vander Wal JS, Klurfeld DM, et al. (2004) Evening ready-to-eat cereal consumption contributes to weight management. *J Am Coll Nutr* **23**, 316–321.

## 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 <sup>2</sup> )	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 <i>ad libitum</i> Breakfast		After <i>ad libitum</i> Breakfast		30 min After <i>ad libitum</i> Breakfast	
	Mean	SEM	Mean	SEM	Mean	SEM
Serum glucose (mmol/l)						
BS0	4.7 <sup>a</sup>	0.3	6.0 <sup>ab</sup>	0.7	6.9 <sup>b</sup>	0.5
BS10	4.4 <sup>a</sup>	0.2	5.6 <sup>a</sup>	0.6	6.6 <sup>a</sup>	0.8
BS30	4.5 <sup>a</sup>	0.3	5.5 <sup>ab</sup>	0.5	7.0 <sup>c</sup>	0.7
Plasma insulin (pmol/l)						
BS0	63.6 <sup>a</sup>	5.8	311.5 <sup>ab</sup>	90.7	504.6 <sup>b</sup>	68.0
BS10	69.5 <sup>a</sup>	8.3	254.5 <sup>b</sup>	64.4	423.2 <sup>b</sup>	50.7
BS30	63.8 <sup>a</sup>	4.7	244.7 <sup>b</sup>	56.2	506.9 <sup>c</sup>	79.4

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

<sup>a,b,c</sup> 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 preceded 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. <sup>a,b</sup> Mean values with different letters were significantly different for the night before.

## **Acknowledgements**

No funding was received for the present study. A.H.H.L., D.R.C., T.G.C., S.D.R.G., K.D.T. and O.C.W. conceptualised and designed the research. A.H.H.L., G.M.D. and O.C.W. conducted the research, while A.H.H.L. and G.M.D. analysed the data. A.H.H.L. and O.C.W. wrote the paper and had primary responsibility for the final content. All authors read, edited and approved the final manuscript. The authors have no conflicts of interest to declare.