PRE-SLEEP PROTEIN CONSUMPTION HAS NO IMPACT ON NEXT-DAY APPETITE, ENERGY INTAKE AND METABOLISM IN OLDER INDIVIDUALS.

By

STEPHEN MICHAEL MOREHEN, BSc.

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CONTENTS

ABSTRACT

Background: Appetite, energy intake and dietary protein intake tend to decrease with age and may contribute towards the development of sarcopenia. Pre-sleep protein stimulates overnight muscle anabolism in older adults and may potentially mitigate sarcopenia progression. However, protein is the most satiating macronutrient and acutely affects appetite, energy intake and metabolic rate. Therefore, the aim of this study was to investigate the effects of pre-sleep protein ingestion on next-day appetite, and next-morning energy intake and metabolism in older populations. **Methods:** 12 participants (8M,4F, aged 71.3 ± 4.2 yrs) were recruited for a single-blind randomized cross-over design study. Participants completed 3 experimental visits, during which they consumed one of three beverages; casein protein (CAS; 40g protein, 168kcal), maltodextrin (MD; 0g protein, 168kcal) or a water placebo (WP; 0g protein, 0kcal) prior to sleep. Next-morning metabolic rate was assessed using indirect calorimetry. Ad libitum energy intake was assessed at a buffet-style breakfast, and subjective ratings of appetite were obtained prior-to and over 24 h after protein ingestion. **Results:** There was no effect of CAS consumption on subjective appetite at any point during the experimental trial compared with MD or WP. Relative energy intake at breakfast (CAS: 6.8 ± 3.1 $kcal.kg^{-1}$, MD: 6.9 ± 3.2 $kcal.kg^{-1}$, WP: 7.2 ± 3.4 $kcal.kg^{-1}$) and next-morning resting metabolic rate (CAS: 1874.4 ± 468 $kcal.day^{-1}$, MD: 1645.4 ± 424.4 $kcal.day^{-1}$, WP: 1805.6 ± 399.8 $kcal.day^{-1}$) were also unaffected. **Conclusion:** Our findings suggest that pre-sleep protein can be implemented by older adults to augment daily protein consumption without impacting next-day appetite or energy intake. Further research should assess whether these findings are applicable in chronically ill and hospitalised populations who are at higher risk of sarcopenia and associated comorbidities.

INTRODUCTION

Sarcopenia

Age-related declines in skeletal muscle (sarcopenia) and strength (dynapenia) are associated with increased functional dependency and an increased risk of frailty, falls, metabolic disease and early death [1-5]. The rapid global expansion in the number of older individuals is expected to pose an unprecedented burden on national healthcare systems [6, 7]. Indeed, current estimates for the cost of sarcopenia and muscle weakness to healthcare services in the UK alone are upwards of £2.5bn annually [8] and furthermore, in 2000 approximately $18.5bn was spent treating the disease in the US – equating to 1.5% of the entire annual national healthcare costs [7]. Sarcopenia is characterized by a reduction in muscle fibre cross-section, particularly of type 2 muscle fibres [9], a decline in motor unit numbers, and a failure to reinnervate denervated muscle fibres [10]. Sarcopenia is exacerbated by a number of factors secondary to chronological ageing, per se, including inactivity [11], reduced energy/protein intake [12] and the presence of chronic inflammation [13]. Whilst reversing chronological ageing is impossible, factors related to secondary ageing can be targeted by interventions.

Anabolic Resistance

Maintenance of skeletal muscle mass is dictated by a fine balance between rates of muscle protein synthesis (MPS) and breakdown (MPB). MPS is effectively stimulated by resistance-based exercise and the provision of dietary protein through constituent essential amino acids [14]. Dietary protein has been identified as a key nutrient for older individuals [15], essential for maintenance of skeletal muscle through stimulation of MPS [16] and, to a lesser extent, suppression of MPB [17]. In older age the MPS response to low-to-moderate dose dietary protein provision is diminished [18], due to an “anabolic resistance” of older muscle to stimuli which normally elicit robust MPS responses in younger muscles. Ingestion of protein-

containing food evokes a state of hyperaminoacidemia, where amino acids are readily and freely available for incorporation into bodily tissues (including muscle). In younger muscles, amino acid-induced MPS results in a positive shift in net protein balance (the algebraic difference between MPS and MPB) and, over time, an accretion or maintenance of skeletal muscle protein mass [19]. In older individuals, however, an increase in MPS does not occur for the same given concentration of amino acids and is only stimulated once a higher dose of protein has been ingested [20, 21]. Although the exact cause of this muscle anabolic resistance is yet to be established, it has been suggested that age-associated factors such as chronic inflammation [22], a reduced level of habitual activity [23] and disruption to intracellular signalling pathways [21] are all contributory [18]. Additionally, recent research has identified a potential role of the gut microbiome in modulating synthetic responses to dietary protein [24], whilst other studies have suggested that impaired muscle capillarisation in old age can exacerbate age-related decrements in MPS through reduced nutrient delivery capability in skeletal muscle [25, 26]. Although the exact driving forces of the phenomenon remain elusive and continually growing, anabolic resistance is considered a significant driving factor in the aetiology of sarcopenia [27].

Protein intake in older populations

Based on evidence that 0.4g/kg/body mass of high-quality protein (per meal) is required to maximally stimulate postprandial MPS in older individuals [28], it has been suggested that dietary protein recommendations should exceed the current RDA of 0.8g/kg/body mass [29], with suggestions ranging from 1.2-2.0g/kg/body mass per day [30]. Indeed, protein intakes which exceed current recommendations are associated with greater long-term lean mass retention in the elderly [12] and may prevent the onset of sarcopenia [31]. Furthermore, higher protein intakes confer benefits beyond muscle mass and function as they are shown to also

contribute to greater cardiovascular health [32, 33]. Importantly, many healthy community-dwelling older individuals meet or exceed the current dietary protein RDA, but consume dietary protein in an uneven pattern across the day (i.e. typically low-protein breakfast and high-protein dinner) [34, 35]. Thus, in addition to an increase in daily protein intake, it is suggested that older individuals aim to distribute dietary protein evenly across daily meals in order to maximally stimulate MPS at each mealtime [36]. More frequent consumption of meals containing between 30 and 45g protein/meal is strongly associated with leg lean mass and strength in older individuals [37]. Furthermore, Ten Haaf *et al*. [38] show that older individuals with a greater distribution of protein consumption throughout the day tend to have higher gait speeds than those with less of a distribution. Similarly, albeit in a younger population, Mamerow *et al.* (2015) demonstrated that three equally split 30g doses of protein stimulated MPS to a greater extent than doses split to mimic a typical pattern of daily protein consumption i.e. 10g, 15g and 65g at breakfast, lunch and dinner respectively [39]. Interestingly, there are divergent findings from Kim *et al*. (2017) which report no effect of protein distribution on functional outcomes such as one-rep-max knee extension, hand-grip strength and gait speed [40]. These findings are supported by Murphy *et al.* (2018) who found that splitting a total dose (breakfast, lunch, dinner, bedtime snack) of 1.3g/kg/day into even (4 x 25%) or uneven (7%/17%/72%/4%) distribution patterns did not affect the overall MPS response [41]. The role of anabolic resistance may provide an explanation for the dissonance among current research, as even distribution patterns may result in levels of protein being consumed at each meal-time being insufficient to stimulate MPS.

Pre-sleep protein

In addition to optimizing dietary protein delivery during the day-time, the overnight period has recently been proposed as a 'window of opportunity' to stimulate MPS in older individuals

[42]. In this regard, ingestion of pre-sleep protein is considered an effective strategy to increase MPS overnight whilst sleeping and is shown to augment adaptive responses to resistance training in skeletal muscle, allowing for greater recovery and performance [42], although previous research regarding these particular effects have focused on primarily younger [43] and athletic populations [44]. Pre-sleep intake of food and/or drink essentially allows for the inclusion of another "mealtime" during the day, wherein a dose of protein may be ingested. In the absence of pre-sleep protein and assuming typical evening/morning mealtimes of 6-8pm/6-8am respectively, this leaves a 10-12-hour period during which no protein is being consumed, digested and, consequently, a catabolic shift in net protein balance as rates of MPB exceed MPS.

It has been demonstrated that 40g of casein protein ingested prior to sleep is successfully digested, absorbed and synthesized into skeletal muscle in older individuals, and that a state of sleep does not compromise any of these processes [45, 46]. Consuming protein before sleep has also been shown to improve markers of recovery from team sports participation in younger individuals [47]. Additional studies in younger individuals have found that a protein-rich pre-bed time snack may also confer benefits for weight management and whole-body metabolic health through increasing next morning resting metabolic rate and fat oxidation [48]. Sarcopenia is often masked by a concomitant increase in adiposity [49] and as such, increases in RMR and reductions in appetite may serve to combat this increased adiposity via higher energy expenditure and reduced energy intake, respectively. This is pertinent as sarcopenia and obesity share many aetiological roots meaning that a reduction in adiposity may elicit reductions in sarcopenic characteristics and vice versa [50]. On the other hand, a reduction in appetite and subsequent reductions in energy (and therefore protein) intakes may actually exacerbate the already-reduced appetite and energy intake that is present due to the

aforementioned “anorexia of ageing”. In populations of healthy older individuals, for whom weight-loss is not necessary, it would be desirable for pre-sleep protein to have no effect on appetite and energy intake, as this would allow it to be implemented without impacting energy and protein intake the following day. Furthermore, an individual’s RMR has been shown to influence the size of a self-determined meal, with a higher RMR causing a higher energy content meal [51]. In addition to this, RMR is positively associated with subjective measurements of appetite in both lean and obese cohorts (albeit from a younger population) [52]. This emphasises the need to investigate whether pre-sleep protein ingestion has any effect on next morning RMR in older cohorts as this may subsequently affect appetite and energy intake at breakfast time. At this time, the effect of pre-sleep protein ingestion on next morning metabolic health parameters has yet to be investigated in older individuals.

The anorexia of ageing

Many older adults actually consume the same relative proportion of protein in their daily energy intakes as younger individuals (15.3 ± 2.2 % of total energy intake as protein in 19-30 year olds compared to 15.9 ± 1.7% in >71 year olds) - thus, as energy requirements decline with age, a progressive decline in protein intake also occurs [53]. Protein has high satiating properties and, as discussed, can be used to promote weight loss in young individuals through reducing subsequent energy intake and spontaneous food consumption [54]. Given that breakfast protein content is typically low (15% of daily protein intake) in older individuals compared to lunch (20%) and dinner (65%) [40], and therefore high-protein breakfasts are desirable to increase daily protein intake, it is important to understand how pre-sleep protein affects next morning appetite and energy/protein intake in this population. In younger individuals, a pre-bedtime protein snack did not to impair next morning appetite or energy intake [55]. This may not, however, be the case in older individuals who already experience

reductions in appetite, termed the "anorexia of ageing" [56], brought on by physiological (impaired sensory perception, reduced chewing capability due to poor dentition), psychosocial (loneliness, cost) and endocrine factors (ghrelin, leptin, glucagon-like peptide-1 (GLP-1) and cholecystokinin (CCK)) [57-59]. Furthermore, the satiating effects of protein intake may differ between young and old, due to potential differences in the rate of gastric emptying [60] and the rate of gastric emptying may be dependent on the time-of-day of protein ingestion. For example, night-time circadian rhythms and lying down during sleep are reported to delay gastric emptying and postprandial amino acid bioavailability [61, 62], with possible consequences for next morning appetite and energy intake in older individuals.

Next-morning appetite

If ingestion of pre-sleep protein engenders a decline in appetite the succeeding morning then individuals may opt to consume less, or even no breakfast and consequently miss out on typical breakfast-based sources of protein such as milk, eggs and yoghurt. This may effectively "cancel out" the muscle anabolic advantage of pre-sleep protein ingestion, rendering the intervention counteractive to the maintenance of skeletal muscle mass with advancing age. Therefore, although pre-sleep protein consumption has been proven to increase overnight MPS in older individuals [45], possible counteractive effects must be determined before nutritional strategies to combat sarcopenia can effectively recommend the implementation of pre-sleep protein. Furthermore, the ingested protein source plays a role in satiety and skeletal muscle anabolism, due to divergent digestive properties and amino acid profiles. Milk proteins have been reported to increase satiety and suppress short-term food intake compared to other sources [63], but the contribution of complete milk proteins vs. milk constituents, whey and casein, is still unclear [64-66]. Casein is considered to be an ideal pre-sleep protein source for muscle anabolism in older individuals, due to its relatively slow digestive properties compared with whey, which

allows for a sustained elevation in plasma amino acid concentrations, and consequently rates of MPS, for the duration of sleep [67, 68].

AIMS & HYPOTHESES

The aim of the present study was to determine whether the potentially satiating effects of a pre-sleep casein protein beverage influence next-morning appetite and energy intake in older individuals compared to an energy-matched, carbohydrate control and a non-energetic water placebo. We hypothesized that pre-sleep casein protein and an energy-matched carbohydrate treatment would increase feelings of satiety in the immediate post-prandial period compared with water, but would not adversely affect next morning appetite, ad libitum energy intake or metabolic rate in older individuals. As such, these findings would lend support to the idea that pre-sleep protein ingestion can be practically implemented, without counteractive effects on appetite and metabolic rate, alongside recommendations for higher-protein intake with all daily meals to promote skeletal muscle anabolism and maintenance in older individuals.

MATERIALS AND METHODS

Participants

12 healthy older participants (8 males, 4 females, aged 71.3 ± 4.2 yrs, height: 1.72 ± 0.08m, weight: 71.1 ± 13.4kg, BMI: 23.75 ± 3.43kg/m2, mean ± SD) were recruited from the local community in Birmingham, UK. Participants were excluded from study participation if they performed regular structured training more than three times a week, smoked, had Type I/II diabetes, or any medical condition that could cause discomfort in response to food ingestion. Furthermore, participants were excluded if they presented an eating disorder, allergy or food intolerance to any of the ingredients present in the study treatments, standardised dinners or ad-libitum breakfast buffet. Participants provided written informed consent and general health

questionnaires before the start of any experimental trials. Ethical approval for the present study was granted by the University of Birmingham Research Ethics Committee (#ERN_18-1221) and the study was completed in accordance with the Declaration of Helsinki (with the exception of registration on an open-trials database).

Study Design

Following a preliminary visit to assess participants' study eligibility, informed consent, anthropometric characteristics, physical function and dietary habits, participants underwent 3 experimental trials in a randomized single-blind crossover fashion. The evening prior to each experimental visit participants were provided with a standardised dinner and pre-sleep treatment beverage. The following morning participants attended the laboratory at ~0730h to undergo resting metabolic rate measurements, blood sampling and consume an ad libitum breakfast buffet. Subjective measurements of appetite were completed the evening prior to and throughout the experimental trial day. Experimental trials were identical apart from the pre-sleep treatment beverage consumed and were separated by ~7-10 days. All study visits took place at the School of Sport, Exercise and Rehabilitation Sciences of the University of Birmingham. An overview of the experimental design is provided in Figure 1.

Preliminary Visit

Participants met with the principal researcher prior to enrolment to discuss the study procedures and confirm their eligibility via a general health questionnaire and food preference form before informed consent was obtained. Following this, height and weight was obtained via a stadiometer and digital weighing scale (both: Seca ®, Birmingham, UK), respectively. A short physical performance battery (SPPB) [69] was completed and participants given scores (1-4) for balance, gait speed and leg strength. Participants were excluded if their combined score was

<9 as this suggested a level of functional dependency beyond that which was deemed to be indicative of a "healthy" older individual. Before leaving the laboratory, participants were provided with a food diary to be completed for three days (consecutive or non-consecutive), including two weekdays and one weekend day.

Experimental Visits

Standardization of evening meal

Food diaries were analysed using Dietplan (Dietplan 7, Forestfield software Ltd, UK) and the macronutrient breakdown subsequently determined. A standardised dinner (50%/32%/18% carbohydrate/fat/protein) was provided to be consumed the night before each experimental visit. Quantities of different foods were manipulated for each participant so that the energy content of the experimental dinner closely matched their habitual average energy content at dinner.

Pre-sleep treatment beverages

Participants were asked to consume 1 of 3 pre-sleep beverages in a randomised cross-over fashion at 2200h the evening prior to the experimental trial visits. Beverages consisted of either 400mL of water placebo (WP), 48g of casein protein powder (MyProtein, Northwich, UK) to deliver 40g of protein (CAS), or 42g of maltodextrin powder (MyProtein, Northwich, UK) matched for the total energy content of the CAS (MD). Participants were provided with a shaker to take home and instructed to mix the CAS or MD powder with 400 mL of water. Non-caloric vanilla flavouring (MyProtein, Northwich, UK) was added to the treatment beverages to improve palatability. A 40g dose of CAS was chosen based on evidence from Kouw *et al.* (2017) showing that a 40g dose of protein effectively stimulated overnight MPS in older adults [45]. The nutritional content of each beverage is presented in Table 2.

Treatment	Energy (kcal)	Carbohydrate (g)	Protein (g)	Fat (g)
CAS	168	1.92	40	0.72
MD	168	42	0	0
WP	0	0	0	0

Table 1 - Nutritional content of bedtime beverages. (CAS: Casein Protein, MD: Maltodextrin, WP: Water Placebo)

Subjective Measurement of Appetite, Thirst and Sleep Quality

Feelings of appetite and thirst were assessed using a 100mm visual analogue scale (VAS) covering 12 questions in the following domains; hunger, fullness, desire to eat and thirst. Two opposing statements accompanied each question and were interspersed by a 100mm horizontal line, for example the line for "How hungry do you feel?" extended from "I am not hungry at all" to "I have never been hungrier". Participants were instructed to mark the 100mm VAS at the point which accurately represented their current feeling. The distance between the mark and beginning of the 100mm VAS was measured to determine a score between 0-100. A VAS was completed immediately prior to sleep prior to the experimental trials, to provide a baseline measurement in the absence of a pre-sleep beverage being ingested. For each experimental trial, a VAS was completed at the following 12 consecutive time points: immediately before (2200h), after (2200h) and 30 min after (2230h) pre-sleep treatment beverage consumption, upon waking (0600-0630h), upon arrival at the laboratory (~0730h), immediately post-RMR measurement (~0810h), immediately pre-breakfast (~0830h), immediately post-breakfast (~0900h), 30 min post-breakfast (~0930h), immediately pre-lunch, immediately pre-dinner and immediately before bedtime. VAS are shown to have high validity and reproducibility in the measurement of subjective appetite [70].

Sleep quality was determined using the Leeds Sleep Evaluation Questionnaire (LSEQ) [71], which consists of 10 questions divided into four domains (getting to sleep, quality of sleep, waking from sleep and behaviour following wakefulness). A 100 mm VAS was used to determine the response to each question, and an average score was calculated for each domain. Participants completed one baseline LSEQ upon waking in the week leading up to the experimental trial as a baseline measure, and on the morning of each of the three experimental trials.

Metabolic Measurements

Upon arrival at the laboratory for each experimental trial, participants rested for 30 min in a supine position on a bed in a quiet, temperature regulated (22-24°C), dimly lit room. Expired gasses were collected over the final 20 min of the measurement period using a mouthpiece attached to a Douglas bag. A gas analyser was used to ascertain the percentage of O_2 and CO_2 in the expired air. Volume and temperature of expired air was assessed by a dry gas meter, and barometric pressure of the laboratory determined in which gas analysis was taking place. Participants were instructed to lie as still as possible, breathe normally, not to speak and to remain awake. The Weir formula [72] was then used to calculate resting metabolic rate and accordingly, resting energy expenditure.

Ad Libitum Energy Intake

An ad libitum breakfast was provided to participants during each experimental visit. To determine energy intake and macronutrient composition, all breakfast items were weighed before and after consumption, and analysed using Dietplan software (Dietplan 7, Forestfield software Ltd, U.K). Participants were seated in a comfortable, quiet environment and instructed to eat as much or as little as they desired. Participants were unaware that their food consumption

was being measured in order to avoid any confounding factors (such as demand characteristics or alterations to eating habits). Breakfast composition is outlined in Table 2.

	Nutritional Content (per 100g)			
Food/Drink	**Energy (kcal)**	**Carbohydrate (g)**	**Protein (g)**	**Fat (g)**
White Bread	239	45.6	8.1	2.2
Cornflakes	378	84	7.0	0.9
Yoghurt	89	11.8	3.9	2.8
Raspberry Jam	251	61.5	< 0.5	< 0.5
Clover Spread	587	1	0.7	64.5
Semi-Skimmed Milk	50	4.8	3.6	1.8
Water	0	0	0	0

Table 2 - Nutritional content of food and drink available to participants at the ad libitum breakfast.

Blood Sampling and Analyses

Venous blood samples were obtained from an antecubital forearm vein at each experimental visit immediately before, immediately after and 30 min after consumption of the ad libitum breakfast. A total of 10ml of blood was obtained and decanted evenly between serum-separator and EDTA vacutainers (BD, Oxford, UK), centrifuged at 3000 RPM for 10 min at 4°C, and stored at -80°C until later analysis. Plasma glucose was assessed using a fully automated analyser machine (rx Daytona+, Randox Laboratories Ltd., County Antrim, UK). Serum insulin was analysed using a commercially available ELISA kit (Quantikine ELISA, R&D Systems, Bio-Techne Corporation, Minnesota, USA), carried out according to the manufacturer's instructions.

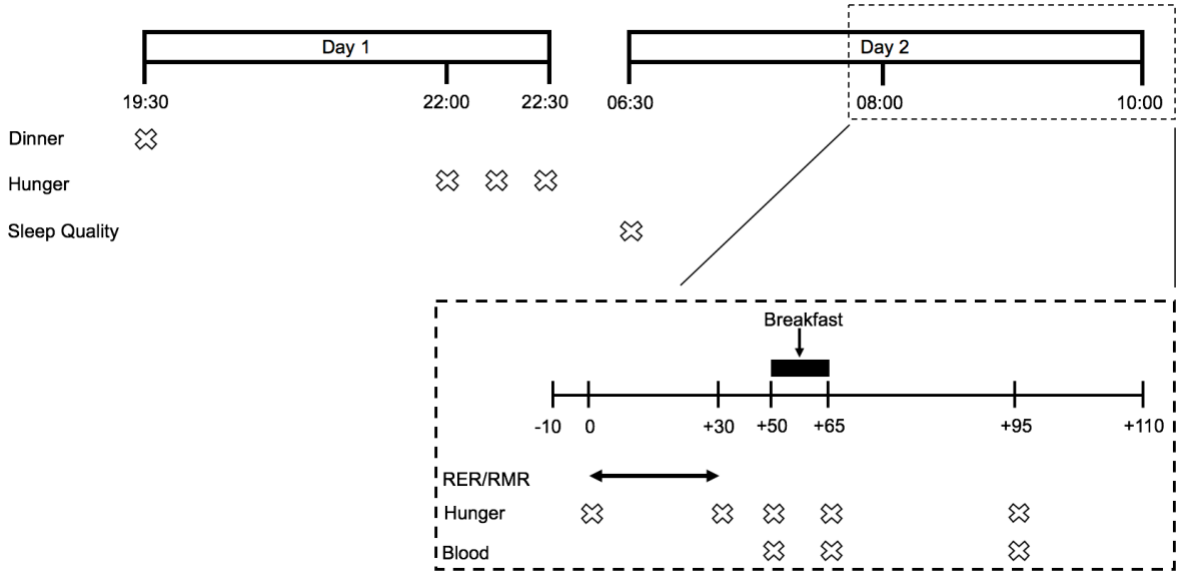

Figure 1. Timeline of experimental visits.

Statistical Analysis

All statistical analyses were completed using IBM SPSS Statistics software package version 26 (IBM Corporation). One-way repeated measures ANOVAs were conducted to examine differences in RER, RMR, subjective ratings of sleep quality and relative energy intake at breakfast. Two-way repeated measures ANOVAs assessed interactions between time-point and condition for subjective hunger scores and glycaemic responses following consumption of breakfast. Pearson's correlational analysis was performed to determine whether pre-breakfast ratings of hunger correlated with actual energy intake at breakfast. Bonferroni post-hoc analysis was conducted to identify specific differences where a main effect or interaction was found. Alpha level was set to $p<0.05$. All data is reported as mean ± standard error and error bars on figures are indicative of standard error.

RESULTS

Appetite

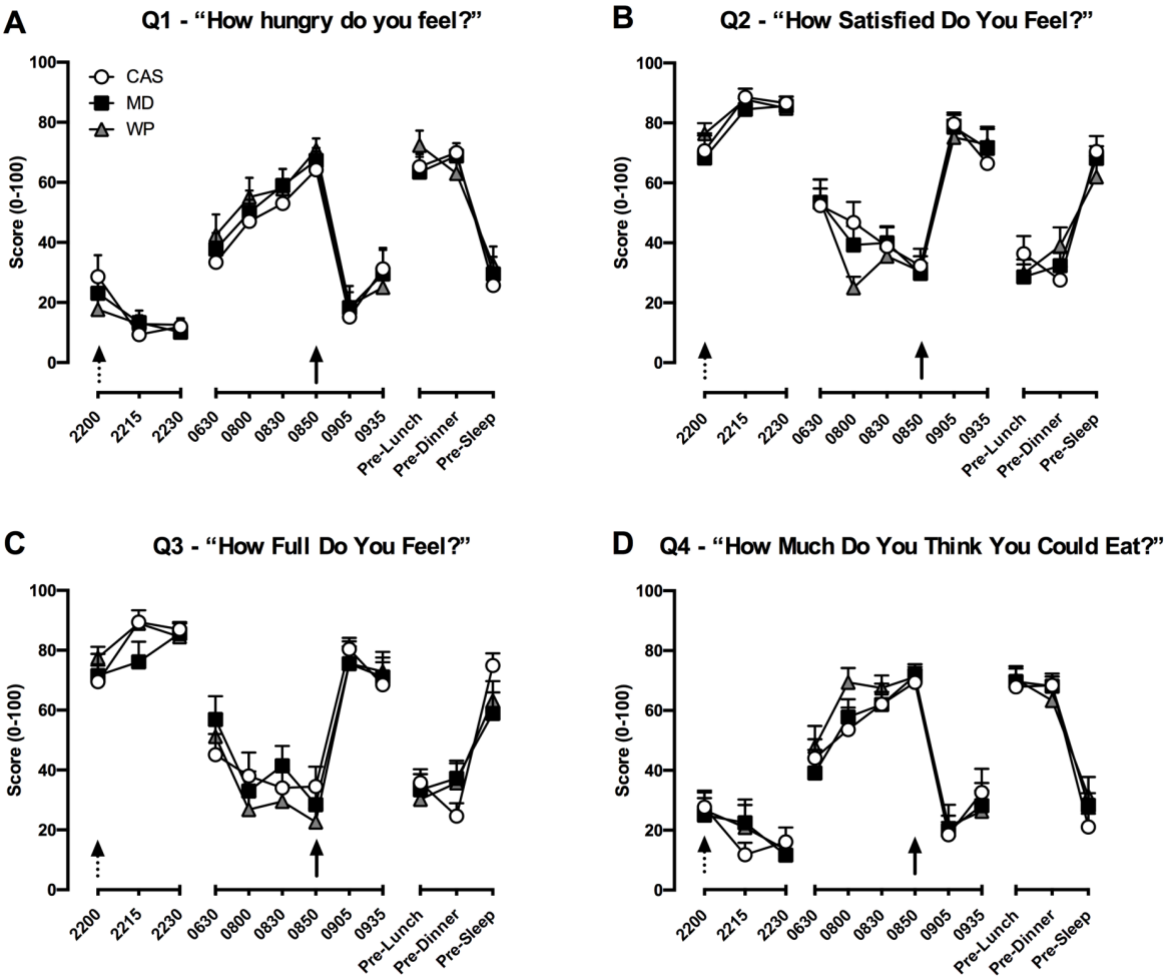

Figure 2 - Subjective ratings of hunger (1A), satiety (1B), fullness (1C) and desire to eat (1D) from visual analogue scales at various time-points throughout the duration of each experimental trial. No significant differences between conditions at any time-point. A significant effect of time was present across all 4 questions ($p<.001$). Arrows at 2200 and 0850 indicate consumption of pre-sleep beverage and ad libitum breakfast, respectively. Error bars represent standard error. CAS: Casein Protein, MD: Maltodextrin, WP: Water Placebo.

Energy Intake

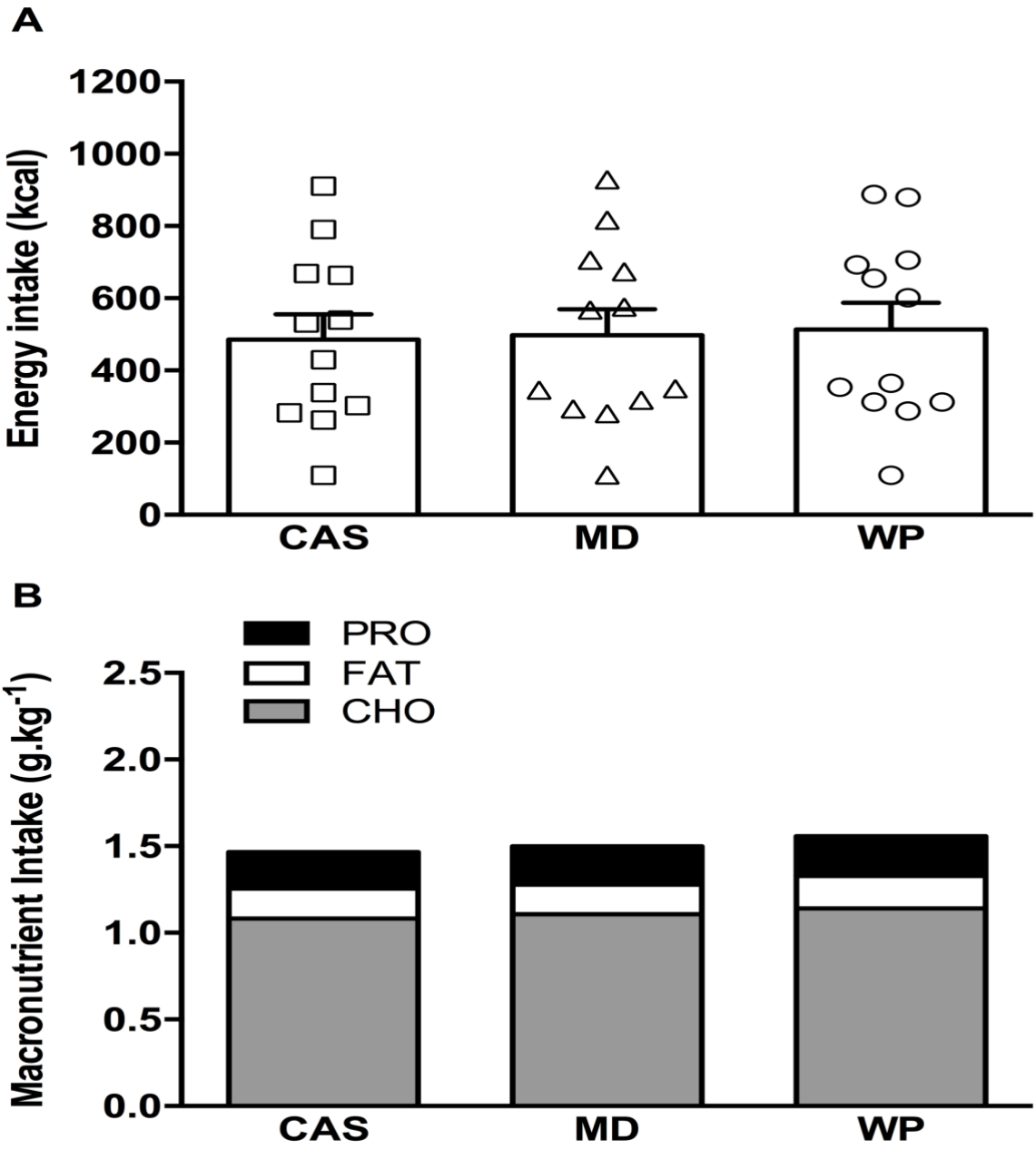

Figure 3 – (**A**) Gross energy intake and(**B**) relative macronutrient intake during ad libitum breakfast. No significant differences between conditions. Error bars represent standard error. PRO: Protein, FAT: Fat, CHO: Carbohydrate. CAS: Casein Protein, MD: Maltodextrin, WP: Water Placebo.

Absolute intake of fat, carbohydrate and protein from the breakfast meal was expressed relative to subjects' bodyweight. There were no significant differences across the three treatment

conditions in absolute total energy intake (Figure 3A) or relative consumption of carbohydrate, fat and protein (Figure 3B), across the three treatment conditions and also compared to the average nutritional content of habitual breakfast intake recorded in the 3-day food diary, taken as a baseline measurement. Furthermore, there was no significant relationship between pre-breakfast subjective rating of hunger and actual energy intake at breakfast, both in each individual condition and also in all conditions combined.

Metabolic measurements

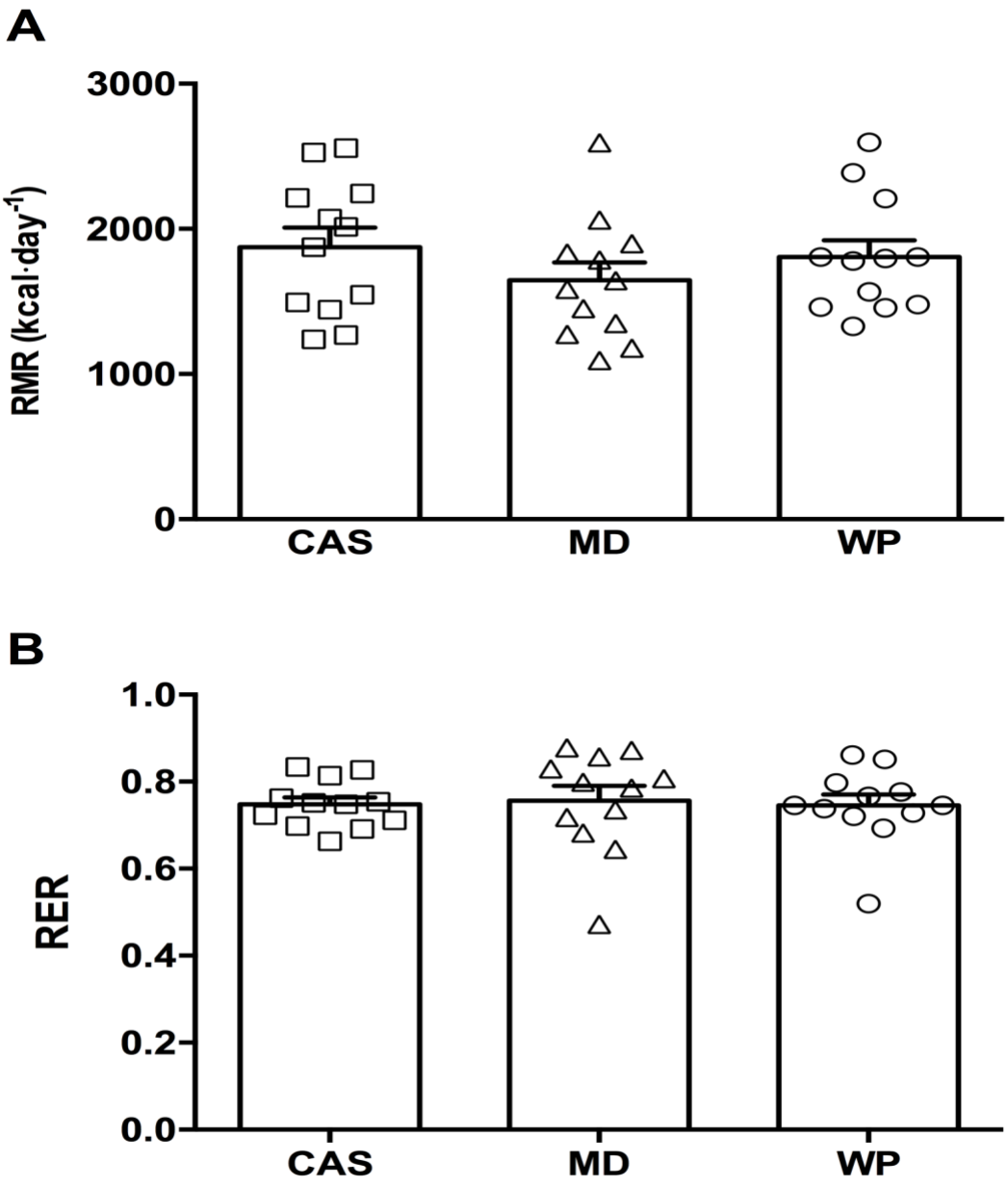

Figure 4.(A) Resting metabolic rate (RMR) and(B) respiratory exchange ratio (RER) as calculated from indirect calorimetry in a fasted, relaxed state. No significant differences between conditions. Error bars represent standard error. Individual data points are shown for each data series. CAS: Casein Protein, MD: Maltodextrin, WP: Water Placebo.

There were no significant differences in next-morning RER or RMR between the three pre-sleep beverage conditions (Figure 4A and 4B, respectively).

Sleep Quality

No significant differences were found between pre-sleep beverage conditions across all four domains of the LSEQ. Furthermore, there was no difference between baseline sleep quality and sleep quality during any of the experimental visits, suggesting that consumption of a pre-sleep beverage did not impact sleep quality in the present study (Table 3).

	LSEQ DOMAIN			
CONDITION	**GTS**	**QOS**	**WFS**	**BFW**
BL	49.7 ± 14.7	44.0 ± 15.2	43.2 ± 13.8	50.3 ± 14.5
CAS	50.8 ± 14.6	41.3 ± 19.1	51.1 ± 10.5	50.1 ± 15.5
MD	50.4 ± 14.6	41.4 ± 13.3	54.0 ± 10.2	53.3 ± 18.2
WP	49.5 ± 14.1	43.5 ± 21.4	54.5 ± 18.3	53.7 ± 20.6

Table 3 - Subjective sleep quality assessed using the Leeds Sleep Evaluation Questionnaire (LSEQ). Mean ± SD. No significant differences between any treatment conditions or baseline. GTS: Getting to sleep, QOS: Quality of sleep, WFS: Waking from sleep, BFW: Behaviour following wakefulness. BL: Baseline, CAS: Casein protein, MD: Maltodextrin, WP: Water placebo.

Plasma glucose and serum insulin

For both insulin and glucose measurements, a significant effect of time was observed ($P < .001$ for both) but no effect of condition was present. Furthermore, there was no significant condition by time interaction for insulin or glucose measurements (Table 4).

CONDITION	PLASMA GLUCOSE CONCENTRATION (MMOL/L)			SERUM INSULIN CONCENTRATION (PMOL/L)		
	Pre	**Post**	**30Post**	**Pre**	**Post**	**30Post**
CAS	5.69 ± 0.43*^	6.72 ± 1.2	7.03 ± 1.94	54.07 ± 16.69*^	192.44 ± 172.46^	350.74 ± 132*
MD	6.00 ± 0.62*^	6.86 ± 1.1^	8.06 ± 1.83*	52.14 ± 12.94 *^	177.98 ± 77.94^	391.69 ± 151.87*
WP	5.81 ± 0.49^	6.51 ± 1.22	7.80 ± 2.14	52.74 ± 15.62*^	150.99 ± 114.78^	369.92 ± 149.85*

Table 4 - Plasma glucose concentration and insulin response to breakfast consumption. Significant main effect of time ($p<0.001$ for both). No main effect of condition and no time x condition interaction was present for both insulin and glucose measurements. No significant differences between conditions. ***** = significantly different to Post, ^ = significantly different to 30Post, all $p<0.05$). CAS: Casein Protein, MD: Maltodextrin, WP: Water

DISCUSSION

Nutritional strategies to increase the daily protein intake of older populations are imperative in the ongoing battle against sarcopenia [73]. Use of protein supplements in older adults has been shown to improve physical performance [74]. Furthermore, a three-year longitudinal study in over 2000 70-79 year olds found that individuals in the highest 20% for daily protein intake lost 40% less lean body mass over the duration over the study than those in the lowest 20% [12]. Similarly, a study found that elderly women with an intake of 1.2-1.76g/kg/day of protein suffered from a lower number of health complications over a 10-year follow-up than those consuming <0.8g/kg/day [75]. Clearly, consuming protein intakes above the current RDA is beneficial for a multitude of health outcomes in older populations. Protein intake within healthy community-dwelling older adults does tend to exceed current recommendations [34, 35]. Whilst this is a positive starting point, closer inspection reveals that the distribution of protein intake throughout the day is typically uneven, with the highest protein content coming from the evening meal and smaller quantities being obtained at breakfast time [35]. A multitude of studies suggest that a ≥30g of protein is required to effectively stimulate MPS in older individuals [76-79]. As such a push towards a more even daily protein distribution may be necessary to ensure that MPS is stimulated at each mealtime rather than just dinner and, to a lesser extent, lunch.

Promising evidence from research investigating pre-sleep protein [42, 45, 47, 67, 80] suggests that the pre-bed/overnight period provides additional hours within the day, so-to-speak, within which MPS can be successfully stimulated [45], contributing towards a greater positive net protein balance in older adults. Nevertheless, it is imperative to consider possible effects that pre-sleep protein ingestion may have on appetite and metabolism the following morning. Protein is highly satiating and if pre-sleep protein were to engender a reduction in appetite prior

to breakfast consumption this may lead to a lower energy and protein intake at breakfast time, a meal at which protein intake is typically lower than the amount required to stimulate MPS in older adults [28, 40]. Theoretically, this scenario could effectively render a pre-sleep protein strategy redundant as the increase in pre-sleep protein is countered by a reduction in breakfast-time protein. This research has shown that this is not the case. The novel findings of the present study comprehensively demonstrate that pre-sleep protein does not adversely affect next-day appetite and energy intake and, as such, can be implemented as a strategy to increase daily protein intake and stimulate muscle anabolism in older individuals.

There is consistent evidence that protein is more satiating, gram-for-gram, than fat or carbohydrate [81] and as such features in weight management strategies as a method of reducing appetite and subsequent energy intake throughout the day [54, 82-84]. A review by Bendtsen *et al.* (2013) suggested that whey protein is more satiating than casein in the acute setting (up to 100 mins after ingestion), whereas casein may be more satiating when appetite is assessed over a longer period of time (up to 300 mins after ingestion)[85]. Explanation for this comes from divergent digestive profiles of the two proteins. Casein is known as a "slow" digesting protein because it coagulates in the acidic environment of the stomach, delaying gastric emptying and leading to a prolonged, moderate level of hyperaminoacidemia. Conversely, whey is quickly digested as it remains soluble in stomach acid and thus elicits a higher, but transient, level of hyperaminoacidemia [86, 87]. This state of increased circulating amino acids is a primary stimulant of MPS [88] and hence explains why casein is currently favoured as the source of protein for pre-sleep supplementation [42], as it elevates amino acid concentrations for longer over a period of time when further food ingestion is not possible (i.e. during sleep). Evidence shows that plasma amino acid concentrations peak ~4h post-ingestion of 40g of casein [45]; this prolonged hyperaminoacidemia engendered by pre-sleep casein

ingestion may therefore modulate next morning appetite and energy intake. Importantly, the presence of free amino acids regulate satiety through interaction with the enteroendocrine receptors which stimulates the release of gastrointestinal hormones, CCK and GLP-1, which signal the brain to reduce sensations of appetite [89]. Hall *et al.* (2003) report how 180 minutes after ingestion of a 48g dose of casein, the concentrations of GLP-1 and CCK remain elevated at ~2.5x and ~3x baseline levels [86]. It is possible, then, that concentrations of these hormones may still be higher than baseline the next morning if casein is ingested prior to sleep, and therefore still exerting an effect on the appetite. Research is warranted to ascertain this as these findings could have implications for appetite and subsequent energy (and protein) intake.

With advancing age, a number of factors (including hormone activity, reductions in metabolism, loneliness, cost) contribute towards a reduction in appetite [57-59], thus the potentially satiating properties of protein consumed before sleep could be theorised to impact appetite and energy intake the following morning in older individuals, whom already have a lower appetite. Various research in younger populations has found no effect of pre-sleep protein on next morning appetite compared to other placebo or other beverages [48, 55, 90]. Contrastingly, Ormsbee *et al.* (2016) [91] found that chocolate milk consumed before bed did have a small effect on next-morning appetite in young females - perhaps due to the combination of carbohydrate and protein rather than one macronutrient in isolation as in other pre-sleep protein research [45, 46, 92, 93]. Furthermore, Groen *et al.* (2012) found next-morning appetite to be reduced in elderly men following intragastric administration of 40g of casein during sleep. However, in their research, the protein was administered after subjects had fallen asleep at 0200hrs and not prior to sleep meaning the time between protein ingestion/digestion and appetite measurement was approximately 3 hours less than in our protocol. Whilst necessary for this particular study, intragastric administration is not a feasible

option for the wider population. A simple, commercially available, casein supplement as implemented in the present study is a more pragmatic strategy for the ingestion of pre-sleep protein.

In the present study, no effect of pre-sleep protein on next-morning appetite was found. Notably, however, satiety increased similarly across all three conditions 30 min post-treatment ingestion suggesting that satiety may be attributable to fluid volume rather than content. Ingestion of the beverages (400ml in all three conditions) will have caused an equal amount of gastric distension as the fluid reached the stomach and this distension activates circuits in the brain which signal sensations of satiety [94]. Appetite was measured via VAS a total of 12 times throughout each experimental visit, including before consumption of a lunch and dinnertime meal the day after consuming the pre-sleep beverage. No differences were found between conditions at any of these 12 time-points, which is in agreement with our hypothesis that there would be no effect of condition on appetite and satiety sensations. A 2011 survey by the NPD group found that 10-11% of US adults aged over 55 don't consume breakfast [95] and evidence shows that protein intake at breakfast is typically below the threshold for MPS stimulation in older adults [40]. These data would be compounded if pre-sleep protein adversely affected appetite the following day, our findings clearly demonstrate that this is not the case.

Visual analogue scales are commonly used to measure appetite [70]. However, it is important to note that they may not be effective predictors of actual energy intake. A systematic review by Holt *et al.* (2017) found that over 50% of the 462 studies included in their review failed to show a direct statistical comparison between subjective ratings of appetite and energy intake [96]. Our findings echo this as we found no relationship between pre-breakfast VAS hunger

ratings and energy intake at breakfast. With that being said, as well as appetite scores, no significant differences were found in total energy intake and across treatments, when expressed in absolute values and relative to bodyweight. Furthermore, energy intake during the ad libitum breakfast did not significantly differ from average habitual breakfast energy intake obtained from food diaries. When the macronutrient content of the food consumed at breakfast was assessed, no differences were found in relative quantities of fat, carbohydrate or protein consumed between conditions. To date, only two other studies have accurately assessed next-morning energy intake and perceived appetite following pre-sleep protein consumption. In agreement with our findings, Lay *et al.* (2018) and Kouw *et al.* (2017) found no effect of pre-sleep protein on next-morning energy intake at an ad libitum breakfast [45, 55]. Our findings extend upon this by demonstrating that appetite remained unaffected throughout the remainder of the next-day. Future research should ensure actual energy intake is assessed alongside appetite for the entirety of the following day, not just breakfast, as appetite is not necessarily indicative of energy intake [96]. Research has, however, shown relationships between VAS scores for appetite and the "hunger hormones" which regulate these sensations [97-99]. Although not measured in the present study, the reported relationships between subjective appetite and hunger hormones suggest that next-morning concentrations and fluctuations of ghrelin, leptin, CCK and GLP-1 in our participants were also likely unaffected by pre-sleep protein - as indicated by the lack of difference in measures of subjective appetite.

Notably, although not significantly different, breakfast energy intake tended to be higher outside of experimental trials (as reported in the food diaries), compared to at the ad libitum breakfast consumed during experimental visits. One explanation for this may be the limited food choices participants were presented with for the ad libitum breakfast during experimental trials. The breakfast in the present study was chosen to represent content of an "average"

breakfast and also because all components were easily controlled and measurable. Other research has employed wider variety in an ad libitum breakfast buffet to assess energy intake, such as Giezenaar *et al.* (2015) [60] who offered 16 food and 3 drink choices versus 5 food and 2 drink choices in our study. Future research, with sufficient time and resources, should consider using a wider variety of choice to better reflect the choice that participants would have in their own homes. Furthermore, as breakfast is a target area for increasing protein intake [39], it would be worthwhile to incorporate high-protein breakfast options (eggs, bacon etc) into future research to determine whether the consumption of pre-sleep protein impacts protein intake the following day. Whilst there was no difference in protein intake in the present study, there was limited protein available for consumption by participants with the bread providing the most protein per 100g (8.1g).

RMR has been shown to modulate appetite and energy intake [51, 100]. As such, it is important to consider whether pre-sleep protein affects next-morning RMR as this may subsequently affect sensations of appetite and eating behaviours. Ingestion of casein protein prior to sleep has been shown to elicit increases in next morning RMR in young males [48]. However, in the study of Madzima *et al.* (2014) [48], it is unclear whether subjects were set a standardized bedtime as they were only instructed to consume treatment beverages thirty minutes prior to sleep. Therefore, subjects may have consumed the beverage relatively late in the day, allowing less time for any satiating effects to "wash out", leading to an elevated metabolic rate. Research in female athletes [91] found similar results but once again, Ormsbee *et al.* (2016) [91] state that participants consumed the pre-sleep beverage 7-9 hours prior to the morning visit. Conversely, our findings suggest that 40g of pre-sleep casein did not have any effect on next morning RMR or RER in our cohort of 65-80-year olds, or at least that any metabolic effect that had occurred during the 9 hours between treatment consumption and measurement of

RMR, had worn off. Research suggests that following an evening meal, it takes approximately 8 hours to return to basal metabolic rate [101], which would offer an explanation as to why we saw no differences between conditions where other research (with potentially shorter "wash-out" periods) did find differences. In agreement with our findings, Lay *et al*. (2018) found that pre-sleep consumption of 40g of casein had no effect on next-morning RMR in overweight middle-aged men, which also concurs with Kinsey and colleagues (2016) who found no alterations to next-morning RMR in obese men [92]. RMR makes up around 50-70% of total daily energy expenditure and fat free mass contributes to approximately 70% of RMR [102]. It is therefore intuitive to expect that RMR would be higher in lean individuals, as in studies by Madzima *et al.* (2014) [48] and Ormsbee *et al.* (2016) [91], compared with obese and older individuals, and also offers explanation for an age-associated decline in RMR [103] likely due to age-associated muscle loss [104].

A lack of sleep modulates next-morning RMR [105], has been shown to impact appetite-regulating hormones and increase caloric intake [106-108] and also inhibits anabolic pathways, in turn contributing to sarcopenia progression [109]. It was therefore important to determine whether the consumption of pre-sleep protein had any negative effects on sleep quality in the present study – as this could have had confounding effects on subjective appetite, RMR and energy intake. With that being said, subjective measurements of sleep quality across all four domains of the LSEQ were unaffected by treatment and, furthermore, no significant deviations from habitual baseline sleep quality were observed in any treatment. In some instances, individual participants commented that consuming 400ml of fluid in close proximity to bedtime caused an increase in nocturia, beyond what was regularly experienced, although this was not consistent across all participants. Nocturia increases with age and is a common occurrence in many older adults [110]. As such, consuming liquid before bed may be off-putting to some

older individuals and could lead to reduced adherence to implemented pre-sleep protein strategies through worry of exacerbating existing issues with nocturia-associated sleep disturbances. A solution to this issue could involve obtaining pre-sleep protein in semi-solid and solid form. Leyh *et al.* (2018) found no difference in next-morning metabolic response or hunger when casein was consumed prior to sleep in a semi-solid whole-food form (cottage cheese) versus energy and protein-matched liquid supplement, in healthy young women [111]. Research is warranted to ascertain whether this finding is replicated in older adults as semi-solid and solid pre-sleep protein ingestion may be a more viable and pragmatic strategy to increase daily protein intake without having to consume a significant volume of fluid in close proximity to bedtime.

It is suggested that age-dependent alterations in hormone regulation play a role in the multi-faceted and complex pathogenesis of sarcopenia [112]. The insulinaemic response to food ingestion is associated with sensations of satiety [99] and as such, and modulating effects of pre-sleep protein on the insulin response to food intake may in turn impact energy intake. Furthermore, insulin is an anabolic hormone and acts by not only inhibiting MPB but also by stimulating MPS in the absence of adequate amino acids [113]. Ageing tends to bring concomitant increases in insulin resistance and poor glycaemic control [114], which is independently associated with reduced strength in older individuals [115]. It was therefore important to determine whether ingestion of pre-sleep protein had any effect on glycaemic responses to food consumption the following morning. Herein, we found no such effects, which aligns with our other outcome measures in which pre-sleep protein had no effect on appetite, energy intake and metabolism. Fasting glucose and insulin were similar in all three conditions, as were the insulin responses immediately after and 30 min after breakfast consumption. Furthermore, fasting measures of insulin resistance were not modulated by pre-sleep beverage

condition, although this was only measured acutely in this study. These results suggest that implementation of a pre-sleep protein strategy would not impact the glycaemic response to consumption of a typical mixed-macronutrient breakfast meal in older adults.

Populations at a much higher risk or muscle loss, frailty and sarcopenia are older individuals who are chronically ill and/or hospitalised [116], rather than the healthy individuals used in the present study. Whilst progression of sarcopenia is typical with advancing age, acute bouts of inactivity, e.g. through illness, injury or hospitalisation, can accelerate declines in skeletal muscle size/strength, subsequently expediting sarcopenia onset [117]. Therefore, an adequate daily protein intake is critical in these at-risk populations in an attempt to mitigate losses in muscle mass/function [79, 118]. As such, research should seek to extend our findings into additional populations so ascertain whether our results are mirrored in those more at risk of sarcopenia. If this is the case, pre-sleep protein would present a cheap, feasible and accessible strategy of increasing protein intake in ill and hospitalised individuals, attenuating declines in muscle mass and strength due to inactivity.

CONCLUSION AND PRACTICAL IMPLICATIONS

The results of the present study suggest that 40g of casein protein ingested as a beverage prior to sleep does not adversely affect sleep quality or next-morning indices of appetite, energy intake or metabolism in older individuals. As such, implementation of pre-sleep protein strategies to increase an individual's daily protein consumption is a viable option and should not elicit reductions in energy (and subsequently protein) intake at breakfast time. Consuming a beverage in close proximity to bedtime may be undesirable for those suffering from nocturia, in this case alternative semi-solid and solid sources (such as cottage cheese) could be explored, though research is needed to ensure that these sources do not modulate appetite – as in the present study. Furthermore, assessing energy and protein intake, appetite and activity

throughout the entirety of the day preceding experimental visits will elucidate whether pre-sleep protein has any effect on whole-day behaviours – ensuring that the strategy has no confounding effects on overall daily energy and protein balance. Finally, future research should look to extend our findings into different populations such as those experiencing increased inactivity due to illness or hospitalisation as pre-sleep protein may provide a strategy of mitigating declines in muscle mass and strength.

REFERENCES

1. Landi, F., et al., *Sarcopenia and mortality among older nursing home residents.* J Am Med Dir Assoc, 2012. **13**(2): p. 121-6.
2. Dufour, A.B., et al., *Sarcopenia definitions considering body size and fat mass are associated with mobility limitations: the Framingham Study.* J Gerontol A Biol Sci Med Sci, 2013. **68**(2): p. 168-74.
3. Xue, Q.L., et al., *Prediction of risk of falling, physical disability, and frailty by rate of decline in grip strength: the women's health and aging study.* Arch Intern Med, 2011. **171**(12): p. 1119-21.
4. Lee, J., et al., *Associations of Sarcopenia and Sarcopenic Obesity With Metabolic Syndrome Considering Both Muscle Mass and Muscle Strength.* J Prev Med Public Health, 2016. **49**(1): p. 35-44.
5. Landi, F., et al., *Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study.* Clin Nutr, 2012. **31**(5): p. 652-8.
6. Ethgen, O., et al., *The Future Prevalence of Sarcopenia in Europe: A Claim for Public Health Action.* Calcif Tissue Int, 2017. **100**(3): p. 229-234.
7. Janssen, I., et al., *The healthcare costs of sarcopenia in the United States.* Journal of the American Geriatrics Society, 2004. **52**(1): p. 80-5.
8. Pinedo-Villanueva, R., et al., *Health Care Costs Associated With Muscle Weakness: A UK Population-Based Estimate.* Calcif Tissue Int, 2019. **104**(2): p. 137-144.
9. Nilwik, R., et al., *The decline in skeletal muscle mass with aging is mainly attributed to a reduction in type II muscle fiber size.* Exp Gerontol, 2013. **48**(5): p. 492-8.
10. Piasecki, M., et al., *Failure to expand the motor unit size to compensate for declining motor unit numbers distinguishes sarcopenic from non-sarcopenic older men.* J Physiol, 2018. **596**(9): p. 1627-1637.
11. Mijnarends, D.M., et al., *Physical activity and incidence of sarcopenia: the population-based AGES-Reykjavik Study.* Age Ageing, 2016. **45**(5): p. 614-20.
12. Houston, D.K., et al., *Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study.* Am J Clin Nutr, 2008. **87**(1): p. 150-5.
13. Wahlin-Larsson, B., et al., *Mechanistic Links Underlying the Impact of C-Reactive Protein on Muscle Mass in Elderly.* Cell Physiol Biochem, 2017. **44**(1): p. 267-278.
14. Atherton, P.J. and K. Smith, *Muscle protein synthesis in response to nutrition and exercise.* J Physiol, 2012. **590**(5): p. 1049-57.
15. Wolfe, R.R., *The role of dietary protein in optimizing muscle mass, function and health outcomes in older individuals.* Br J Nutr, 2012. **108 Suppl 2**: p. S88-93.
16. Morton, R.W., C. McGlory, and S.M. Phillips, *Nutritional interventions to augment resistance training-induced skeletal muscle hypertrophy.* Front Physiol, 2015. **6**: p. 245.
17. Biolo, G., et al., *An abundant supply of amino acids enhances the metabolic effect of exercise on muscle protein.* American Journal of Physiology-Endocrinology and Metabolism, 1997. **273**(1): p. E122-E129.
18. Smeuninx, B., et al., *Age-Related Anabolic Resistance of Myofibrillar Protein Synthesis Is Exacerbated in Obese Inactive Individuals.* J Clin Endocrinol Metab, 2017. **102**(9): p. 3535-3545.

19. Bohe, J., et al., *Human muscle protein synthesis is modulated by extracellular, not intramuscular amino acid availability: a dose-response study.* J Physiol, 2003. **552**(Pt 1): p. 315-24.
20. Katsanos, C.S., et al., *Aging is associated with diminished accretion of muscle proteins after the ingestion of a small bolus of essential amino acids.* Am J Clin Nutr, 2005. **82**(5): p. 1065-73.
21. Cuthbertson, D., et al., *Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle.* FASEB J, 2005. **19**(3): p. 422-4.
22. Walrand, S., et al., *Physiopathological mechanism of sarcopenia.* Clin Geriatr Med, 2011. **27**(3): p. 365-85.
23. Breen, L., et al., *Two weeks of reduced activity decreases leg lean mass and induces "anabolic resistance" of myofibrillar protein synthesis in healthy elderly.* J Clin Endocrinol Metab, 2013. **98**(6): p. 2604-12.
24. Ni Lochlainn, M., R.C.E. Bowyer, and C.J. Steves, *Dietary Protein and Muscle in Aging People: The Potential Role of the Gut Microbiome.* Nutrients, 2018. **10**(7).
25. Snijders, T., et al., *Muscle fibre capillarization is a critical factor in muscle fibre hypertrophy during resistance exercise training in older men.* J Cachexia Sarcopenia Muscle, 2017. **8**(2): p. 267-276.
26. Prior, S.J., et al., *Sarcopenia Is Associated With Lower Skeletal Muscle Capillarization and Exercise Capacity in Older Adults.* J Gerontol A Biol Sci Med Sci, 2016. **71**(8): p. 1096-101.
27. Breen, L. and S.M. Phillips, *Skeletal muscle protein metabolism in the elderly: Interventions to counteract the 'anabolic resistance' of ageing.* Nutr Metab (Lond), 2011. **8**: p. 68.
28. Moore, D.R., et al., *Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men.* J Gerontol A Biol Sci Med Sci, 2015. **70**(1): p. 57-62.
29. Bauer, J., et al., *Evidence-based Recommendations for Optimal Dietary Protein Intake in Older People: A Position Paper From the PROT-AGE Study Group.* Journal of the American Medical Directors Association, 2013.
30. Baum, J.I., I.Y. Kim, and R.R. Wolfe, *Protein Consumption and the Elderly: What Is the Optimal Level of Intake?* Nutrients, 2016. **8**(6).
31. Morais, J.A., S. Chevalier, and R. Gougeon, *Protein turnover and requirements in the healthy and frail elderly.* J Nutr Health Aging, 2006. **10**(4): p. 272-83.
32. Hu, F.B., et al., *Dietary protein and risk of ischemic heart disease in women.* Am J Clin Nutr, 1999. **70**(2): p. 221-7.
33. Obarzanek, E., P.A. Velletri, and J.A. Cutler, *Dietary protein and blood pressure.* JAMA, 1996. **275**(20): p. 1598-603.
34. Cardon-Thomas, D.K., et al., *Dietary Protein in Older Adults: Adequate Daily Intake but Potential for Improved Distribution.* Nutrients, 2017. **9**(3).
35. Tieland, M., et al., *Dietary protein intake in community-dwelling, frail, and institutionalized elderly people: scope for improvement.* European journal of nutrition, 2011.
36. Paddon-Jones, D. and B.B. Rasmussen, *Dietary protein recommendations and the prevention of sarcopenia.* Curr Opin Clin Nutr Metab Care, 2009. **12**(1): p. 86-90.
37. Loenneke, J.P., et al., *Per meal dose and frequency of protein consumption is associated with lean mass and muscle performance.* Clin Nutr, 2016. **35**(6): p. 1506-1511.

38. Ten Haaf, D.S.M., et al., *Protein Intake and Distribution in Relation to Physical Functioning and Quality of Life in Community-Dwelling Elderly People: Acknowledging the Role of Physical Activity.* Nutrients, 2018. **10**(4).
39. Mamerow, M.M., et al., *Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults.* J Nutr, 2014. **144**(6): p. 876-80.
40. Kim, I.Y., et al., *Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults: A randomized-controlled trial.* Clin Nutr, 2018. **37**(2): p. 488-493.
41. Murphy, C.H., et al., *Hypoenergetic diet-induced reductions in myofibrillar protein synthesis are restored with resistance training and balanced daily protein ingestion in older men.* Am J Physiol Endocrinol Metab, 2015. **308**(9): p. E734-43.
42. Snijders, T., et al., *The Impact of Pre-sleep Protein Ingestion on the Skeletal Muscle Adaptive Response to Exercise in Humans: An Update.* Front Nutr, 2019. **6**: p. 17.
43. Snijders, T., et al., *Protein Ingestion before Sleep Increases Muscle Mass and Strength Gains during Prolonged Resistance-Type Exercise Training in Healthy Young Men.* J Nutr, 2015. **145**(6): p. 1178-84.
44. Wall, B.T., J.P. Morton, and L.J. van Loon, *Strategies to maintain skeletal muscle mass in the injured athlete: nutritional considerations and exercise mimetics.* Eur J Sport Sci, 2015. **15**(1): p. 53-62.
45. Kouw, I.W., et al., *Protein Ingestion before Sleep Increases Overnight Muscle Protein Synthesis Rates in Healthy Older Men: A Randomized Controlled Trial.* J Nutr, 2017. **147**(12): p. 2252-2261.
46. Holwerda, A.M., et al., *Physical Activity Performed in the Evening Increases the Overnight Muscle Protein Synthetic Response to Presleep Protein Ingestion in Older Men.* J Nutr, 2016. **146**(7): p. 1307-14.
47. Abbott, W., et al., *Presleep Casein Protein Ingestion: Acceleration of Functional Recovery in Professional Soccer Players.* Int J Sports Physiol Perform, 2019. **14**(3): p. 385-391.
48. Madzima, T.A., et al., *Night-time consumption of protein or carbohydrate results in increased morning resting energy expenditure in active college-aged men.* Br J Nutr, 2014. **111**(1): p. 71-7.
49. Baumgartner, R.N., *Body composition in healthy aging.* Ann N Y Acad Sci, 2000. **904**: p. 437-48.
50. Zamboni, M., S. Rubele, and A.P. Rossi, *Sarcopenia and obesity.* Curr Opin Clin Nutr Metab Care, 2019. **22**(1): p. 13-19.
51. Caudwell, P., et al., *Resting metabolic rate is associated with hunger, self-determined meal size, and daily energy intake and may represent a marker for appetite.* Am J Clin Nutr, 2013. **97**(1): p. 7-14.
52. Gibbons, C., et al., *Validation of a new hand-held electronic data capture method for continuous monitoring of subjective appetite sensations.* Int J Behav Nutr Phys Act, 2011. **8**: p. 57.
53. Fulgoni, V.L., 3rd, *Current protein intake in America: analysis of the National Health and Nutrition Examination Survey, 2003-2004.* Am J Clin Nutr, 2008. **87**(5): p. 1554S-1557S.
54. Weigle, D.S., et al., *A high-protein diet induces sustained reductions in appetite, ad libitum caloric intake, and body weight despite compensatory changes in diurnal plasma leptin and ghrelin concentrations.* Am J Clin Nutr, 2005. **82**(1): p. 41-8.
55. Lay, A.H.H., et al., *A bedtime milk snack does not impact RMR, substrate utilisation and appetite the following morning in mildly overweight males.* Br J Nutr, 2018. **119**(12): p. 1355-1365.

56. Morley, J.E., *Anorexia of ageing: a key component in the pathogenesis of both sarcopenia and cachexia.* J Cachexia Sarcopenia Muscle, 2017. **8**(4): p. 523-526.
57. Giezenaar, C., et al., *Ageing Is Associated with Decreases in Appetite and Energy Intake--A Meta-Analysis in Healthy Adults.* Nutrients, 2016. **8**(1).
58. Pilgrim, A.L., et al., *An overview of appetite decline in older people.* Nurs Older People, 2015. **27**(5): p. 29-35.
59. Atalayer, D. and N.M. Astbury, *Anorexia of aging and gut hormones.* Aging Dis, 2013. **4**(5): p. 264-75.
60. Giezenaar, C., et al., *Lesser suppression of energy intake by orally ingested whey protein in healthy older men compared with young controls.* Am J Physiol Regul Integr Comp Physiol, 2015. **309**(8): p. R845-54.
61. Holwerda, A.M., et al., *Body Position Modulates Gastric Emptying and Affects the Post-Prandial Rise in Plasma Amino Acid Concentrations Following Protein Ingestion in Humans.* Nutrients, 2016. **8**(4): p. 221.
62. Vaughn, B.V., S. Rotolo, and H.L. Roth, *Circadian rhythm and sleep influences on digestive physiology and disorders.* ChronoPhysiology and Therapy, 2014. **4**: p. 67-77.
63. Luhovyy, B.L., T. Akhavan, and G.H. Anderson, *Whey proteins in the regulation of food intake and satiety.* J Am Coll Nutr, 2007. **26**(6): p. 704S-12S.
64. Abou-Samra, R., et al., *Effect of different protein sources on satiation and short-term satiety when consumed as a starter.* Nutr J, 2011. **10**: p. 139.
65. Bowen, J., et al., *Energy intake, ghrelin, and cholecystokinin after different carbohydrate and protein preloads in overweight men.* J Clin Endocrinol Metab, 2006. **91**(4): p. 1477-83.
66. Greco, E., et al., *The Role of Source of Protein in Regulation of Food Intake, Satiety, Body Weight and Body Composition.* J Nutr Health Food Eng, 2017. **6**(6).
67. Trommelen, J. and L.J. van Loon, *Pre-Sleep Protein Ingestion to Improve the Skeletal Muscle Adaptive Response to Exercise Training.* Nutrients, 2016. **8**(12).
68. Kinsey, A.W. and M.J. Ormsbee, *The health impact of nighttime eating: old and new perspectives.* Nutrients, 2015. **7**(4): p. 2648-62.
69. Guralnik, J.M., et al., *A short physical performance battery assessing lower extremity function: association with self-reported disability and prediction of mortality and nursing home admission.* J Gerontol, 1994. **49**(2): p. M85-94.
70. Flint, A., et al., *Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies.* Int J Obes Relat Metab Disord, 2000. **24**(1): p. 38-48.
71. Parrott, A.C. and I. Hindmarch, *Factor analysis of a sleep evaluation questionnaire.* Psychol Med, 1978. **8**(2): p. 325-9.
72. Weir, J.B., *New methods for calculating metabolic rate with special reference to protein metabolism.* J Physiol, 1949. **109**(1-2): p. 1-9.
73. Cruz-Jentoft, A.J., et al., *Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS).* Age Ageing, 2014. **43**(6): p. 748-59.
74. Tieland, M., et al., *Protein supplementation improves physical performance in frail elderly people: a randomized, double-blind, placebo-controlled trial.* J Am Med Dir Assoc, 2012. **13**(8): p. 720-6.
75. Vellas, B.J., et al., *Changes in nutritional status and patterns of morbidity among free-living elderly persons: a 10-year longitudinal study.* Nutrition, 1997. **13**(6): p. 515-9.

76. Paddon-Jones, D. and H. Leidy, *Dietary protein and muscle in older persons.* Curr Opin Clin Nutr Metab Care, 2014. **17**(1): p. 5-11.
77. Deutz, N.E. and R.R. Wolfe, *Is there a maximal anabolic response to protein intake with a meal?* Clin Nutr, 2013. **32**(2): p. 309-13.
78. Luiking, Y.C., et al., *Postprandial muscle protein synthesis is higher after a high whey protein, leucine-enriched supplement than after a dairy-like product in healthy older people: a randomized controlled trial.* Nutr J, 2014. **13**: p. 9.
79. Thalacker-Mercer, A.E. and M.J. Drummond, *The importance of dietary protein for muscle health in inactive, hospitalized older adults.* Ann N Y Acad Sci, 2014. **1328**: p. 1-9.
80. Groen, B.B., et al., *Intragastric protein administration stimulates overnight muscle protein synthesis in elderly men.* Am J Physiol Endocrinol Metab, 2012. **302**(1): p. E52-60.
81. Astrup, A., *The satiating power of protein--a key to obesity prevention?* Am J Clin Nutr, 2005. **82**(1): p. 1-2.
82. Luhovyy, B.L., T. Akhavan, and G.H. Anderson, *Whey Proteins in the Regulation of Food Intake and Satiety.* Journal of the American College of Nutrition, 2007. **26**(6): p. 704S-712S.
83. Rigamonti, A.E., et al., *Whey Proteins Reduce Appetite, Stimulate Anorexigenic Gastrointestinal Peptides and Improve Glucometabolic Homeostasis in Young Obese Women.* Nutrients, 2019. **11**(2).
84. Leidy, H.J., et al., *The role of protein in weight loss and maintenance.* Am J Clin Nutr, 2015. **101**(6): p. 1320S-1329S.
85. Bendtsen, L.Q., et al., *Effect of dairy proteins on appetite, energy expenditure, body weight, and composition: a review of the evidence from controlled clinical trials.* Adv Nutr, 2013. **4**(4): p. 418-38.
86. Hall, W.L., et al., *Casein and whey exert different effects on plasma amino acid profiles, gastrointestinal hormone secretion and appetite.* Br J Nutr, 2003. **89**(2): p. 239-48.
87. Luiking, Y.C., et al., *Protein type and caloric density of protein supplements modulate postprandial amino acid profile through changes in gastrointestinal behaviour: A randomized trial.* Clin Nutr, 2016. **35**(1): p. 48-58.
88. Wolfe, R.R., *Regulation of muscle protein by amino acids.* J Nutr, 2002. **132**(10): p. 3219S-24S.
89. Hajishafiee, M., V. Bitarafan, and C. Feinle-Bisset, *Gastrointestinal Sensing of Meal-Related Signals in Humans, and Dysregulations in Eating-Related Disorders.* Nutrients, 2019. **11**(6).
90. Kinsey, A.W., et al., *Influence of night-time protein and carbohydrate intake on appetite and cardiometabolic risk in sedentary overweight and obese women.* Br J Nutr, 2014. **112**(3): p. 320-7.
91. Ormsbee, M.J., et al., *Nighttime feeding likely alters morning metabolism but not exercise performance in female athletes.* Appl Physiol Nutr Metab, 2016. **41**(7): p. 719-27.
92. Kinsey, A.W., et al., *The Effect of Casein Protein Prior to Sleep on Fat Metabolism in Obese Men.* Nutrients, 2016. **8**(8).
93. Madzima, T.A., et al., *Pre-Sleep Consumption of Casein and Whey Protein: Effects on Morning Metabolism and Resistance Exercise Performance in Active Women.* Nutrients, 2018. **10**(9).
94. Wang, G.J., et al., *Gastric distention activates satiety circuitry in the human brain.* Neuroimage, 2008. **39**(4): p. 1824-31.

95. *31 Million U.S. Consumers Skip Breakfast Each Day, Reports NPD*. 2011 [cited 2019 16th August]; Available from: https://www.npd.com/wps/portal/npd/us/news/press-releases/pr_111011b/.
96. Holt, G.M., et al., *Systematic literature review shows that appetite rating does not predict energy intake.* Crit Rev Food Sci Nutr, 2017. **57**(16): p. 3577-3582.
97. Erdmann, J., et al., *Food intake and plasma ghrelin response during potato-, rice- and pasta-rich test meals.* Eur J Nutr, 2007. **46**(4): p. 196-203.
98. Flint, A., et al., *Associations between postprandial insulin and blood glucose responses, appetite sensations and energy intake in normal weight and overweight individuals: a meta-analysis of test meal studies.* Br J Nutr, 2007. **98**(1): p. 17-25.
99. Martens, M.J., et al., *Satiating capacity and post-prandial relationships between appetite parameters and gut-peptide concentrations with solid and liquefied carbohydrate.* PLoS One, 2012. **7**(7): p. e42110.
100. Blundell, J.E., et al., *The biology of appetite control: Do resting metabolic rate and fat-free mass drive energy intake?* Physiol Behav, 2015. **152**(Pt B): p. 473-8.
101. Westerterp, K.R., *Diet induced thermogenesis.* Nutr Metab (Lond), 2004. **1**(1): p. 5.
102. Cunningham, J.J., *Body composition as a determinant of energy expenditure: a synthetic review and a proposed general prediction equation.* Am J Clin Nutr, 1991. **54**(6): p. 963-9.
103. Frisard, M.I., et al., *Aging, resting metabolic rate, and oxidative damage: results from the Louisiana Healthy Aging Study.* J Gerontol A Biol Sci Med Sci, 2007. **62**(7): p. 752-9.
104. Bosy-Westphal, A., et al., *The age-related decline in resting energy expenditure in humans is due to the loss of fat-free mass and to alterations in its metabolically active components.* J Nutr, 2003. **133**(7): p. 2356-62.
105. Spaeth, A.M., D.F. Dinges, and N. Goel, *Resting metabolic rate varies by race and by sleep duration.* Obesity (Silver Spring), 2015. **23**(12): p. 2349-56.
106. Greer, S.M., A.N. Goldstein, and M.P. Walker, *The impact of sleep deprivation on food desire in the human brain.* Nat Commun, 2013. **4**: p. 2259.
107. Hanlon, E.C. and E. Van Cauter, *Quantification of sleep behavior and of its impact on the cross-talk between the brain and peripheral metabolism.* Proc Natl Acad Sci U S A, 2011. **108 Suppl 3**: p. 15609-16.
108. Brondel, L., et al., *Acute partial sleep deprivation increases food intake in healthy men.* Am J Clin Nutr, 2010. **91**(6): p. 1550-9.
109. Piovezan, R.D., et al., *The impact of sleep on age-related sarcopenia: Possible connections and clinical implications.* Ageing Res Rev, 2015. **23**(Pt B): p. 210-20.
110. Kujubu, D.A. and S.R. Aboseif, *Evaluation of nocturia in the elderly.* Perm J, 2007. **11**(1): p. 37-9.
111. Leyh, S.M., et al., *Pre-sleep protein in casein supplement or whole-food form has no impact on resting energy expenditure or hunger in women.* Br J Nutr, 2018. **120**(9): p. 988-994.
112. Short, K.R., et al., *Decline in skeletal muscle mitochondrial function with aging in humans.* Proc Natl Acad Sci U S A, 2005. **102**(15): p. 5618-23.
113. Chow, L.S., et al., *Mechanism of insulin's anabolic effect on muscle: measurements of muscle protein synthesis and breakdown using aminoacyl-tRNA and other surrogate measures.* Am J Physiol Endocrinol Metab, 2006. **291**(4): p. E729-36.
114. Muller, D.C., et al., *The effect of age on insulin resistance and secretion: a review.* Semin Nephrol, 1996. **16**(4): p. 289-98.

115. Abbatecola, A.M., et al., *Discovering pathways of sarcopenia in older adults: a role for insulin resistance on mitochondria dysfunction.* J Nutr Health Aging, 2011. **15**(10): p. 890-5.
116. Welch, C., et al., *Acute Sarcopenia Secondary to Hospitalisation - An Emerging Condition Affecting Older Adults.* Aging Dis, 2018. **9**(1): p. 151-164.
117. Bell, K.E., et al., *Muscle Disuse as a Pivotal Problem in Sarcopenia-related Muscle Loss and Dysfunction.* J Frailty Aging, 2016. **5**(1): p. 33-41.
118. Murphy, C.H., S.Y. Oikawa, and S.M. Phillips, *Dietary Protein to Maintain Muscle Mass in Aging: A Case for Per-meal Protein Recommendations.* J Frailty Aging, 2016. **5**(1): p. 49-58.