Title: Effects of pre-meal whey protein consumption on acute food intake and energy balance over a 48-hour period.

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Abstract

The effects of pre-meal whey protein consumption on acute food intake and subsequent energy balance measured over 48-h was investigated in males of healthy-weight (HW) or living with overweight and obesity (OV/OB). On two separate trial days, following a controlled breakfast (09:00) and lunch (13:00), 12 HW and 12 OV/OB males consumed either whey protein (20g) or flavoured water beverages (16:40), and *ad libitum* test meal (17:00). A controlled 48-h assessment of energy intake and expenditure was used to determine any compensatory behaviour. Test meal energy intake reduced 15.9% in HW (P=0.003), and 17.8% in OV/OB (P=0.005) following whey protein, compared to placebo. We report no between-group differences and no changes in compensatory behaviour. A small dose of whey protein reduces energy intake at the next meal, without upregulating compensatory behaviours in both HW and OV/OB males. However, chronic effects on body composition and weight loss remain to be elucidated.

Study Highlights:

- Whey protein (20 g) reduced energy intake in both HW (193.4 kcal, 15.9%) and OV/OB (215.81 kcal, 17.8%) when consumed 20 min prior to mealtime, compared to placebo.
- Energy deficits induced in an *ad libitum* test meal did not upregulate compensatory behaviours over the following 48 hours.
- A slowed eating rate may influence food intake following premeal whey ingestion.

Key Words: Appetite, Energy balance, Obesity, Whey protein.

Author Contributions: The authors' responsibilities were as follows — DK, GT, DP and DB designed the research; DK conducted the research; DK, GT, and DP analysed the data; DK, GT, DP and DB wrote the manuscript; DK had primary responsibility for the final content; and all authors read and approved the final manuscript. Declarations of interest: none.

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1. Introduction

Dietary protein intake induces satiety and reduces food intake to a greater extent than other macronutrients (Poppitt *et al*., 1998; Speakman, 2022). Milk proteins are of specific interest in the management of overweight and obesity due to strong associations between high dairy consumption and low body mass (Phillips *et al*., 2003; Lu *et al*., 2016). Indeed, whey protein has been reported to suppress appetite and subsequent food intake in comparison to placebo or isocaloric doses of casein, egg or soy protein in healthy individuals and people with obesity (Akhavan *et al*., 2010; Pal & Ellis, 2010; Poppitt *et al*., 2011; Zafar *et al*., 2013). This may be due to the abundance of branched chain amino acids (BCAAs) and bioactive peptides in whey protein, providing a wide range of physiologic functions including delayed gastric emptying and stimulation of appetite satiating hormones (Madureira *et al*., 2010; Stanstrup *et al*., 2014; King *et al*., 2018). Physiologically, 20 min appears to be the minimum interval for postabsorptive effects of the preload to influence energy intake (Booth *et al*., 1976), with inter-meal intervals (IMI) of between 20-120 min reported to reduce food intake in adults (Almiron-Roig *et al*., 2013). The smallest

efficacious dose of whey protein required to suppress food intake was 20 g when IMI was 30 min (Akhavan *et al*., 2010).

Despite the acute appetite supressing effects of whey protein, when no concomitant dietary or exercise intervention is undertaken, the effects of chronic supplementation of whey protein on body composition are equivocal (Pal & Ellis, 2010; Baer *et al*., 2011). Baer *et al.* (2011) reported reductions in body mass and waist circumference following 23 weeks of whey protein supplementation (56 g/d) in people with obesity when compared to isoenergetic (1670 kJ/d) carbohydrate. In contrast, Pal and Ellis (2010) reported no change in body composition after 12-week supplementation (54 g/d) when compared to isoenergetic glucose supplementation. This may, in part, be attributed to compensatory adaptations in other components contributing to energy balance. Previous short-term feeding studies have failed to identify whether the energy deficits reported were sustained in the longer-term, with no quantification of energy expended through physical activity and diet induced thermogenesis (DIT). Furthermore, basal metabolic rate (BMR) and daily energy requirements are typically estimated using physical activity questionnaires for which validity is questionable (Neilson *et al*., 2008).

The objectives of this study were to determine the effects of whey protein consumption (20 g) 20 minutes prior to an *ad libitum* test meal on acute food intake and energy balance over the subsequent 48-hours in males of healthy-weight or with overweight and obesity. Therefore, this study is designed to advance our understanding of the appetite suppressing effects of whey protein and identify

compensatory behaviour following whey protein ingestion, which could optimise body mass management strategies. We hypothesized that a significant reduction in food intake would be observed following whey ingestion at the *ad libitum* meal, but that compensatory adjustments in behaviour would negate the energy deficit over the subsequent 2 days. Secondly, we hypothesized that a greater degree of compensation would be observed in the people living with overweight and obesity.

2. Methods

2.1 Participants

The study population consisted of 12 healthy-weight males (HW, 20.0-24.99 kg/m^2) and 12 males living with overweight or obesity (OV/OB, >25kg/m^2) aged 18-65 years, without diagnosed metabolic/autoimmune disease, common food allergens, intolerances, or dietary restrictions. Breakfast skippers, smokers, dieters and those currently taking prescribed or over-the-counter medications that influence appetite or gastric motility were excluded. Restrained eaters were identified for exclusion by a score of >12 on the cognitive restraint scale of the 51 Item Three Factor Eating Questionnaire (Stunkard & Messick, 1985). Female participants were excluded due to the time constraints of not being able to control for the effect of the menstrual cycle on dietary and physical activity behaviour (Buffenstein *et al*., 1995). Participants were recruited from Northumbria University and external local organisations through poster advertisement, and local areas of Newcastle-upon-Tyne through radio broadcast advertisements. Participants were financially compensated (£20 shopping voucher). The present study was conducted in accordance with the Declaration of Helsinki and the procedures were approved by

117 the Northumbria University Ethics Committee (REF: HLSDK090916). Written
118 informed consent was obtained from all participants prior to enrolment and they had
119 the right to withdraw at any time.

120

121 2.2 Pre-trial procedures

122 For pre-trial procedures participants arrived at the Northumbria University laboratory
123 following an overnight fast (10-12 h) but allowed water *ad libitum*. Participants
124 removed footwear and excessive clothing to permit accurate stature, body mass and
125 BMI measurements (Seca, Hamburg, Germany). Resting metabolic rate (RMR) was
126 assessed by indirect calorimetry using an online gas analyser (Oxycon Pro,
127 CareFusion, USA). Participants lay supine for 30 minutes while metabolic gas
128 exchange parameters of oxygen consumption ($\dot{V}O_2$) and expired carbon dioxide
129 ($\dot{V}CO_2$) were collected for 20 min. Alcohol and caffeine use and strenuous exercise
130 were prohibited for 24 h and 48 h, respectively as Rocha *et al.* (2006) has shown
131 that even a light bout of intensity activity can influence energy balance in the days
132 following exercise. RMR was calculated using the abbreviated Weir equation (((3.94
133 * $\dot{V}O_2$) + (1.106 * $\dot{V}CO_2$)) * 1.44) (Weir, 1949).

134

135 On the same visit, participants were familiarised to the *ad libitum* test meal
136 procedures and university food laboratory. Participants were separated into
137 individual feeding booths whilst personal possessions such as smart phones and
138 computers were prohibited to minimise distractions, and the potential viewing of time
139 or food cues. A uniform, homogenous meal of pasta (Tesco fusili pasta, UK) and
140 tomato sauce (Lloyd Grossman, UK) (100g; 614 kJ, 145 kcal, 4% fat, 82%

carbohydrate, 14% protein) was served until the participant signalled that they were sufficiently full and satisfied. Interactions between the principal investigator were minimised but bowls were removed from the participant before the contents were fully consumed and replenished. Food intake was recorded by weighing the bowls before and after consumption, meal-time duration was also recorded.

Two days preceding experimental trials, Actiheart accelerometers (Actiheart, CamNtech, United Kingdom) were fitted to participants, providing valid assessments of energy expenditure via a combination of heart rate monitoring and movement registration (Lof *et al*., 2013). The accelerometer attached onto two ECG electrodes (3M Health Care, Canada) placed at 12-lead positions V1 and V4 and worn for 4 days, collecting data in 1-minute intervals. Participants also received weighing scales to assist accurate reporting of daily food intake for diary reporting and were guided in their use. Weighing measurements of all ingredients was carried out twice in repetition to ensure accurate reporting. Participants were asked to replicate dietary choices in the 24 h prior to each main trial. To assist this standardisation, pre-packaged meals (Tesco cottage pie, UK) and snacks (Nature Valley bar, UK) were provided to be consumed in the evening prior to each trial with the aim of normalising appetite perceptions, glucose metabolism and gut hormone parameters (Chandarana *et al*., 2009).

2.3 Protocol

Participants were studied on three separate occasions with 7 d between each study visit. Trials were conducted in a randomized, single-blind, crossover design. Trial

sequences were randomly assigned with the use of a computerised random-number generator (www.randomization.com). Participants were instructed to avoid consuming any alcoholic beverages and conducting any strenuous physical exercise 24 h prior to the study day. A schematic of the main trial protocol is presented in Figure 1. Participants arrived at 08:45 following an overnight fast and completed visual analogue scales (VAS) to rate subjective hunger, fullness, prospective fullness, desire to eat, thirst, mood and nausea on a 100 mm continuum (Flint *et al.*, 2000). At 09:00, a fixed-nutrient breakfast meal containing cereal (Cheerios, Nestle, UK) and whole milk (Tesco, UK), equivalent to 15% of RMR, was consumed within 15 minutes. At 13:00 a fixed-nutrient lunch meal of chicken soup (Heinze, UK), crisps (Kettle Foods, UK) and oat bar (Nature Valley, UK), equal to 35% of RMR, was consumed within 15 minutes. VAS were recorded every 30 minutes throughout the study day.

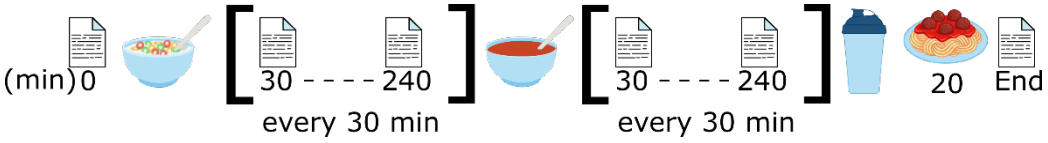

Figure 1. Schematic of experimental trial protocol. Document symbol denotes VAS completion, food images denote breakfast/lunch/evening meal, drinks bottle denotes beverage consumption.

At 16:40, participants received either whey protein (20 g, Lacprodan©, Arla Foods Ingredients Group, Denmark) or flavoured water placebo to be consumed as quickly as possible and within 1 minute. The mixture of whey protein was achieved using protein shakers (Smart Shake, UK) with a standardised vigorous mixing time of 15

188 seconds. Drinks were served in a ready-made 150 ml opaque bottle with calorie-free
189 citrus flavoured sweetener (20 ml, Fun One, Germany) added to both beverages to
190 standardise taste and palatability which was tested previously (King *et al*., 2018).
191 VAS were recorded to measure subjective ratings for how pleasant, salty, bitter,
192 sweet, creamy, thick, sticky, fruity, and refreshing the preload tasted. Nutritional
193 composition of the Lacprodan© whey protein powder is shown in Table 1.

194

195 At 17:00, participants commenced consumption of the *ad libitum* test meal, as
196 described above. VAS were recorded once fullness was signalled. Upon departure
197 from the laboratory, participants were required to complete diet diaries for 48 hours
198 using the provided weighing scales. Nutrition analysis was performed on software
199 (Microdiet, Downlee systems LTD, UK) along with item packaging, to determine the
200 composition and nutritional content of foods consumed.

201

Table 1. Nutritional composition of Lacprodan© whey protein concentrate

Chemical / Nutritional Specification	Value
Energy per 100 g	1583 kJ / 377 kcal
Lactose	2.0 %
Fat	2-6 %
Ash	2.5 %
Moisture	5.5 %
Sodium (Na)	0.2 %
Magnesium (Mg)	0.1 %
Phosphorus (P)	0.3 %
Calcium (Ca)	0.4 %
Iron (Fe)	20 ppm

ppm, parts per million

202

2.4 Statistical approach, analysis and power

Sample size calculation was determined to identify the minimal clinically important difference set to 180 kcal. Previous research suggests a within-subjects standard deviation of 120 kcal for *ad libitum* food intake in healthy-weight individuals and people living with obesity (Seimon *et al*., 2013). Therefore, using a 2-tailed *p*-value of 0.05, 10 healthy-weight, and 10 people with overweight or obesity were needed to reject the null hypothesis that the population means are equal with a power of 90%. The sample size calculation was processed using the software Power and Sample Size Calculations (PS) (Dupont & Plummer, 1998). To account for potential drop-out, a sample size of 12 healthy-weight males and 12 males with overweight or obesity were targeted.

All hypotheses were specified *a priori*. All data were analysed using the Statistical Package for the Social Sciences (SPSS 24, IBM, United States) and reported as means and their standard deviation (mean ± SD). Tests of normality and sphericity were performed using the Shapiro-Wilk test, and Mauchly's test, respectively. Composite appetite score (mm) was calculated as [desire to eat + hunger + (100 − fullness) + prospective food consumption]/4 (Anderson *et al*., 2002). Paired *t*-tests were used to examine differences between trials for HW and OV/OB groups for outcomes including food intake and physical activity energy expenditure. To identify the effect of the intervention between groups, a mixed-model ANOVA was conducted (within-subjects' variables [Treatment]; between-subjects' variables [Group]). Area under the curve was calculated using the trapezoid method. Relationships between variables were assessed using Pearson's linear correlations

assuming normality of data. A *p*-value ≤0.05 was regarded as being statistically significant. The analytic plan was pre-specified, and any data-driven analyses are clearly identified and discussed appropriately.

3. Results

3.1 Participants

Twenty-four males completed the study (Table 2). With the exception of body mass and BMI, there were no significant differences in baseline characteristics. All preloads were tolerated by participants.

TABLE 2. Participant characteristics categorised by body mass status

Characteristic	HW group (n =12)	OV/OB group (n =12)
Age (y)	29.3 ± 10.3	36.2 ± 12.5
Stature (m)	1.8 ± 0.1	1.8 ± 0.1
Body mass (kg)	77.0 ± 11.5*	94.8 ± 17.9*
BMI (kg/m^2)	22.8 ± 2.2*	29.6 ± 6.9*
RMR (kcal/day)	1941 ± 410	2112 ± 195
Restraint Score	8.1 ± 1.2	9.3 ± 1.1
PAL	1.27 ± 0.1	1.25 ± 0.1

Data presented as $\bar{x}$ ± SD. BMI, Body mass index; RMR, Resting metabolic rate; PAL, Physical activity level. Physical Activity Level (PAL) was calculated by dividing participants' total daily energy expenditure by RMR. * denotes significant difference between groups (p < 0.05).

3.2 Pre-laboratory standardisation

Self-reported dietary intake during the 24 h prior to each main trial was similar for both HW (Whey 2119.0 ± 553.7 kcal; Control 2223.8 ± 500.4 kcal, $t_{(11)}$ = -.869, 95% CI -369.9 to 160.5, p = .403) and OV/OB (Whey 1988.1 ± 523.7; Control 2034.8 ±

454.1, $t_{(10)}$ = -.544, 95% CI -237.9 to 144.6, p = .598) groups. Similarly, no differences were observed in physical activity energy expenditure (PAEE) for the 24 h preceding each trial in both HW (Whey 415.8 ± 74.2 kcal; Control 443.6 ± 104.7 kcal, $t_{(10)}$ = -.763, 95% CI -109.0 to 53.4, p = .463) and OV/OB males (Whey 410.5 ± 300.5 kcal; Control 476.2 ± 241.2 kcal, $t_{(9)}$ = -.853, 95% CI -239.9 to 108.5, p = .416).

3.3 Laboratory standardisation

Figures 2 and 3 show time-course changes in self-reported ratings of hunger, fullness, desire to eat, and prospective food intake after breakfast and lunch, respectively, in HW and OV/OB participants. All fasting self-reported ratings of appetite were similar upon arrival at the laboratory prior to breakfast during whey and control trials for HW and OV/OB participants (p > 0.05). Standardisation of all appetite sensations was achieved with no differences in total postprandial AUC following breakfast and lunch during whey and control trials in both HW and OV/OB males (p > 0.05), as presented in Table 3. Composite appetite scores were also similar following breakfast and lunch meals in HW and OV/OB males during both trials (p > 0.05; Figure 2).

Table 3. Postprandial areas under the curve (AUCs) for self-reported ratings of appetite following control and whey trials post-breakfast and post-lunch in HW and OV/OB males.

$AUC_{0\text{-}240min}$ ($cm \cdot min^{-1}$)	**HW Group (n=12)**		**OV/OB Group (n=12)**	
	Control	**Whey**	**Control**	**Whey**

Breakfast				
Hunger	1387 ± 384	1365 ± 284 (-1.6%)	915 ± 408	1031 ± 422 (12.7%)
Fullness	823 ± 436	774 ± 394 (-5.9%)	887 ± 325	853 ± 332 (-3.8%)
DTE	1419 ± 370	1441 ± 273 (1.6%)	1065 ± 384	1154 ± 342 (8.4%)
PI	1511 ± 322	1512 ± 287 (0.0%)	1368 ± 332	1432 ± 332 (4.7%)
Lunch				
Hunger	1274 ± 429	1213 ± 315 (-4.8%)	798 ± 478	917 ± 464 (14.8%)
Fullness	947 ± 339	1011 ± 284 (6.8%)	1141 ± 356	1088 ± 301 (-4.6%)
DTE	1451 ± 408	1311 ± 308 (-9.6%)	921 ± 491	1044 ± 474 (13.3%)
PI	1514 ± 412	1377 ± 277 (-9.1%)	1162 ± 387	1293 ± 443 (11.4%)

Data are presented as $\bar{x}$ ± SD, percentages represent change as a percentage of the control trial. DTE, desire to eat; PI, prospective food intake.

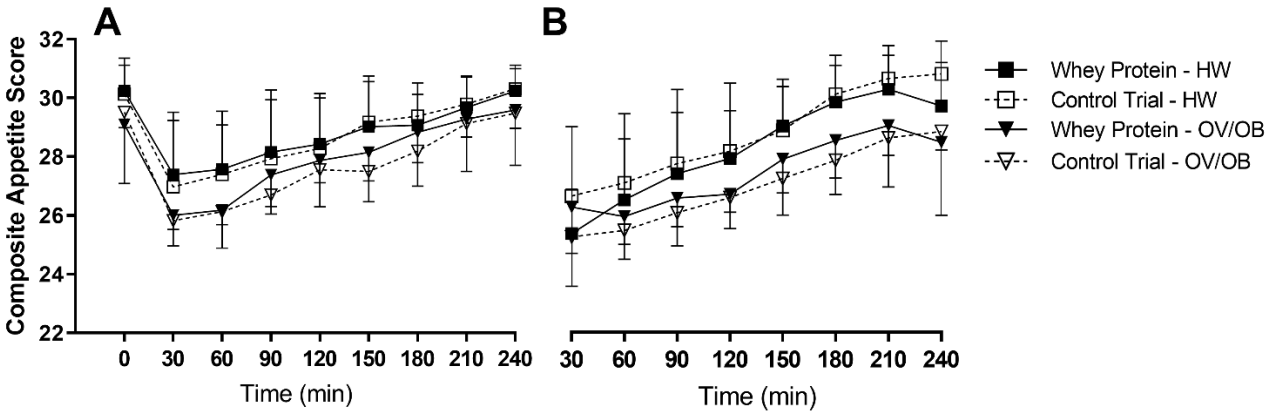

Figure 2. Time-course changes in composite appetite scores ([desire to eat + hunger + (100 - fullness) + prospective consumption]/4) following breakfast (A) and lunch (B) during whey and control trials in HW and OV/OB individuals (*n* = 12 for HW and OV/OB). Data are presented as mean ± SD. No between group differences were observed (p>0.05).

3.4 Laboratory phase

Figure 3 illustrates group mean and individual change in energy intake at the test meal in HW and OV/OB males following whey and control preloads. When compared to control trials, a reduction in test meal energy intake was observed following whey

preloads of 15.9% in HW (Whey 1023.2 ± 545.8 kcal; Control 1216.5 ± 483.5 kcal, 95% CI -302.7 to -84.0, p = .003) and 17.8% in OV/OB (Whey 997.9 ± 248.2 kcal; Control 1213.7 ± 267.6 kcal, 95% CI -352.4 to -79.2, p = .005) males. Total meal consumption time was greater following whey preloads in both HW (Whey 15.8 ± 2.7 min; Control 14.08 ± 3.2 min, MD = 1.67 min, $t_{(11)}$ = 3.708, p = .003) and OV/OB (Whey 19.58 ± 1.35 min; Control 17.83 ± 1.36 min, MD = 1.75 min, $t_{(11)}$ = 3.656, p = .004) groups. Upon the cessation of whey and control test meals, composite appetite scores were similar in HW (Whey 23.0 ± 0.3; Control 23.1 ± 0.7 kcal, $t_{(11)}$ = -.489, p = .635) and OV/OB (Whey 23.4 ± 0.7; Control 23.4 ± 0.7, $t_{(11)}$ = .032, p = .975) groups. No significant differences were observed in measures of food intake between HW and OV/OB groups (p > 0.05).

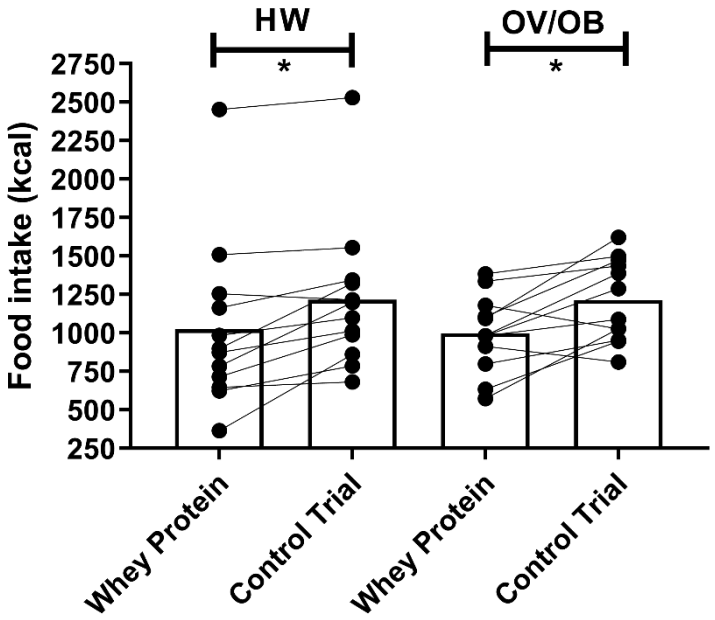

Figure 3. Caloric intake at the *ad libitum* test meal for HW and OV/OB individuals following whey protein and placebo trials (n = 12 for HW and OV/OB). Data presented mean bar and individual before and after plot. * denotes whey and control trials different (HW, p = 0.003; OV/OB, p = 0.005).

3.5 Post-laboratory phase

In the evening of the test meal, there were no compensatory behaviours in food intake in response to whey and control trials, with similar caloric intake reported for HW (Whey 310.9 ± 268.5 kcal; Control 363.1 ± 210.3 kcal, MD = -52.10, $t_{(11)}$ = -.886, 95% CI -183.2 to 78.9, p = .397) and OV/OB groups (Whey 405.6 ± 449.3 kcal; Control 442.9 ± 495.0 kcal, MD = -37.38, $t_{(11)}$ = -.312, 95% CI -304.7 to 229.9, p = .762). Similarly, no compensatory behaviour in PAEE was observed in the evening following the discharge of participants from laboratory for HW (Whey 203.8 ± 36.4 kcal; Control 218.5 ± 52.3 kcal, $t_{(9)}$ = -1.014, 95% CI -77.7 to 29.6, p = .337) and OV/OB (Whey 258.1 ± 159.7 kcal; Control 255.7 ± 113.6 kcal, $t_{(7)}$ = .032, 95% CI -178.9 to 183.7, p = .976) males.

On the first day post-trial, daily caloric intake following whey and control trials was similar for HW (Whey 2203.0 ± 586.1 kcal; Control 2229.2 ± 447.6 kcal, $t_{(11)}$ = -.312, 95% CI -309.8 to 257.4, p = .843) and OV/OB males (Whey 2484.9 ± 927.3 kcal; Control 2600.7 ± 1282.1 kcal, $t_{(9)}$ = -.384, 95% CI -797.7 to 566.3, p = .710). Similarly, no differences were observed in PAEE in the day following main trials in HW (Whey 429.7 ± 137.5 kcal; Control 447.1 ± 154.5 kcal, $t_{(10)}$ = -.518, 95% CI -101.7 to 63.3, p = .616) and OV/OB (Whey 615.3 ± 183.9 kcal; Control 585.7 ± 312.5 kcal, $t_{(8)}$ = -.155, 95% CI -339.5 to 296.6, p = .880) males.

On the second day following trials, no differences between conditions were observed for caloric intake in HW (Whey 2350.4 ± 508.5 kcal; Control 2092.1 ± 647.8 kcal, $t_{(11)}$

323 = 1.136, 95% CI -242.3 to 759.0, p = .280) or OV/OB (Whey 2315.5 ± 716.7 kcal;
324 Control 2536.2 ± 1252.3 kcal, $t_{(9)}$ = -1.208, 95% CI -634.1 to 192.6, p = .258)
325 individuals. Likewise, no differences were observed between trials for PAEE in HW
326 (Whey 439.0 ± 128.6 kcal; Control 426.1 ± 83.5 kcal, $t_{(7)}$ = .224, 95% CI -34.6 to
327 41.8, p = .829) and OV/OB (Whey 461.9 ± 131.3 kcal; Control 466.6 ± 295.8 kcal, $t_{(6)}$
328 = -.158, 95% CI -294.8 to 259.1, p = .880) males.

329

330 **4. Discussion**

331 We report a statistically significant and meaningful reduction in food intake following
332 whey protein ingestion in both HW (193.4 kcal, 15.9%) and OV/OB (215.81 kcal,
333 17.8%), when compared to placebo. Considering that 68 kcal was consumed as part
334 of the whey protein preload, the net energy deficits achieved during the test meal
335 were 125.4 kcal and 147.81 kcal for HW and OV/OB, equating to 86 g and 102 g of
336 pasta respectively. Interestingly, our analysis on self-reported diet diaries and
337 physical activity accelerometery detected no significant differences in compensatory
338 behaviour over the following 2 days.

339

340 There is strong evidence from earlier studies that a whey protein preload reduces
341 food intake at a subsequent test meal in healthy, lean populations (Hall *et al*., 2003;
342 Anderson & Moore, 2004; Akhavan *et al*., 2010; Astbury *et al*., 2010; Zafar *et al*.,
343 2013; Chungchunlam *et al*., 2017) with few conflicting studies (Chungchunlam *et al*.,
344 2009). However, in studies including people living with overweight and obesity, the
345 satiating effect of whey protein is more unclear, reporting reduced food intake
346 (Bowen *et al*., 2006; Zafar *et al*., 2013), or no effect (Bowen *et al*., 2007; Poppitt *et*

al., 2011). When compared to a glucose preload, two studies reported significant reductions in food intake in overweight cohorts (BMI; 30.7 ± 2.5 kg/m^2; 30.1 ± 1.1 kg/m^2) by 15.6% and 10% following the ingestion of 25 g and 50 g whey protein 180 min prior to a test meal, respectively (Bowen *et al*., 2006; Zafar *et al*., 2013). Conversely, Bowen *et al*. (2007) observed increased VAS-rated fullness following 50 g whey when compared to fructose, but no significant difference in energy intake when ingested 240 min prior to a buffet meal in people with obesity (32.5 ± 0.6 kg/m^2). Similarly, Poppitt *et al*. (2011) reported that 20 g whey ingestion suppressed immediate postprandial measures of satiety, however the effects were short-term and not sufficient to significantly impact on subsequent food intake when measured 2 h later.

Therefore, to the authors knowledge this is the first study to investigate the effects of a low dose of whey protein on food intake when ingested only 20 min before an *ad libitum meal* in males with overweight and obesity. The anorexigenic effect we report may be due to the release of several gut peptides including cholecystokinin (CCK), glucagonlike peptide 1 (GLP-1), peptide YY (PYY) and insulin, along with the suppression of acylated ghrelin (Hall *et al*., 2003; Batterham *et al*., 2006; Blom *et al*., 2006; El Khoury *et al*., 2006; Burton-Freeman, 2008; Foster-Schubert *et al*., 2008), although the evidence for the latter is inconsistent (Cummings, 2006; Lejeune *et al*., 2006). The present experimental protocol did not allow for the investigation of these possible mechanisms which is a limitation, although it is possible that the small inter-meal interval may have aligned the test meal alongside peak GLP-1 and insulin concentrations, resulting in reduced energy intake (Bowen *et al*., 2006; Ma *et al*., 2009). However, postprandial hormone responses do not always translate into a

more satiating effect of a given protein (Veldhorst *et al*., 2009; Juvonen *et al*., 2011). Furthermore, ingestion of whey protein preloads < 90 min prior to feeding have been shown to slow gastric emptying, as assessed by the plasma concentrations of oral paracetamol consumed with the meal (Ma *et al*., 2009; Akhavan *et al*., 2014). In the present investigation, the reduction in food intake following whey ingestion may also be attributed to the reduced eating rate in both HW (19%) and OV/OB (21.0%). Indeed, evidence suggests that a slowed eating rate reduces energy intake (Bolhuis *et al*., 2014) and similar responses have been observed when whey protein is ingested after resistance exercise in males (Monteyne *et al*., 2018) but not females (Martin *et al*., 2007).

Our results suggest that a low dose of whey protein, consumed 20 min before a meal, elicits a meaningful energy deficit in both HW and people living with OV/OB , without compensatory changes in food intake and physical activity over the following 2 days. Therefore, in theory, weight loss strategies could incorporate whey protein as a tool to achieve long-term energy deficits. However, the feasibility of such a strategy remains unclear since little is known about the effects of chronic whey protein ingestion on food intake and body composition in people with overweight or obesity. Future research should focus on identifying whether the effect of whey protein preloading on energy intake persists with regular exposure, similar to its effects on reducing postprandial glycaemia over 4 weeks (Ma *et al*., 2015). Furthermore, unlike previous studies administering carbohydrate control groups (Pal & Ellis, 2010; Baer *et al*., 2011), comparisons between whey protein and no intervention would capture the effects of the caloric burden ingested within the preload within free-living conditions.

The current investigation was robust in its design to ensure standardisation of appetite perceptions and gut hormone parameters between trials. This design allows direct observation of energy intake at the breakfast, lunch and evening test meal in controlled environments, overcoming the issue of misreporting of intake. Where this wasn't possible for the evening meal prior to study days, participants received take away meals and snacks for ease of replication. Furthermore, the homogenous, uniform tomato-based pasta meal prevented confounding factors often observed in buffet-style test meals. Large variation in energy intake has been reported despite two separate buffet test meals with identical feeding conditions in the same individuals (Stensel, 2010), which may be due to preferences for expensive, palatable foods that may not be readily available in everyday life. However, our introduction of dietary reporting in the subsequent 2 days under free-living conditions relies on the compliance of the individual who may alter dietary habits, such as under-reporting by up to 30% in overweight cohorts (Lichtman *et al*., 1992).

In conclusion, our findings show a meaningful reduction in food intake following a small whey protein preload in both healthy-weight males or those with overweight and obesity. There were no compensatory dietary and physical activity behaviours identified over the 2 days of post-intervention monitoring, suggesting whey protein is an effective tool for inducing an energy deficit in males. This could be effective in the long term, but future research is required to assess the effect of chronic whey protein supplementation on food intake and changes in body composition.

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