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Citation: King, David, Peart, Daniel, Broom, David and Tew, Garry (2022) Effects of pre-meal whey protein consumption on acute food intake and energy balance over a 48-hour period. *Journal of Functional Foods*, 99. p. 105308. ISSN 1756-4646

Published by: Elsevier

URL: <https://doi.org/10.1016/j.jff.2022.105308>
<<https://doi.org/10.1016/j.jff.2022.105308>>

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

3

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20

21

22 **Abstract**

23 The effects of pre-meal whey protein consumption on acute food intake and
24 subsequent energy balance measured over 48-h was investigated in males of
25 healthy-weight (HW) or living with overweight and obesity (OV/OB). On two separate
26 trial days, following a controlled breakfast (09:00) and lunch (13:00), 12 HW and 12
27 OV/OB males consumed either whey protein (20g) or flavoured water beverages
28 (16:40), and *ad libitum* test meal (17:00). A controlled 48-h assessment of energy
29 intake and expenditure was used to determine any compensatory behaviour. Test
30 meal energy intake reduced 15.9% in HW (P=0.003), and 17.8% in OV/OB
31 (P=0.005) following whey protein, compared to placebo. We report no between-
32 group differences and no changes in compensatory behaviour. A small dose of whey
33 protein reduces energy intake at the next meal, without upregulating compensatory
34 behaviours in both HW and OV/OB males. However, chronic effects on body
35 composition and weight loss remain to be elucidated.

36 **Study Highlights:**

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

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

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

50 **Funding:** This research did not receive any specific grant from funding agencies in
51 the public, commercial, or not-for-profit sectors.

52

53 **1. Introduction**

54 Dietary protein intake induces satiety and reduces food intake to a greater extent
55 than other macronutrients (Poppitt *et al.*, 1998; Speakman, 2022). Milk proteins are
56 of specific interest in the management of overweight and obesity due to strong
57 associations between high dairy consumption and low body mass (Phillips *et al.*,
58 2003; Lu *et al.*, 2016). Indeed, whey protein has been reported to suppress appetite
59 and subsequent food intake in comparison to placebo or isocaloric doses of casein,
60 egg or soy protein in healthy individuals and people with obesity (Akhavan *et al.*,
61 2010; Pal & Ellis, 2010; Poppitt *et al.*, 2011; Zafar *et al.*, 2013). This may be due to
62 the abundance of branched chain amino acids (BCAAs) and bioactive peptides in
63 whey protein, providing a wide range of physiologic functions including delayed
64 gastric emptying and stimulation of appetite satiating hormones (Madureira *et al.*,
65 2010; Stanstrup *et al.*, 2014; King *et al.*, 2018). Physiologically, 20 min appears to be
66 the minimum interval for postabsorptive effects of the preload to influence energy
67 intake (Booth *et al.*, 1976), with inter-meal intervals (IMI) of between 20-120 min
68 reported to reduce food intake in adults (Almiron-Roig *et al.*, 2013). The smallest

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

71

72 Despite the acute appetite suppressing effects of whey protein, when no concomitant
73 dietary or exercise intervention is undertaken, the effects of chronic supplementation
74 of whey protein on body composition are equivocal (Pal & Ellis, 2010; Baer *et al.*,
75 2011). Baer *et al.* (2011) reported reductions in body mass and waist circumference
76 following 23 weeks of whey protein supplementation (56 g/d) in people with obesity
77 when compared to isoenergetic (1670 kJ/d) carbohydrate. In contrast, Pal and Ellis
78 (2010) reported no change in body composition after 12-week supplementation (54
79 g/d) when compared to isoenergetic glucose supplementation. This may, in part, be
80 attributed to compensatory adaptations in other components contributing to energy
81 balance. Previous short-term feeding studies have failed to identify whether the
82 energy deficits reported were sustained in the longer-term, with no quantification of
83 energy expended through physical activity and diet induced thermogenesis (DIT).
84 Furthermore, basal metabolic rate (BMR) and daily energy requirements are typically
85 estimated using physical activity questionnaires for which validity is questionable
86 (Neilson *et al.*, 2008).

87

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

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

99

100 **2. Methods**

101 2.1 Participants

102 The study population consisted of 12 healthy-weight males (HW, 20.0-24.99 kg/m²)
103 and 12 males living with overweight or obesity (OV/OB, >25kg/m²) aged 18-65 years,
104 without diagnosed metabolic/autoimmune disease, common food allergens,
105 intolerances, or dietary restrictions. Breakfast skippers, smokers, dieters and those
106 currently taking prescribed or over-the-counter medications that influence appetite or
107 gastric motility were excluded. Restrained eaters were identified for exclusion by a
108 score of >12 on the cognitive restraint scale of the 51 Item Three Factor Eating
109 Questionnaire (Stunkard & Messick, 1985). Female participants were excluded due
110 to the time constraints of not being able to control for the effect of the menstrual
111 cycle on dietary and physical activity behaviour (Buffenstein *et al.*, 1995).

112 Participants were recruited from Northumbria University and external local
113 organisations through poster advertisement, and local areas of Newcastle-upon-
114 Tyne through radio broadcast advertisements. Participants were financially
115 compensated (£20 shopping voucher). The present study was conducted in
116 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%

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

146

147 Two days preceding experimental trials, Actiheart accelerometers (Actiheart,
148 CamNtech, United Kingdom) were fitted to participants, providing valid assessments
149 of energy expenditure via a combination of heart rate monitoring and movement
150 registration (Lof *et al.*, 2013). The accelerometer attached onto two ECG electrodes
151 (3M Health Care, Canada) placed at 12-lead positions V1 and V4 and worn for 4
152 days, collecting data in 1-minute intervals. Participants also received weighing scales
153 to assist accurate reporting of daily food intake for diary reporting and were guided in
154 their use. Weighing measurements of all ingredients was carried out twice in
155 repetition to ensure accurate reporting. Participants were asked to replicate dietary
156 choices in the 24 h prior to each main trial. To assist this standardisation, pre-
157 packaged meals (Tesco cottage pie, UK) and snacks (Nature Valley bar, UK) were
158 provided to be consumed in the evening prior to each trial with the aim of normalising
159 appetite perceptions, glucose metabolism and gut hormone parameters
160 (Chandarana *et al.*, 2009).

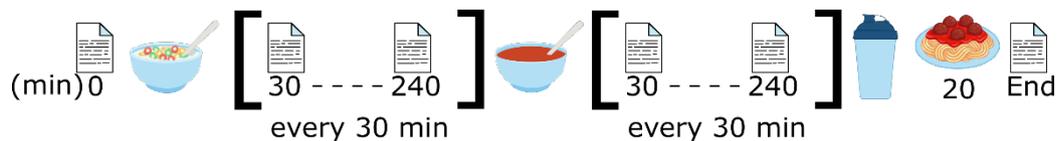
161

162 2.3 Protocol

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

165 sequences were randomly assigned with the use of a computerised random-number
 166 generator (www.randomization.com). Participants were instructed to avoid
 167 consuming any alcoholic beverages and conducting any strenuous physical exercise
 168 24 h prior to the study day. A schematic of the main trial protocol is presented in
 169 Figure 1. Participants arrived at 08:45 following an overnight fast and completed
 170 visual analogue scales (VAS) to rate subjective hunger, fullness, prospective
 171 fullness, desire to eat, thirst, mood and nausea on a 100 mm continuum (Flint *et al.*,
 172 2000). At 09:00, a fixed-nutrient breakfast meal containing cereal (Cheerios, Nestle,
 173 UK) and whole milk (Tesco, UK), equivalent to 15% of RMR, was consumed within
 174 15 minutes. At 13:00 a fixed-nutrient lunch meal of chicken soup (Heinze, UK), crisps
 175 (Kettle Foods, UK) and oat bar (Nature Valley, UK), equal to 35% of RMR, was
 176 consumed within 15 minutes. VAS were recorded every 30 minutes throughout the
 177 study day.

178



179

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

183

184 At 16:40, participants received either whey protein (20 g, Lacprodan©, Arla Foods
 185 Ingredients Group, Denmark) or flavoured water placebo to be consumed as quickly
 186 as possible and within 1 minute. The mixture of whey protein was achieved using
 187 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

203 2.4 Statistical approach, analysis and power

204 Sample size calculation was determined to identify the minimal clinically important
205 difference set to 180 kcal. Previous research suggests a within-subjects standard
206 deviation of 120 kcal for *ad libitum* food intake in healthy-weight individuals and
207 people living with obesity (Seimon *et al.*, 2013). Therefore, using a 2-tailed *p*-value of
208 0.05, 10 healthy-weight, and 10 people with overweight or obesity were needed to
209 reject the null hypothesis that the population means are equal with a power of 90%.
210 The sample size calculation was processed using the software Power and Sample
211 Size Calculations (PS) (Dupont & Plummer, 1998). To account for potential drop-out,
212 a sample size of 12 healthy-weight males and 12 males with overweight or obesity
213 were targeted.

214

215 All hypotheses were specified *a priori*. All data were analysed using the Statistical
216 Package for the Social Sciences (SPSS 24, IBM, United States) and reported as
217 means and their standard deviation (mean \pm SD). Tests of normality and sphericity
218 were performed using the Shapiro-Wilk test, and Mauchly's test, respectively.
219 Composite appetite score (mm) was calculated as [desire to
220 eat + hunger + (100 – fullness) + prospective food consumption]/4 (Anderson *et al.*,
221 2002). Paired *t*-tests were used to examine differences between trials for HW and
222 OV/OB groups for outcomes including food intake and physical activity energy
223 expenditure. To identify the effect of the intervention between groups, a mixed-model
224 ANOVA was conducted (within-subjects' variables [Treatment]; between-subjects'
225 variables [Group]). Area under the curve was calculated using the trapezoid method.
226 Relationships between variables were assessed using Pearson's linear correlations

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

230

231 3. Results

232 3.1 Participants

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

236

237 **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

238 Data presented as $\bar{x} \pm SD$. BMI, Body mass index; RMR, Resting metabolic rate; PAL,
 239 Physical activity level. Physical Activity Level (PAL) was calculated by dividing participants'
 240 total daily energy expenditure by RMR. * denotes significant difference between groups ($p <$
 241 0.05).

242

243 3.2 Pre-laboratory standardisation

244 Self-reported dietary intake during the 24 h prior to each main trial was similar for
 245 both HW (Whey 2119.0 ± 553.7 kcal; Control 2223.8 ± 500.4 kcal, $t_{(11)} = -0.869$, 95%
 246 CI -369.9 to 160.5, $p = .403$) and OV/OB (Whey 1988.1 ± 523.7; Control 2034.8 ±

247 454.1, $t_{(10)} = -.544$, 95% CI -237.9 to 144.6, $p = .598$) groups. Similarly, no
 248 differences were observed in physical activity energy expenditure (PAEE) for the 24
 249 h preceding each trial in both HW (Whey 415.8 ± 74.2 kcal; Control 443.6 ± 104.7
 250 kcal, $t_{(10)} = -.763$, 95% CI -109.0 to 53.4, $p = .463$) and OV/OB males (Whey $410.5 \pm$
 251 300.5 kcal; Control 476.2 ± 241.2 kcal, $t_{(9)} = -.853$, 95% CI -239.9 to 108.5, $p = .416$).

252

253 3.3 Laboratory standardisation

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

264

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

AUC _{0-240min} (cm·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%)

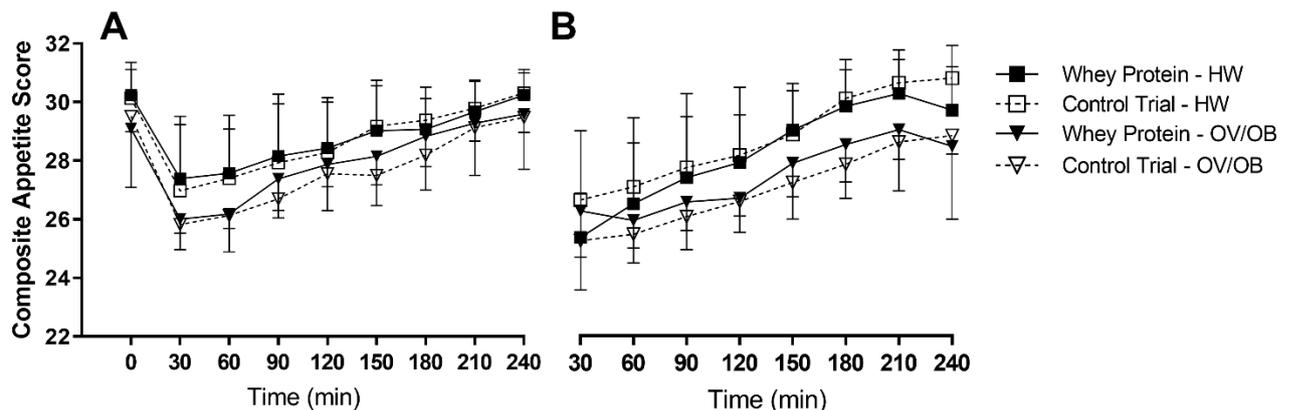
Lunch

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%)

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

270

271



272

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

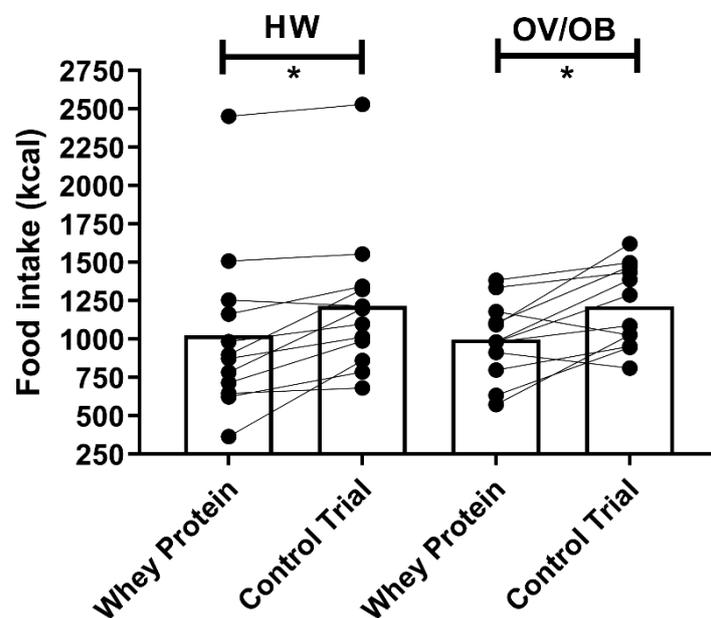
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278 3.4 Laboratory phase

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

282 preloads of 15.9% in HW (Whey 1023.2 ± 545.8 kcal; Control 1216.5 ± 483.5 kcal,
 283 95% CI -302.7 to -84.0, $p = .003$) and 17.8% in OV/OB (Whey 997.9 ± 248.2 kcal;
 284 Control 1213.7 ± 267.6 kcal, 95% CI -352.4 to -79.2, $p = .005$) males. Total meal
 285 consumption time was greater following whey preloads in both HW (Whey 15.8 ± 2.7
 286 min; Control 14.08 ± 3.2 min, MD = 1.67 min, $t_{(11)} = 3.708$, $p = .003$) and OV/OB
 287 (Whey 19.58 ± 1.35 min; Control 17.83 ± 1.36 min, MD = 1.75 min, $t_{(11)} = 3.656$, $p =$
 288 .004) groups. Upon the cessation of whey and control test meals, composite appetite
 289 scores were similar in HW (Whey 23.0 ± 0.3; Control 23.1 ± 0.7 kcal, $t_{(11)} = -.489$, $p =$
 290 .635) and OV/OB (Whey 23.4 ± 0.7; Control 23.4 ± 0.7, $t_{(11)} = .032$, $p = .975$) groups.
 291 No significant differences were observed in measures of food intake between HW
 292 and OV/OB groups ($p > 0.05$).

293



294

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

299

300 3.5 Post-laboratory phase

301 In the evening of the test meal, there were no compensatory behaviours in food
302 intake in response to whey and control trials, with similar caloric intake reported for
303 HW (Whey 310.9 ± 268.5 kcal; Control 363.1 ± 210.3 kcal, MD = -52.10, $t_{(11)} = -.886$,
304 95% CI -183.2 to 78.9, $p = .397$) and OV/OB groups (Whey 405.6 ± 449.3 kcal;
305 Control 442.9 ± 495.0 kcal, MD = -37.38, $t_{(11)} = -.312$, 95% CI -304.7 to 229.9, $p =$
306 .762). Similarly, no compensatory behaviour in PAEE was observed in the evening
307 following the discharge of participants from laboratory for HW (Whey 203.8 ± 36.4
308 kcal; Control 218.5 ± 52.3 kcal, $t_{(9)} = -1.014$, 95% CI -77.7 to 29.6, $p = .337$) and
309 OV/OB (Whey 258.1 ± 159.7 kcal; Control 255.7 ± 113.6 kcal, $t_{(7)} = .032$, 95% CI -
310 178.9 to 183.7, $p = .976$) males.

311

312 On the first day post-trial, daily caloric intake following whey and control trials was
313 similar for HW (Whey 2203.0 ± 586.1 kcal; Control 2229.2 ± 447.6 kcal, $t_{(11)} = -.312$,
314 95% CI -309.8 to 257.4, $p = .843$) and OV/OB males (Whey 2484.9 ± 927.3 kcal;
315 Control 2600.7 ± 1282.1 kcal, $t_{(9)} = -.384$, 95% CI -797.7 to 566.3, $p = .710$).
316 Similarly, no differences were observed in PAEE in the day following main trials in
317 HW (Whey 429.7 ± 137.5 kcal; Control 447.1 ± 154.5 kcal, $t_{(10)} = -.518$, 95% CI -
318 101.7 to 63.3, $p = .616$) and OV/OB (Whey 615.3 ± 183.9 kcal; Control 585.7 ± 312.5
319 kcal, $t_{(8)} = -.155$, 95% CI -339.5 to 296.6, $p = .880$) males.

320

321 On the second day following trials, no differences between conditions were observed
322 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 \pm 716.7 kcal;
324 Control 2536.2 \pm 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 \pm 128.6 kcal; Control 426.1 \pm 83.5 kcal, $t_{(7)} = .224$, 95% CI -34.6 to
327 41.8, $p = .829$) and OV/OB (Whey 461.9 \pm 131.3 kcal; Control 466.6 \pm 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 accelerometry 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*

347 *al.*, 2011). When compared to a glucose preload, two studies reported significant
348 reductions in food intake in overweight cohorts (BMI; 30.7 ± 2.5 kg/m²; 30.1 ± 1.1
349 kg/m²) by 15.6% and 10% following the ingestion of 25 g and 50 g whey protein 180
350 min prior to a test meal, respectively (Bowen *et al.*, 2006; Zafar *et al.*, 2013).
351 Conversely, Bowen *et al.* (2007) observed increased VAS-rated fullness following 50
352 g whey when compared to fructose, but no significant difference in energy intake
353 when ingested 240 min prior to a buffet meal in people with obesity (32.5 ± 0.6
354 kg/m²). Similarly, Poppitt *et al.* (2011) reported that 20 g whey ingestion suppressed
355 immediate postprandial measures of satiety, however the effects were short-term
356 and not sufficient to significantly impact on subsequent food intake when measured 2
357 h later.

358

359 Therefore, to the authors knowledge this is the first study to investigate the effects of
360 a low dose of whey protein on food intake when ingested only 20 min before an *ad*
361 *libitum meal* in males with overweight and obesity. The anorexigenic effect we report
362 may be due to the release of several gut peptides including cholecystinin (CCK),
363 glucagonlike peptide 1 (GLP-1), peptide YY (PYY) and insulin, along with the
364 suppression of acylated ghrelin (Hall *et al.*, 2003; Batterham *et al.*, 2006; Blom *et al.*,
365 2006; El Khoury *et al.*, 2006; Burton-Freeman, 2008; Foster-Schubert *et al.*, 2008),
366 although the evidence for the latter is inconsistent (Cummings, 2006; Lejeune *et al.*,
367 2006). The present experimental protocol did not allow for the investigation of these
368 possible mechanisms which is a limitation, although it is possible that the small inter-
369 meal interval may have aligned the test meal alongside peak GLP-1 and insulin
370 concentrations, resulting in reduced energy intake (Bowen *et al.*, 2006; Ma *et al.*,
371 2009). However, postprandial hormone responses do not always translate into a

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

382

383 Our results suggest that a low dose of whey protein, consumed 20 min before a
384 meal, elicits a meaningful energy deficit in both HW and people living with OV/OB ,
385 without compensatory changes in food intake and physical activity over the following
386 2 days. Therefore, in theory, weight loss strategies could incorporate whey protein as
387 a tool to achieve long-term energy deficits. However, the feasibility of such a strategy
388 remains unclear since little is known about the effects of chronic whey protein
389 ingestion on food intake and body composition in people with overweight or obesity.
390 Future research should focus on identifying whether the effect of whey protein
391 preloading on energy intake persists with regular exposure, similar to its effects on
392 reducing postprandial glycaemia over 4 weeks (Ma *et al.*, 2015). Furthermore, unlike
393 previous studies administering carbohydrate control groups (Pal & Ellis, 2010; Baer
394 *et al.*, 2011), comparisons between whey protein and no intervention would capture
395 the effects of the caloric burden ingested within the preload within free-living
396 conditions.

397

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

412

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

420

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