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**METABOLIC, ENDOCRINE AND APPETITE-RELATED RESPONSES TO ACUTE  
AND DAILY MILK SNACK CONSUMPTION IN HEALTHY, ADOLESCENT  
MALES**

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## Abstract

2 Comprising of two experiments, this study assessed the metabolic, endocrine and appetite-  
3 related responses to acute and chronic milk consumption in adolescent males (15-18 y).  
4 Eleven adolescents [mean  $\pm$  SD age: 16.5  $\pm$  0.9 y; BMI: 23.3  $\pm$  3.3 kg/m<sup>2</sup>] participated in the  
5 acute experiment and completed two laboratory visits (milk vs. fruit-juice) in a randomized  
6 crossover design, separated by 7-d. Seventeen adolescents [age: 16.1  $\pm$  0.9 y; BMI: 21.8  $\pm$  3.7  
7 kg/m<sup>2</sup>] completed the chronic experiment. For the chronic experiment, a parallel design with  
8 two groups was used. Participants were randomly allocated and consumed milk (n = 9) or  
9 fruit-juice (n = 8) for 28-d, completing laboratory visits on the first (baseline, day-0) and last  
10 day (follow-up, day-28) of the intervention phase. On laboratory visits (for both  
11 experiments), measures of appetite, metabolism and endocrine responses were assessed at  
12 regular intervals. In addition, eating behavior was quantified by ad libitum assessment under  
13 laboratory conditions and in the free-living environment by weighed food record. Acute milk  
14 intake stimulated glucagon (P = .027 [16.8 pg·mL; 95% CI: 2.4, 31.3]) and reduced ad  
15 libitum energy intake relative to fruit-juice (P = .048 [-651.3 kJ; 95% CI: -1294.1, -8.6]), but  
16 was comparable in the free-living environment. Chronic milk intake reduced free-living  
17 energy intake at the follow-up visit compared to baseline (P = .013 [-1910.9 kJ; 95% CI: -  
18 554.6, -3267.2]), whereas the opposite was apparent for fruit-juice. Relative to baseline,  
19 chronic milk intake increased the insulin response to both breakfast (P = .031) and mid-  
20 morning milk consumption (P = .050) whilst attenuating blood glucose (P = .025). Together,  
21 these findings suggest milk consumption impacts favorably on eating behavior in adolescent  
22 males, potentially through integrated endocrine responses.

23 **Keywords: Milk, Fruit-Juice, Appetite, Adolescents, Snack, Energy Intake**

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## 32 **Introduction**

33 Snacking has become commonplace and characterizes a major element of modern eating  
34 behavior, yet is often considered to contribute to the current obesity epidemic (Chapelot,  
35 2011). Snacking is defined as an episode of food consumption occurring outside the context  
36 of typical main meals, including all food and beverage items (Chapelot, 2011). Snack foods  
37 are readily available in a variety of settings, including the school environment (Savidge,  
38 Macfarlane, Ball, Worsley, & Crawford, 2007), and therefore snacking is highly prevalent,  
39 particularly among children and adolescents. For example, 98% of 12- to 17-y-old students in  
40 a recent UK study reported consuming one or more snacks daily, and this was greatest among  
41 male adolescents (Macdiarmid et al., 2009). Consequently, snacking contributes significantly  
42 to daily energy and nutritional intake in young people (Ovaskainen et al., 2006), which is  
43 potentially problematic and may lead to overconsumption of calories, free-sugars and  
44 nutrient-poor, energy-dense foods. Indeed, while the health effects associated with such  
45 dietary behaviours are well known (Chapelot, 2011) the promotion of more healthful snacks  
46 could benefit overall dietary intake, nutritional status and actually act as a marker for  
47 healthier eating habits.

48 From a child and adolescent perspective, fruit-juice drinks, sugar-sweetened  
49 beverages and milks are frequently reported as common beverage snack items consumed  
50 between main meals (Duffey et al., 2012). Despite fruit-juice providing vitamins, minerals  
51 and antioxidants, and sugar-sweetened beverages which hold a negligible nutritive value,  
52 high rates of consumption appear to promote weight gain in children and adolescents  
53 (Dennison, 1996; Dennison, Rockwell, & Baker, 1997; Woodward-Lopez, Kao, & Ritchie,  
54 2011). Interestingly, the opposite may stand true for milk-based beverages (Dror, 2014).  
55 Indeed, emerging evidence suggests that milk-based beverages protect against adiposity in  
56 children and adolescents (Abreu et al., 2014; Barba, Troiano, Russo, Venezia, & Siani, 2005;  
57 Moore, Singer, Qureshi, & Bradlee, 2008), and the replacement of sugar-sweetened  
58 beverages with milk or water, but not fruit-juice, is inversely associated with body fatness  
59 throughout the transition from childhood to adolescence (Zheng et al., 2015). Relative to  
60 fruit-juice drinks and sugar-sweetened beverages, milk-based beverages are recognized as a  
61 nutrient-dense foodstuff and contain a host of constituents that improve the overall nutritional  
62 quality of the child and adolescent diet (Fiorito, Mitchell, Smiciklas-Wright, & Birch, 2006).  
63 Outside of the health-related benefits, recent evidence also indicates that high rates of milk

64 consumption are positively associated with academic performance and motivation for  
65 learning in adolescents compared to sugar-sweetened beverage intake (Kim et al., 2016).

66 Efforts to establish the relationship between milk and adiposity have identified several  
67 plausible mechanisms, all of which may be attributed to the nutritional composition of milk.  
68 Literature from cell and adult studies indicate that dairy calcium stimulates adipocyte  
69 lipolysis (Zemel, Shi, Greer, Dirienzo, & Zemel, 2000), increases energy expenditure (Zemel,  
70 et al., 2000), fat oxidation and faecal fat excretion (Melanson, Donahoo, Dong, Ida, & Zemel,  
71 2005; van Loon, Saris, Verhagen, & Wagenmakers, 2000). Beyond calcium, milk proteins  
72 (whey and casein, and their products of digestion) may act to potentiate peptides from  
73 gastrointestinal, pancreatic and adipose tissue origin (Anderson & Moore, 2004; Bowen,  
74 Noakes, & Clifton, 2006; Schneeman, Burton-Freeman, & Davis, 2003), increasing  
75 perceptions of satiety (Dove et al., 2009; Gilbert et al., 2011) and thus reducing energy intake  
76 (Dove, et al., 2009). In addition, medium chain triglycerides, conjugated linoleic acid and  
77 lactose may also be implicated in the role of milk-based foods on reducing energy intake  
78 (Aziz & Anderson, 2007). Taken together, it appears that milk-based beverages have a unique  
79 potential to influence elements of energy balance. In this sense, milk contains a host of  
80 components and bioactive constituents that act individually, and probably synergistically, to  
81 impart beneficial effects on body mass regulation through actions related to appetite, eating  
82 behavior and metabolism. It is prudent to highlight, however, that the majority of this appetite  
83 and metabolic research has been conducted in adult populations, and at present there remains  
84 a dearth of mechanistic information in children and adolescents.

85 According to the acute literature (Birch, McPhee, Bryant, & Johnson, 1993;  
86 Mehrabani, Salehi-Abargouei, Asemi, Feizi, & Safavi, 2014; Zandstra, Mathey, Graaf, & van  
87 Staveren, 2000), mid-morning dairy snack consumption [ice-cream (Birch, et al., 1993),  
88 yogurt (Zandstra, et al., 2000) and milk (Mehrabani, et al., 2014)] reduces energy intake and  
89 increases energy expenditure (Apolzan et al., 2006) in children and adolescents (3- to 15-y-  
90 old). However, these studies are primarily limited to acute child investigations utilizing  
91 dissimilar preloads (differing according to volume and energetic content) and single energy  
92 intake assessment (laboratory based *ad libitum* assessment). Moreover, no quantitative  
93 measures of subjective appetite and/or appetite- and metabolism-related peptides were  
94 included which may have provided valuable insights concerning the mechanisms impacting  
95 on appetite and eating behaviour, and thus remains to be examined. Without a better  
96 understanding of the mechanisms impacting on appetite and eating behavior following dairy

97 consumption, it remains challenging to reconcile the potential effects of different dairy foods  
98 on energy regulation in children and adolescents. Consequently, this study investigated the  
99 effect of acute and chronic (28-d) mid-morning milk snack consumption on subsequent  
100 metabolic, endocrine and appetite-related responses.

101

## 102 **Materials and Methods**

### 103 *Experimental Design*

104 A randomized crossover design was implemented with two experimental conditions to  
105 investigate the acute effects of milk consumption on subsequent energy intake, circulating  
106 concentrations of glucagon-like peptide-1 (GLP-1<sub>7-36</sub>), glucagon, insulin, leptin and blood  
107 glucose, energy expenditure and subjective appetite. Experimental visits consisted of mid-  
108 morning milk (< 2% fat) and an isoenergetic and isovolumetric serving of fruit-juice, each  
109 separated by 7-days. To investigate the effects of chronic milk consumption on the  
110 abovementioned metabolic, endocrine and appetite-related responses, a parallel design with  
111 two intervention groups was used. Participants were randomly allocated to groups, and  
112 received either daily mid-morning milk (<2% fat) or an isoenergetic and isovolumetric fruit-  
113 juice for 28 days. Participants made two experimental visits to the nutrition and metabolism  
114 laboratory, which were scheduled on the first (day-0, baseline) and last (day-28, follow up)  
115 days of the intervention phase. Participants were matched according to age ( $16.1 \pm 1.1$  vs.  
116  $16.4 \pm 0.7$  y), body mass ( $69.4 \pm 18.3$  vs.  $68.2 \pm 10.5$  kg), body mass index ([BMI]  $22.0 \pm 5.0$   
117 vs.  $21.6 \pm 2.5$  kg·m<sup>2</sup>) and habitual calcium intake ( $814.5 \pm 118.4$  vs.  $836.0 \pm 274.9$  mg·d)  
118 intake. Habitual calcium intakes were estimated using a validated food frequency  
119 questionnaire for determining calcium and vitamin D intake in adolescents (Taylor et al.,  
120 2009). All testing was completed during school-term time.

121

### 122 *Participants*

123 Participants were recruited from a local secondary school in the North-East of England, after  
124 attendance at an initial information seminar. Adolescent males between 15 and 18 y of age  
125 were eligible to participate. Eleven male adolescents (mean  $\pm$  SD; age:  $16.5 \pm 0.8$  y; body  
126 mass:  $73.4 \pm 11.5$  kg; stature:  $1.8 \pm 0.1$  m; BMI:  $23.3 \pm 3.3$  kg/m<sup>2</sup>) were recruited for the

127 acute experiment and another 19 different participants (mean  $\pm$  SD; age:  $16.1 \pm 0.9$  y; body  
128 mass:  $68.8 \pm 13.9$  kg; stature:  $1.8 \pm 0.0$  m; BMI:  $21.8 \pm 3.7$  kg/m<sup>2</sup>; habitual calcium intake:  
129  $790 \pm 217$  mg·d) for the daily experiment. All participants were free of milk-related allergies,  
130 diabetes or other metabolic disorders (and medication) known to affect taste, smell and  
131 appetite.

132 The Faculty of Health and Life Sciences Ethics Committee at Northumbria University  
133 reviewed the experimental procedures and approved the study. The study was conducted in  
134 accordance with the Declaration of Helsinki of 1975, as revised in 2013. All participants  
135 provided written informed parental consent and student assent before any study-related  
136 procedures were performed. This trial was registered at [clinicaltrials.gov](https://clinicaltrials.gov): NCT02487342.

137

#### 138 *Pre-Trial Standardisation*

139 Participants were instructed to record all food and fluid consumption 24 h preceding the first  
140 visit for both the acute and chronic experiments, using a self-report, weighed food diary.  
141 Participants were also advised to refrain from caffeine and alcohol consumption ( $\geq 24$ -h) and  
142 strenuous physical activity ( $\geq 24$ -h) before each experimental visit (days-0 and -28 of the  
143 daily experiment). Participants were requested to replicate these dietary and exercise  
144 behaviors for subsequent experimental visits. Between waking and arrival at the nutrition and  
145 metabolism laboratory, consumption of water only was permitted. Participants were  
146 requested to record, document and replicate morning water consumption for subsequent  
147 experimental visits.

148

#### 149 *Experimental Protocol*

150 On experimental visits for both the acute and chronic experiments (days-0 and -28 of the  
151 daily experiment), participants arrived at the nutrition and metabolism laboratory at 0745 h.  
152 After 30 min rest, a baseline fingertip-capillary blood sample was drawn, expired gas sample  
153 (300 s resting sample) collected, and participants completed a series of baseline subjective  
154 appetite visual analogue scales (VAS). A standardized cereal and milk breakfast meal was  
155 provided at 0830 h. Participants were given 15 min to consume the entire contents of the  
156 breakfast meal.

157 Participants subsequently remained at rest in the laboratory for 180 min in an  
158 environment free from food cues. This period started upon the first mouthful of the breakfast  
159 meal. Further samples of fingertip-capillary blood and subjective appetite VAS were  
160 collected at 30 min intervals during the 180 min. Expired gas samples (300 sec) were  
161 collected at 25-30, 55-60, 85-90, 115-120, 145-150, and 175-180 min. Mid-morning snacks  
162 were administered at 90 min. At 180 min, a homogenous *ad libitum* pasta meal was provided.  
163 Participants were instructed to eat until comfortably full and satisfied. On completion of the  
164 *ad libitum* pasta meal, participants were free to leave the laboratory and returned to school via  
165 chaperon. They were asked to record any further food and fluid items consumed during the  
166 remainder of the day utilizing a combined weighed self-reported food record and 24 h dietary  
167 recall technique. The intervals between test meals were selected to be representative of a  
168 typical school day. During the intervention phase for the daily experiment, participants were  
169 instructed to maintain their usual feeding and physical activity practices.

170

#### 171 *Test Meals*

##### 172 *Breakfast*

173 Breakfast consisted of semi-skimmed milk (Tesco, UK) and Kellogg's Rice Krispies  
174 (Kelloggs, Manchester, UK), in a cereal to milk ratio of 30 g: 125 mL. The quantity issued  
175 was designed to provide 10% of the participants estimated daily energy requirement for  
176 protein, fat and carbohydrate (14%, 14% and 72%, respectively) as used previously (Astbury,  
177 Taylor, French, & Macdonald, 2014).

##### 178 *Mid-Morning Snacks*

179 For both experiments, mid-morning snack items consisted of milk (< 2% fat, Tesco, UK), and  
180 an orange fruit-juice (Tesco, UK). All items were isovolumetric (217 mL) and isoenergetic  
181 (427 kJ), yet differed according to macro-nutrient composition (**Table 1**). Milk was selected  
182 as the dairy preload as this represents the most commonly consumed dairy food for this sex  
183 and age group (Green, Turner, Stevenson, & Rumbold, 2015). The volume selected was  
184 based on nationally representative consumption patterns (Bates, Lennox, & Swan, 2010), and  
185 is similar to other snack-based studies (Almiron-Roig, Grathwohl, Green, & Erkner, 2009;  
186 Mehrabani, et al., 2014). All packaging labels were removed and snack items were served in  
187 appropriate opaque serving containers.

188 For the acute experiment, participants completed two experimental conditions and  
189 were issued with one of the two snacks in a counterbalanced randomized manner. For the  
190 chronic experiment, a parallel design with two intervention groups was employed whereby  
191 participants were randomly allocated to groups and received either daily milk or fruit-juice  
192 for the 28-d intervention phase. During the intervention phase, the lead investigator visited  
193 participants on the school campus on a daily basis. A temporary nutrition laboratory was  
194 setup within the school where participants arrived between 1100-1115 h daily to consume  
195 their mid-morning snack. Participants were registered on a daily basis to monitor compliance,  
196 and snacks were consumed in the presence of the researcher (during weekdays only). On  
197 Fridays, participants were provided with the snacks for weekend periods. Participants were  
198 requested to consume the snacks once daily between 1100-1115 h, and return empty  
199 containers the following Monday (as a measure of compliance).

#### 200 *Pasta Meal*

201 Lunchtime food intake (180 min) was evaluated by means of a homogenous pasta meal and  
202 comprised of pasta (Tesco, UK), tomato sauce (Tesco, UK), cheddar cheese (Tesco, UK) and  
203 olive oil (Tesco, UK) and provided 859 kJ of energy per 100 g portion (205 kcal; energy  
204 contributions 14% protein, 52% carbohydrate and 34% fat). The test lunch was offered *ad*  
205 *libitum*. Participants were initially provided with 400 g of the pasta meal. Research staff  
206 continuously refilled participant's bowls (before the dish became empty) until participants  
207 indicated they were comfortably full. Detailed information concerning the nutrient  
208 composition of the pasta meal and the method of cooking has been reported in previous  
209 studies (Gonzalez et al., 2015). In addition, similar pasta meals have been used successfully  
210 in previous adolescent research (Rumbold et al., 2013). Energy intake from the pasta meal  
211 was calculated based on the amount consumed and nutritional composition as indicated by  
212 the manufacturer. To facilitate this, research staff covertly weighed the meal prior to serving,  
213 and immediately following meal termination.

214

#### 215 *Subjective Appetite*

216 Subjective measures of appetite were assessed using validated 100 mm, paper based VAS  
217 (Flint, Raben, Blundell & Astrup, 2000). Scales were anchored with diametrically opposed  
218 feelings of extremity, and addressed hunger ('how hungry do you feel?'), gut fullness ('how

219 full do you feel?’), prospective food consumption (‘how much do you think you can eat?’),  
220 and satisfaction (‘how satisfied do you feel?’). Subjective measures of appetite were reported  
221 immediately prior to each fingertip-capillary blood sample.

222

### 223 *Gas Analysis*

224 To collect gas samples, a mouthpiece attached to a two-way, non-rebreathing valve (model  
225 2730, Hans Rudolph, Kansas City, Missouri) was used. Gas samples, collected in Douglas  
226 Bags, were analyzed for concentrations of oxygen and carbon dioxide using a paramagnetic  
227 and infrared transducers, respectively (Servomex 5200S, Crowborough, East Sussex, UK). In  
228 addition, bag volume and temperature of expired gas samples were determined using a dry  
229 gas meter (Harvard Apparatus, Edenbridge, Kent, UK) and thermistor (model 810-080, ETI,  
230 Worthing, UK), respectively. Participants inserted the mouthpiece and rested for  
231 approximately 2 min before each expired gas sample (300 sec) was collected. Samples were  
232 collected at 25-30, 55-60, 85-90, 115-120, 145-150, and 175-180 min. Rates of energy  
233 expenditure (kJ), and substrate oxidation were estimated based on caloric equivalents of  
234 carbohydrate utilization and lipid oxidation, using stoichiometric equations as described  
235 (Frayn, 1983) with the assumption that protein oxidation was negligible.

236

### 237 *Free-Living Energy Intake*

238 Participants recorded all food and drink items consumed for the remainder of each  
239 experimental day. This was completed utilizing a combined weighed self-reported food  
240 record and dietary recall, used previously with adolescent populations (Rumbold, St Clair  
241 Gibson, Stevenson, & Dodd-Reynolds, 2011). Participants were requested to give full  
242 comprehensive recordings of all food and drink items consumed, weighing all items prior to  
243 and following consumption (if leftovers were present). Following each study day, the lead  
244 investigator visited the participants on a one-to-one basis on school campus grounds and  
245 completed 24 h recall interviews. Interviews used a two-pass approach (Ashley, Bovee, &  
246 Andersen, 2003), and lasted approximately 15 min per participant. All interviews took place  
247 at the same time each day and were conducted by the same researcher. One trained member  
248 (the lead investigator) of the research team examined all food records utilizing the nutritional  
249 software package Nutritics (Nutritics Professional v3.09, Nutritics, Ireland).

250 *Blood Sampling and Analysis*

251 At seven separate intervals, during laboratory visits (for both the acute and chronic  
252 experiment), fingertip-capillary (0.3 mL) blood samples were drawn into pre-cooled EDTA-  
253 treated microvettes. Samples were collected whilst participants lay in a semi-supine position  
254 at pre-breakfast ( $t = 0$  min) and at 30, 60, 90, 120, 150 and 180 min following breakfast  
255 consumption for the determination of plasma GLP-1<sub>7-36</sub>, glucagon, insulin, and leptin.  
256 Research from our laboratory has previously provided affirmation that fingertip-capillary  
257 blood sampling offers an appropriate methodological and reproducible approach for the  
258 quantification of appetite-related peptides in a resting state (Green, Gonzalez, Thomas,  
259 Stevenson, & Rumbold, 2014; Allsop, Rumbold & Green, 2016). Consequently, pre-  
260 analytical (e.g. sample treatment) and analytical (e.g. sample handling) procedures were  
261 followed as previously described (Green, et al., 2014). Briefly, microvettes contained  
262 aprotinin (33  $\mu\text{L}\cdot\text{mL}$  whole blood) and a di-peptidyl peptidase IV inhibitor (30  $\mu\text{L}\cdot\text{mL}$  whole  
263 blood) for the preservation of GLP-1<sub>7-36</sub> and glucagon.

264 Following blood collection, samples were centrifuged immediately. Microvettes were  
265 spun at 1509  $g$  (3000 rpm) for 10 min in a multispeed micro-centrifuge. Quantitative  
266 assessments of GLP-1<sub>7-36</sub> (pg·mL), glucagon (pg·mL), leptin (pg·mL) and insulin (pmol·L)  
267 were simultaneously determined in 40  $\mu\text{L}$  of plasma by electrochemiluminescence using a  
268 human hormone multiplex assay kit (Sector Imager 2400, MesoScale Discovery, Maryland,  
269 USA). Of note, the addition of protease inhibitors to samples for the preservation of GLP-1<sub>7-36</sub>  
270 and glucagon does not influence measured concentrations of plasma leptin and insulin  
271 (Bielohuby, Popp, & Bidlingmaier, 2012). Samples from each participant were analyzed  
272 within the same run to eliminate inter-assay variation. Intra-assay coefficients of variation  
273 were determined by the repeated measurement of a single baseline fingertip-capillary blood  
274 sample three times. For the acute experiment, average inter-assay coefficients of variation  
275 were 11%, 10%, 12% and 12% for GLP-1<sub>7-36</sub>, glucagon, leptin and insulin, respectively. For  
276 the daily experiment, average inter-assay coefficients of variation were 10%, 8%, 6% and 8%  
277 for GLP-1<sub>7-36</sub>, glucagon, leptin and insulin, respectively.

278 Additional fingertip-capillary (0.02 mL) blood samples for the determination of blood  
279 glucose were drawn into sodium heparinized capillary tubes and transferred into eppendorfs  
280 containing 1 mL haemolysis solution (EKF Diagnostics). Samples were subsequently shaken  
281 to encourage haemolysis, placed on ice and processed immediately. Concentrations of blood

282 glucose were quantified instantaneously by glucose oxidase method (BiosenC\_line, EKF  
283 Diagnostics). Concentrations of blood glucose were quantified instantaneously by the glucose  
284 oxidase method using an automated glucose analyzer (BiosenC\_line, EKF Diagnostics),  
285 based on an electro-chemical measuring principle following the conversion of  $\beta$ -D-glucose to  
286 gluconic acid. Prior to use, the analyzer was calibrated with a solution of known  
287 concentration (12 mmol·L), provided by the manufacturer.

288

### 289 *Sample-Size Estimate and Power*

290 An a priori sample-size estimate was conducted on the basis of the ability to detect a  
291 difference in postprandial plasma glucagon, an appetite-related peptide responsible for  
292 actions including increased satiety and reduced food intake (anorexigenic behaviors). For the  
293 acute experiment, sample-size estimation was conducted based on a pre-determined clinically  
294 significant time-averaged AUC difference for plasma glucagon. Given that the reported  
295 typical percentage error of between-day fingertip-capillary plasma glucagon is 8.2% (Green  
296 et al., 2014), it was estimated that 10 participants would provide > 80% chance of statistically  
297 detecting a difference with  $P < 0.05$ . For the purpose of the chronic study, sample-size  
298 estimation was conducted based on observations from the acute experiment that mid-morning  
299 milk consumption elicits a 16.8 pg·mL greater plasma glucagon response relative to a serving  
300 of fruit-juice, alongside the previously mentioned between-day typical error. Consequently, it  
301 was estimated that 18 participants (nine per group) would provide > 80% chance of  
302 statistically detecting a difference with  $P < 0.05$ .

303

### 304 *Statistical Analysis*

305 Computer software package JMP<sup>®</sup>, version 12.2.0 (SAS Institute Inc., Cary, NC) was used to  
306 perform all statistical analysis. Data were checked for normal distribution with the use of the  
307 Kolmogorov-Smirnov normality test and were log-transformed if appropriate before  
308 statistical analysis. Statistical significance was accepted at an  $\alpha$  level of  $P \leq .05$ . All data are  
309 presented as mean  $\pm$  SEM unless otherwise stated, with effects expressed as mean difference  $\pm$   
310 95% confidence intervals (CI).

311 For the acute experiment, comparisons between experimental visits for baseline  
312 fingertip-capillary variables, baseline subjective appetite, energy expenditure, *ad libitum* and  
313 free-living energy intake were assessed using paired samples t-tests. Area under the curve  
314 (AUC) values were computed for fingertip-capillary variables and subjective appetite using  
315 the trapezoidal rule for the post-breakfast (0-90 min) and post-snack (90-180 min) periods,  
316 and these values were subsequently time averaged. The postprandial period was split into 0-  
317 90 min and 90-180 min as the time points after the breakfast meal and mid-morning snack  
318 may influence the effect of particular appetite-related components (e.g. hormonal, metabolic,  
319 physical or cognitive) (Blundell et al., 2010). Data concerning postprandial time-averaged  
320 AUC estimates of fingertip-capillary variables and subjective appetite were assessed using  
321 paired samples t-tests with 95% confidence intervals (CI) comparing between experimental  
322 visits.

323 To investigate the effect of daily milk or fruit-juice on appetite and eating behavior,  
324 values obtained during the baseline experimental visit (day-0) were compared with  
325 intervention follow up experimental visits (day-28). As the methodological approach of  
326 experimental visits was identical between the acute and daily experiment, statistical  
327 procedures were conducted in the same manner as stated for the acute experiment. This  
328 comprised paired samples t-test analysis to determine differences for fingertip-capillary  
329 variables, subjective appetite, energy expenditure, *ad libitum* and free-living energy intake,  
330 with effects expressed as mean difference  $\pm$  95% CI relative to the baseline experimental visit  
331 (day 0). Reflecting the results at the level of data measurement, the use of CI indicates the  
332 direction of the effect studied (du Prel, Hommel, Rohrig, & Blettner, 2009). If the CI does not  
333 include the value of zero, it can be assumed a directional effect exists (Shakespeare, Gebski,  
334 Thiagarajan, & Jay Lu, 2006). Differences between baseline (day-0) and intervention follow  
335 up experimental visits (day-28) for the milk and fruit-juice group were analyzed using  
336 independent t-tests to determine contrasts between groups for fingertip-capillary variables,  
337 subjective appetite, energy expenditure, *ad libitum* and free-living energy intake, with effects  
338 expressed as mean difference  $\pm$  95% CI.

339

## 340 **Results**

341 In total, all 11 participants completed the acute experiment. Due to difficulties associated  
342 with attendance at the intervention follow up experimental visit (day-28), data for the chronic  
343 experiment are presented for 17 participants [milk group (n = 9) fruit-juice group (n = 8)].

344

#### 345 *Hormonal Variables*

346 In the acute experiment, fasted plasma concentrations of GLP-1<sub>7-36</sub>, glucagon, insulin, leptin  
347 and blood glucose concentrations were comparable between conditions. This remained during  
348 the post-breakfast period (time-averaged AUC<sub>0-90 min</sub>), suggesting that the postprandial  
349 concentrations of these peptides were comparable following breakfast. Following the acute  
350 mid-morning milk snack, however, time-averaged AUC<sub>90-180 min</sub> estimates of glucagon were  
351 greater relative to the fruit-juice snack (95.5 ± 6.4 pg·mL vs. 76.7 ± 7.2 pg·mL, respectively;  
352  $P = .027$  [16.8 pg·mL; 95% CI: 2.4, 31.3]).

353 Following both chronic fruit-juice and milk snack consumption, fasted plasma  
354 concentrations of GLP-1<sub>7-36</sub>, glucagon, insulin, leptin and blood glucose concentrations did  
355 not differ at the intervention follow up (day-28) compared to baseline (day-0) (**Figures 1 &**  
356 **2**). No differences were observed for GLP-1<sub>7-36</sub>, glucagon, insulin, leptin and blood glucose  
357 post-breakfast (AUC<sub>0-90 min</sub>) and post-snack (AUC<sub>90-180 min</sub>) between the intervention follow  
358 up (day-28) and baseline (day-0) following chronic fruit-juice (**Figures 1 & 2**). This finding  
359 remained following chronic milk consumption for GLP-1<sub>7-36</sub>, glucagon, and leptin (**Figures 1**  
360 **& 2**). Time-averaged AUC<sub>0-90 min</sub> insulin, was elevated post-breakfast at the intervention  
361 follow up (day-28) compared to baseline (day-0) after chronic milk consumption (356 ± 54.6  
362 pmol·L vs. 277.5 ± 48.8 pmol·L, respectively;  $P = .031$  [79.4 pmol·L; 95% CI: 149.3, 9.4],  
363 **Figure 2, Panel A**). This remained evident for time-averaged AUC<sub>90-180min</sub> (198.4 ± 38.2  
364 pmol·L vs. 163.5 ± 29.1 pmol·L, respectively;  $P = .050$  [34.9 pmol·L; 95% CI: 66.0, 3.8],  
365 **Figure 2, Panel A**). Consistent with the insulinotropic effect of chronic milk, measures of  
366 time-averaged AUC<sub>90-180 min</sub> glucose concentrations were attenuated at the intervention follow  
367 up (day-28) compared to baseline (day-0) (4.2 ± 0.1 mmol·L vs. 4.5 ± 0.1 mmol·L,  
368 respectively;  $P = .025$  [-0.3 mmol·L; 95% CI: -0.0, -0.4], **Figure 2, Panel C**).

369

#### 370 *Energy Intake*

371 Energy intake at the *ad libitum* pasta meal (**Figure 3 Panel A**) was lower following acute  
372 mid-morning milk consumption compared to fruit-juice (5459.7 ± 503.2 kJ vs. 6111.0 ±  
373 461.2 kJ, respectively;  $P = .048$  [-651.3 kJ; 95% CI: -1294.1, -8.6]), but was no different in  
374 the free-living environment (2936.0 ± 630.5 kJ vs. 3751.2 ± 802.6 kJ, respectively;  $P = .476$

375 [815.1 kJ; 95% CI: 3268.2, -1638.0]. No statistical differences were found for total daily  
376 energy intake following acute mid-morning milk consumption compared to fruit-juice  
377 (10,289 ± 519.0 kJ vs. 11,755.9 ± 885.2 kJ, respectively;  $P = .213$  [1466.4 kJ; 95% CI:  
378 3923.0, -990.3]).

379         Following chronic fruit-juice consumption, energy intake at the intervention follow up  
380 (day-28) *ad libitum* pasta meal (**Figure 4**) was greater and approached statistical significance  
381 compared to baseline (day-0) (6272.0 ± 756.3 kJ vs. 5384.2 ± 413.6 kJ, respectively;  $P = .056$   
382 [887.8 kJ; 95% CI: 1813.7, -37.5]), but was no different in the free-living environment ( $P =$   
383  $.822$  [-195.3 kJ; 95% CI: 1926.8, -2317.3]). Chronic milk consumption appeared to have the  
384 opposite effect. In this sense, energy intake at the intervention follow up (day-28) *ad libitum*  
385 pasta meal (**Figure 4**) remained comparable to baseline (day-0) (4994.4 ± 192.3 kJ vs. 4792.5  
386 ± 308.3 kJ, respectively;  $P = .326$  [201.8 kJ; 95% CI: 646.4, -242.7]), yet was lower in the  
387 free-living environment (3460.7 ± 317.6 kJ vs. 4960.7 ± 781.9 kJ, respectively;  $P = .013$  [-  
388 1910.9 kJ; 95% CI: -554.6, -3267.2]). No statistical differences were found for total daily  
389 energy intake in either group.

390

### 391 *Subjective Appetite*

392 Subjective appetite data is illustrated in **Table 2**. In the acute experiment, fasted hunger,  
393 fullness, prospective food consumption, and satisfaction was comparable between conditions.  
394 This remained true during the post-breakfast period (time-averaged  $AUC_{0-90 \text{ min}}$ ). Following  
395 the acute mid-morning milk snack, time-averaged  $AUC_{90-180 \text{ min}}$  fullness was lower relative to  
396 the fruit-juice snack (20.8 ± 2.6 mm vs. 26.8 ± 4.0 mm, respectively;  $P = .038$  [-6.0 mm; 95%  
397 CI: -11.6, -0.4]). Consistent with a reduction in subjective fullness, time-averaged  $AUC_{90-180}$   
398  $\text{min}$  prospective food consumption was greater for milk relative to the fruit-juice snack (76.9 ±  
399 3.5 mm vs. 72.7 ± 3.2 mm, respectively;  $P = .005$  [4.9 mm; 95% CI: 1.8, 7.9]).

400         Following chronic fruit-juice consumption, fasted hunger, fullness, prospective food  
401 consumption, and satisfaction were all similar at the intervention follow up (day-28)  
402 compared to baseline (day-0). A similar pattern emerged following chronic milk  
403 consumption, however, measures of fasted prospective food consumption were elevated at  
404 the intervention follow up (day-28) compared to baseline (day-0) (75.1 ± 6.9 mm vs. 58.8 ±  
405 3.6 mm, respectively;  $P = .041$  [16.2 mm; 95% CI: 31.6, 0.8]). Post-breakfast time-averaged  
406  $AUC_{0-90 \text{ min}}$  hunger, fullness, prospective food consumption, and satisfaction were comparable  
407 at the intervention follow up (day-28) compared to baseline (day-0) for both groups. This

408 finding was also observed during the post-snack period (time-averaged AUC<sub>90-180 min</sub>) for  
409 both groups.

410

#### 411 *Metabolic Responses*

412 In the acute experiment, fasted energy expenditure, carbohydrate and fat oxidation were  
413 comparable between conditions. Total post-breakfast (0-90 min) and post-snack (90-180 min)  
414 energy expenditure (kJ), carbohydrate (g) and fat (g) oxidation were not different between  
415 conditions. Following both chronic fruit-juice and milk snack consumption, fasted energy  
416 expenditure, carbohydrate and fat oxidation did not differ at the intervention follow up (day-  
417 28) compared to baseline (day-0). Similarly, no differences were observed for total post-  
418 breakfast (0-90 min) and post-snack (90-180 min) energy expenditure (kJ), carbohydrate (g) or  
419 fat (g) oxidation between the intervention follow up (day-28) and baseline (day-0) following  
420 both chronic fruit-juice and milk consumption.

421

#### 422 *Between Group Comparisons*

423 The baseline (day-0) to intervention follow up (day-28) change in fingertip-capillary  
424 variables, subjective appetite, metabolic responses, *ad libitum* and free-living energy intake  
425 were not significantly different ( $P \geq .05$  for all variables) between milk and fruit-juice as  
426 assessed by independent t-test (data not shown).

427

## 428 **Discussion**

429 Accumulating evidence suggests that milk-based dairy foods elicit anti-obesity properties  
430 through actions on eating behaviour and metabolism (Aziz & Anderson, 2007), yet research  
431 concerning the physiological mechanisms impacting on energy regulation among children  
432 and adolescents is sparse. To the best of our knowledge, the present study is therefore the first  
433 to assess the differential effects of acute and chronic (28-d) mid-morning milk consumption  
434 on subsequent metabolic, endocrine and appetite-related responses in adolescent males. The  
435 major findings of this study were that acute mid-morning milk consumption increased  
436 postprandial glucagon secretion and impacts on eating behaviour, reducing subsequent  
437 voluntary energy intake at the next meal (acute experiment), compared with fruit-juice.  
438 Alongside increased postprandial insulin secretion and attenuated blood glucose  
439 concentrations, reductions in eating behaviour were replicated under free-living conditions  
440 when milk was consumed chronically (28-d), whereas the opposite was apparent for fruit-

441 juice consumption. Findings arising from this study begin to provide some mechanistic  
442 insight that may have contributed to these observations.

443         The observation that acute and chronic mid-morning milk consumption reduces  
444 subsequent energy intake supports previous observations in children and adolescents (Birch,  
445 et al., 1993; Mehrabani, et al., 2014; Zandstra, et al., 2000), contributing further to the  
446 understanding that milk-based dairy foods exert the potential to impact favorably on eating  
447 behavior. In this study, energy intake at the *ad libitum* pasta meal following acute mid-  
448 morning milk consumption was 651.3 kJ (155.7 kcal) lower than when consuming fruit-juice,  
449 with no significant differences between conditions thereafter. Following chronic milk  
450 consumption, energy intake at the *ad libitum* pasta meal was comparable between baseline  
451 (day-0) and follow up (day-28) experimental visits, yet free-living energy intake was reduced  
452 by 1910.9 kJ (456.7 kcal), while the opposite was apparent following daily fruit-juice. It has  
453 been suggested that sustained reductions in daily energy intake averaging 460 to 690 kJ (110  
454 to 165 kcal) may offer an effective approach for preventing excess weight accumulation in  
455 children and adolescents (Wang, Gortmaker, Sobol, & Kuntz, 2006). On the basis of our  
456 observations, it would be prudent for future investigations to assess whether milk-based dairy  
457 foods provide application to overweight and obese metabolically diseased pediatric  
458 populations utilizing longer observation periods.

459         The mechanism by which milk consumption affects eating behavior is not properly  
460 understood, however, there are several plausible explanations and constituents of milk that  
461 may act synergistically to elucidate its actions. It is widely recognized that dietary proteins  
462 are more satiating than energetic equivalents of carbohydrate and fat under most conditions,  
463 and suppress eating behavior at the next available opportunity (Anderson & Moore, 2004;  
464 Astrup, 2005; Rolls, Hetherington, & Burley, 1988). In this study, experimental preloads  
465 were isoenergetic (427 kJ) and isovolumetric (217 mL), yet differed according to  
466 macronutrient composition whereby milk contained considerably more protein than the fruit-  
467 juice drink (7.5 g vs. 1.1 g). From a physiological perspective, milk consumption reportedly  
468 lowers the postprandial secretion of blood glucose (Panahi et al., 2013), and may be brought  
469 about due to an insulintropic effect (stimulating the production and activity of insulin)  
470 (Nilsson, Stenberg, Frid, Holst, & Bjorck, 2004; Ostman, Liljeberg Elmstahl, & Bjorck,  
471 2001). The insulintropic response may involve milk proteins, digestion and the release of  
472 plasma amino acids, and appetite-related hormone secretion, all of which are known to  
473 mediate insulin secretion (Nilsson, et al., 2004; Schmid, Schusdziarra, Schulte-Frohlinde,

474 Maier, & Classen, 1989; van Loon, et al., 2000). In addition, the postprandial response of the  
475 appetite- and metabolism-related peptides quantified in this study are profoundly influenced  
476 according to ingested macro- (and micro-) nutrient composition, and also energy content.  
477 Insulin and glucagon for example rise in response to protein feeding (Acheson et al., 2011).  
478 Considering the higher protein content of milk, a heightened insulin and glucagon and  
479 attenuated glucose response may have been expected. Indeed, greater circulating  
480 concentrations of insulin and glucagon have been related to anorexigenic behaviors in the  
481 adult literature, including increased satiety and acutely reduced food intake (Flint et al., 2007;  
482 Parker et al., 2013; Penick & Hinkle, 1961; Woods, Lutz, Geary, & Langhans, 2006).

483         Despite the consistent observation that acute and chronic (28-d) milk consumption  
484 reduced energy intake at the *ad libitum* pasta meal (acute experiment) and in free-living  
485 environment (chronic experiment), these observations were not reflected by differences in  
486 subjective appetite. In fact, our subjective appetite data infers contrasting observations,  
487 whereby milk consumption reduced subjective fullness and increased subjective prospective  
488 food consumption. This finding was unexpected, especially considering evidence from adult  
489 studies suggest acute and daily milk consumption attenuates subjective appetite (Dove, et al.,  
490 2009; Gilbert, et al., 2011). Nonetheless, the uncoupling relationship of VAS to reflect and  
491 forecast subsequent eating behaviour is common in some (Thivel et al., 2012; Thivel et al.,  
492 2011) but not all adolescent studies (Leidy, Ortinau, Douglas & Hoertel, 2013; Leidy et al.,  
493 2015). Consequently, this highlights the necessity to include measures of appetite-related  
494 peptides in younger populations. It could be reasonable to suggest that alterations in appetite-  
495 related peptides may therefore supersede subjective perceptions of food-related emotions to  
496 influence subsequent appetite and eating behavior in adolescents.

497         In the present study, we did not observe any capacity for acute or chronic mid-  
498 morning milk or fruit-juice consumption to impact on measures of energy expenditure or  
499 substrate metabolism. Reasons for this may involve the volume of product that was  
500 distributed in this study. There is evidence in the adolescent literature that single milk  
501 constituents or whole dairy food consumption exerts the ability to affect postprandial  
502 metabolism (Apolzan, et al., 2006). Although only available in abstract form, Apolzan et al.  
503 (2006) evaluated energy expenditure (kcal-min) for 240 min after consumption of a high  
504 energy (40% of participants energy requirement) low calcium non-dairy control ( $45 \pm 2$  mg  
505 Ca), supplemental calcium ( $670 \pm 4$  mg Ca) or a dairy-based product ( $687 \pm 5$  mg Ca) in  
506 overweight adolescent male and females. They observed a greater rate of energy expenditure

507 following the consumption of the dairy-based product compared with the low calcium non-  
508 dairy control, but only in overweight adolescent males. No differences were recorded  
509 following supplemental calcium ingestion, which may suggest additional constituents found  
510 within milk-based dairy foods act to impact on metabolism. To expand, it is well established,  
511 for example, that protein elicits a greater effect on diet induced thermogenesis (20-35% of  
512 energy consumed) compared to calorie matched intakes of carbohydrate (5-15% of energy  
513 consumed) or fat (0-3% of energy consumed) (Panahi, et al., 2013), albeit it in adults.  
514 Nonetheless, despite the higher protein content of the milk (compared to fruit-juice), it may  
515 be that the energy and calcium content of milk used in the present study (~272 mg Ca) was  
516 insufficient to elicit a significant effect on postprandial metabolism and may therefore  
517 demonstrate a dose response, yet the volume selected was based on nationally representative  
518 consumption patterns, and is similar to other snack-based studies. Furthermore, the lack of  
519 any effect may be attributed to the fact our participants were of normal weight.

520         The present study was conducted in a manner that reflected a typical school day, and  
521 thus practical application in a free-living environment. In this sense, the snack items  
522 provided, timing of the mid-morning snack, and volume provided are common for the studied  
523 age and sex. Additionally, the authors' believe various aspects of the methodological  
524 approach were extremely robust (energy and volume matched snacks, rigorous assessment of  
525 subjective appetite, appetite- and metabolism-related peptides, energy expenditure and eating  
526 behavior). The results presented from this study may provide some important practical  
527 implications. Firstly, the findings of this study provide a strong foundation for further  
528 appetite- and metabolism-related research in children and adolescent populations who are  
529 metabolically challenged. Based on our findings, it seems milk is an attractive alternative to  
530 fruit-juice and sugar-sweetened beverages and may begin to provide initial evidence for  
531 stakeholders to help shape the development of future nutrition provision for children and  
532 adolescents. Indeed, the inclusion of milk-based foodstuff as a component of a healthy  
533 balanced diet is recognized extensively, providing a significant contribution of several  
534 essential nutrients (Fiorito, et al., 2006). Thus, encouraging the consumption of milk at mid-  
535 morning, over fruit-juice or other energy-dense food snacks that offer little nutritional benefit  
536 may positively influence young people's eating habits, especially within the school  
537 environment.

538         Caution should be observed when extrapolating the results of this study as the  
539 findings are constrained to adolescent males over a relatively modest duration. Moreover,  
540 there are a few limitations that warrant mention. Firstly, while our study design provided

541 robust experimental control, the provision of a limited highly palatable meal (cheese and  
542 tomato pasta) may have induced an element of overconsumption or progressive dislike for  
543 that matter that (if faced with) may have influenced subsequent eating behaviour and thus  
544 satiety. In children, the amount of food consumed at a laboratory test meal has been shown to  
545 vary in children according to palatability (Keller, Kirzner, Pietrobelli, St-Onge & Faith,  
546 2009), whereby the presentation of highly palatable foods promoted overconsumption at ad  
547 libitum assessments (Fearnbach, Thivel, Meyermann & Keller, 2015; Deighton, Frampton &  
548 Gonzalez 2016). Indeed, this has recently been shown for the meal used in this study  
549 (Deighton et al., 2016). However, the cited study presents evidence that eating behaviour at  
550 pasta meal better represents preceding appetite (Deighton et al., 2016). While it could be  
551 argued that presenting a highly palatable single course ad libitum meal may not reflect true  
552 eating practices it is important to note that the pasta meal utilised in this study is typical of  
553 what is commonly offered in a school setting, and similar meals have been used successfully  
554 in previous within-group adolescent research studies (Rumbold et al., 2013). Secondly, we  
555 did not control for factors such as usual breakfast and snacking habits in our sample of  
556 adolescent males. Although it could be argued this introduces a number of potentially  
557 confounding variables, retrospectively revisiting 24-hour food diaries for pre-trial  
558 standardisation few participants skipped breakfast or did not include snacks among their  
559 habitual dietary behaviors. Nonetheless, future investigation is certainly warranted to help  
560 further establish the longer-term health benefits of milk-based dairy food consumption in  
561 children and adolescents, particularly in those who are metabolically compromised. It would  
562 also be advantageous to follow on from this body of work by investigating female  
563 adolescents and younger populations.

564 To summarize, in an acute setting the consumption of milk influences short-term  
565 energy intake, reducing energy intake at an *ad libitum* pasta meal relative to an isoenergetic  
566 and isovolumetric serving of fruit-juice in adolescent males. This was replicated under free-  
567 living conditions whereby energy intake was reduced following chronic (28-d) mid-morning  
568 milk consumption, whereas the opposite was apparent for daily fruit-juice consumption.  
569 Acute mid-morning milk consumption increased postprandial glucagon secretion. In addition,  
570 chronic (28-d) mid-morning milk consumption elicited an increased postprandial insulin  
571 secretion and attenuates blood glucose concentrations. The findings presented throughout this  
572 study therefore indicate that acute and chronic mid-morning milk consumption can influence  
573 short-term eating behavior in male adolescents, and begin to illustrate that this may be  
574 facilitated through integrated endocrine responses.

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584

585 **References**

- 586 Abreu, S., Santos, R., Moreira, C., Santos, P. C., Vale, S., Soares-Miranda, L., Autran, R.,  
 587 Mota, J., & Moreira, P. (2014). Relationship of milk intake and physical activity to  
 588 abdominal obesity among adolescents. *Pediatr Obes*, 9(1), 71-80. doi:  
 589 10.1111/j.2047-6310.2012.00130.x
- 590 Acheson, K. J., Blondel-Lubrano, A., Oguey-Araymon, S., Beaumont, M., Emady-Azar, S.,  
 591 Ammon-Zufferey, C., Monnard, I., Pinaud, S., Nielsen-Moennoz, C., & Bovetto, L.  
 592 (2011). Protein choices targeting thermogenesis and metabolism. *Am J Clin Nutr*,  
 593 93(3), 525-534. doi: 10.3945/ajcn.110.005850
- 594 Almiron-Roig, E., Grathwohl, D., Green, H., & Erkner, A. (2009). Impact of some  
 595 isoenergetic snacks on satiety and next meal intake in healthy adults. *J Hum Nutr*  
 596 *Diet*, 22(5), 469-474. doi: 10.1111/j.1365-277X.2009.00978.x
- 597 Allsop, S., Rumbold, P. L., & Green, B. P. (2016). The between-day reproducibility of  
 598 fasting, satiety-related analytes, in 8 to 11 year-old boys. *Physiol Behav*, 164(Pt  
 599 A):207-213. doi: 10.1016/j.physbeh.2016.06.002.
- 600 Anderson, G. H., & Moore, S. E. (2004). Dietary proteins in the regulation of food intake and  
 601 body weight in humans. *J Nutr*, 134(4), 974S-979S.
- 602 Apolzan, J. W., Campbell, W. W., Carnell, N. S., Braun, M. M., Teegarden, D., Craig, B. A.,  
 603 Martin, B. R., & Weaver, C. M. (2006). Dairy affects acute thermic effect of food in  
 604 overweight, adolescent boys, but not girls. *The FASEB Journal*, 20(4), A587.
- 605 Ashley, J., Bovee, V., & Andersen, R. (2003). Dietary intake: Recording and analyzing.  
 606 *Obesity: Etiology, Assessment, Treatment and Prevention (ed) RE Andersen*.
- 607 Astbury, N. M., Taylor, M. A., French, S. J., & Macdonald, I. A. (2014). Snacks containing  
 608 whey protein and polydextrose induce a sustained reduction in daily energy intake  
 609 over 2 wk under free-living conditions. *Am J Clin Nutr*, 99(5), 1131-1140. doi:  
 610 10.3945/ajcn.113.075978
- 611 Astrup, A. (2005). The satiating power of protein--a key to obesity prevention? *Am J Clin*  
 612 *Nutr*, 82(1), 1-2.
- 613 Aziz, G., & Anderson, G. (2007). The effects of dairy components on food intake and satiety.  
 614 *Functional dairy products*, 2, 19-45.
- 615 Barba, G., Troiano, E., Russo, P., Venezia, A., & Siani, A. (2005). Inverse association  
 616 between body mass and frequency of milk consumption in children. *Br J Nutr*, 93(1),  
 617 15-19.
- 618 Bates, B., Lennox, A., & Swan, G. (2010). National diet and nutrition survey: headline results  
 619 from year 1 of the rolling programme (2008/2009). *London: Food Standards Agency*.
- 620 Bielohuby, M., Popp, S., & Bidlingmaier, M. (2012). A guide for measurement of circulating  
 621 metabolic hormones in rodents: Pitfalls during the pre-analytical phase. *Mol Metab*,  
 622 1(1-2), 47-60. doi: 10.1016/j.molmet.2012.07.004

- 623 Birch, L. L., McPhee, L. S., Bryant, J. L., & Johnson, S. L. (1993). Children's lunch intake:  
624 effects of midmorning snacks varying in energy density and fat content. *Appetite*,  
625 20(2), 83-94. doi: 10.1006/appe.1993.1011
- 626 Blundell, J., de Graaf, C., Hulshof, T., Jebb, S., Livingstone, B., Lluch, A., Mela, D., Salah,  
627 S., Schuring, E., van der Knaap, H., & Westerterp, M. (2010). Appetite control:  
628 methodological aspects of the evaluation of foods. *Obes Rev*, 11(3), 251-270. doi:  
629 10.1111/j.1467-789X.2010.00714.x
- 630 Bowen, J., Noakes, M., & Clifton, P. M. (2006). Appetite regulatory hormone responses to  
631 various dietary proteins differ by body mass index status despite similar reductions in  
632 ad libitum energy intake. *J Clin Endocrinol Metab*, 91(8), 2913-2919. doi:  
633 10.1210/jc.2006-0609
- 634 Chapelot, D. (2011). The role of snacking in energy balance: a biobehavioral approach. *J*  
635 *Nutr*, 141(1), 158-162. doi: 10.3945/jn.109.114330
- 636 Deighton, K., Frampton J., Gonzalez, J. T. (2016). Test-meal palatability is associated with  
637 overconsumption but better represents preceding changes in appetite in non-obese  
638 males. *Br J Nutr*, 1: 1-9. doi:10.1017/S0007114516002750
- 639 Dennison, B. A. (1996). Fruit juice consumption by infants and children: a review. *J Am Coll*  
640 *Nutr*, 15(5 Suppl), 4S-11S.
- 641 Dennison, B. A., Rockwell, H. L., & Baker, S. L. (1997). Excess fruit juice consumption by  
642 preschool-aged children is associated with short stature and obesity. *Pediatrics*, 99(1),  
643 15-22.
- 644 Dove, E. R., Hodgson, J. M., Puddey, I. B., Beilin, L. J., Lee, Y. P., & Mori, T. A. (2009).  
645 Skim milk compared with a fruit drink acutely reduces appetite and energy intake in  
646 overweight men and women. *Am J Clin Nutr*, 90(1), 70-75. doi:  
647 10.3945/ajcn.2008.27411
- 648 Dror, D. K. (2014). Dairy consumption and pre-school, school-age and adolescent obesity in  
649 developed countries: a systematic review and meta-analysis. *Obes Rev*, 15(6), 516-  
650 527. doi: 10.1111/obr.12158
- 651 du Prel, J. B., Hommel, G., Rohrig, B., & Blettner, M. (2009). Confidence interval or p-  
652 value?: part 4 of a series on evaluation of scientific publications. *Dtsch Arztebl Int*,  
653 106(19), 335-339. doi: 10.3238/arztebl.2009.0335
- 654 Duffey, K. J., Huybrechts, I., Mouratidou, T., Libuda, L., Kersting, M., De Vriendt, T.,  
655 Gottrand, F., Widhalm, K., Dallongeville, J., Hallstrom, L., Gonzalez-Gross, M., De  
656 Henauw, S., Moreno, L. A., & Popkin, B. M. (2012). Beverage consumption among  
657 European adolescents in the HELENA study. *Eur J Clin Nutr*, 66(2), 244-252. doi:  
658 10.1038/ejcn.2011.166
- 659 Fearnbach, S. N., Thivel, D., Meyermann, K., & Keller, K. L. (2015). Intake at a single,  
660 palatable buffet test meal is associated with total body fat and regional fat distribution  
661 in children. *Appetite*, 92, 233-239. doi: <http://dx.doi.org/10.1016/j.appet.2015.05.036>
- 662 Fiorito, L. M., Mitchell, D. C., Smiciklas-Wright, H., & Birch, L. L. (2006). Dairy and dairy-  
663 related nutrient intake during middle childhood. *J Am Diet Assoc*, 106(4), 534-542.  
664 doi: 10.1016/j.jada.2006.01.005
- 665 Flint, A., Gregersen, N. T., Gluud, L. L., Moller, B. K., Raben, A., Tetens, I., Verdich, C., &  
666 Astrup, A. (2007). Associations between postprandial insulin and blood glucose  
667 responses, appetite sensations and energy intake in normal weight and overweight  
668 individuals: a meta-analysis of test meal studies. *Br J Nutr*, 98(1), 17-25. doi:  
669 10.1017/s000711450768297x
- 670 Flint, A., Raben, A., Blundell, J.E., & Astrup, A. (2000). Reproducibility, power and validity  
671 of visual analogue scales in assessment of appetite sensations in single test meal  
672 studies. *Int. J. Obes*, 24, 38-48.

673 Frayn, K. N. (1983). Calculation of substrate oxidation rates in vivo from gaseous exchange.  
674 *J Appl Physiol Respir Environ Exerc Physiol*, 55(2), 628-634.

675 Gilbert, J. A., Joannisse, D. R., Chaput, J. P., Miegueu, P., Cianflone, K., Almeras, N., &  
676 Tremblay, A. (2011). Milk supplementation facilitates appetite control in obese  
677 women during weight loss: a randomised, single-blind, placebo-controlled trial. *Br J*  
678 *Nutr*, 105(1), 133-143. doi: 10.1017/s0007114510003119

679 Gonzalez, J. T., Green, B. P., Brown, M. A., Rumbold, P. L., Turner, L. A., & Stevenson, E.  
680 J. (2015). Calcium ingestion suppresses appetite and produces acute  
681 overcompensation of energy intake independent of protein in healthy adults. *J Nutr*,  
682 145(3), 476-482. doi: 10.3945/jn.114.205708

683 Green, B. P., Gonzalez, J. T., Thomas, K., Stevenson, E., & Rumbold, P. L. (2014).  
684 Agreement between fingertip-capillary and antecubital-venous appetite-related  
685 peptides. *Endocr Connect*, 3(4), 233-242. doi: 10.1530/ec-14-0110

686 Green, B. P., Turner, L., Stevenson, E., & Rumbold, P. L. (2015). Short communication:  
687 Patterns of dairy consumption in free-living children and adolescents. *J Dairy Sci*,  
688 98(6), 3701-3705. doi: 10.3168/jds.2014-9161

689 Keller, K. L., Kirzner, J., Pietrobelli, A., St-Onge, M.-P., & Faith, M. S. (2009). Increased  
690 Sweetened Beverage Intake Is Associated with Reduced Milk and Calcium Intake in  
691 3- to 7-Year-Old Children at Multi-Item Laboratory Lunches. *J Am Diet Assoc*,  
692 109(3), 497-501. doi: <http://dx.doi.org/10.1016/j.jada.2008.11.030>

693 Kim, S. Y., Sim, S., Park, B., Kong, I. G., Kim, J. H., & Choi, H. G. (2016). Dietary Habits  
694 Are Associated With School Performance in Adolescents. *Medicine (Baltimore)*,  
695 95(12), e3096. doi: 10.1097/md.0000000000003096

696 Leidy, H. J., Ortinau, L. C., Douglas, S. M., & Hoertel, H. A. (2013). Beneficial effects of a  
697 higher-protein breakfast on the appetitive, hormonal, and neural signals controlling  
698 energy intake regulation in overweight/obese, "breakfast-skipping," late-adolescent  
699 girls. *Am J Clin Nutr*, 97(4), 677-688. doi: 10.3945/ajcn.112.053116

700 Leidy, H. J., Todd, C. B., Zino, A. Z., Immel, J. E., Mukherjea, R., Shafer, R. S., Ortinau, L.  
701 C., & Braun, M. (2015). Consuming High-Protein Soy Snacks Affects Appetite  
702 Control, Satiety, and Diet Quality in Young People and Influences Select Aspects of  
703 Mood and Cognition. *J Nutr*, 145(7), 1614-1622. doi: 10.3945/jn.115.212092

704 Macdiarmid, J., Loe, J., Craig, L. C., Masson, L. F., Holmes, B., & McNeill, G. (2009). Meal  
705 and snacking patterns of school-aged children in Scotland. *Eur J Clin Nutr*, 63(11),  
706 1297-1304. doi: 10.1038/ejcn.2009.87

707 Mehrabani, S., Salehi-Abargouei, A., Asemi, M., Feizi, A., & Safavi, S. M. (2014). Effect of  
708 Low-fat Milk Consumption Compared to Apple Juice and Water on the Energy Intake  
709 Among 10-12-Year-Old Obese Boys: A Three-way Cross-over Clinical Trial. *Int J*  
710 *Prev Med*, 5(11), 1405-1411.

711 Melanson, E. L., Donahoo, W. T., Dong, F., Ida, T., & Zemmel, M. B. (2005). Effect of low-  
712 and high-calcium dairy-based diets on macronutrient oxidation in humans. *Obes Res*,  
713 13(12), 2102-2112. doi: 10.1038/oby.2005.261

714 Moore, L. L., Singer, M. R., Qureshi, M. M., & Bradlee, M. L. (2008). Dairy intake and  
715 anthropometric measures of body fat among children and adolescents in NHANES. *J*  
716 *Am Coll Nutr*, 27(6), 702-710.

717 Nilsson, M., Stenberg, M., Frid, A. H., Holst, J. J., & Bjorck, I. M. (2004). Glycemia and  
718 insulinemia in healthy subjects after lactose-equivalent meals of milk and other food  
719 proteins: the role of plasma amino acids and incretins. *Am J Clin Nutr*, 80(5), 1246-  
720 1253.

- 721 Ostman, E. M., Liljeberg Elmstahl, H. G., & Bjorck, I. M. (2001). Inconsistency between  
722 glycemic and insulinemic responses to regular and fermented milk products. *Am J*  
723 *Clin Nutr*, 74(1), 96-100.
- 724 Ovaskainen, M. L., Reinivuo, H., Tapanainen, H., Hannila, M. L., Korhonen, T., & Pakkala,  
725 H. (2006). Snacks as an element of energy intake and food consumption. *Eur J Clin*  
726 *Nutr*, 60(4), 494-501. doi: 10.1038/sj.ejcn.1602343
- 727 Panahi, S., Luhovyy, B. L., Liu, T. T., Akhavan, T., El Khoury, D., Goff, H. D., & Anderson,  
728 G. H. (2013). Energy and macronutrient content of familiar beverages interact with  
729 pre-meal intervals to determine later food intake, appetite and glycemic response in  
730 young adults. *Appetite*, 60(1), 154-161. doi: 10.1016/j.appet.2012.09.018
- 731 Parker, J. A., McCullough, K. A., Field, B. C., Minnion, J. S., Martin, N. M., Ghatei, M. A.,  
732 & Bloom, S. R. (2013). Glucagon and GLP-1 inhibit food intake and increase c-fos  
733 expression in similar appetite regulating centres in the brainstem and amygdala. *Int J*  
734 *Obes (Lond)*, 37(10), 1391-1398. doi: 10.1038/ijo.2012.227
- 735 Penick, S. B., & Hinkle, L. E., Jr. (1961). Depression of food intake induced in healthy  
736 subjects by glucagon. *N Engl J Med*, 264, 893-897. doi:  
737 10.1056/nejm196105042641801
- 738 Rolls, B. J., Hetherington, M., & Burley, V. J. (1988). The specificity of satiety: the influence  
739 of foods of different macronutrient content on the development of satiety. *Physiol*  
740 *Behav*, 43(2), 145-153.
- 741 Rumbold, P. L., St Clair Gibson, A., Stevenson, E., & Dodd-Reynolds, C. J. (2011).  
742 Agreement between two methods of dietary data collection in female adolescent  
743 netball players. *Appetite*, 57(2), 443-447. doi: 10.1016/j.appet.2011.06.013
- 744 Rumbold, P. L., St Clair Gibson, A., Stevenson, E. J., King, J. A., Stensel, D. J., & Dodd-  
745 Reynolds, C. J. (2013). Influence of netball-based exercise on energy intake,  
746 subjective appetite and plasma acylated ghrelin in adolescent girls. *Appl Physiol Nutr*  
747 *Metab*, 38(8), 854-861. doi: 10.1139/apnm-2012-0466
- 748 Savige, G., Macfarlane, A., Ball, K., Worsley, A., & Crawford, D. (2007). Snacking  
749 behaviours of adolescents and their association with skipping meals. *Int J Behav Nutr*  
750 *Phys Act*, 4, 36. doi: 10.1186/1479-5868-4-36
- 751 Schmid, R., Schusdziarra, V., Schulte-Frohlinde, E., Maier, V., & Classen, M. (1989). Role  
752 of amino acids in stimulation of postprandial insulin, glucagon, and pancreatic  
753 polypeptide in humans. *Pancreas*, 4(3), 305-314.
- 754 Schneeman, B. O., Burton-Freeman, B., & Davis, P. (2003). Incorporating dairy foods into  
755 low and high fat diets increases the postprandial cholecystokinin response in men and  
756 women. *J Nutr*, 133(12), 4124-4128.
- 757 Shakespeare, T. P., Gebiski, V. J., Thiagarajan, A., & Jay Lu, J. (2006). Development of a  
758 spreadsheet for the calculation of new tools to improve the reporting of the results of  
759 medical research. *Med Inform Internet Med*, 31(2), 121-127. doi:  
760 10.1080/14639230600551397
- 761 Taylor, C., Lamparello, B., Kruczek, K., Anderson, E. J., Hubbard, J., & Misra, M. (2009).  
762 Validation of a food frequency questionnaire for determining calcium and vitamin D  
763 intake by adolescent girls with anorexia nervosa. *J Am Diet Assoc*, 109(3), 479-485,  
764 485 e471-473. doi: 10.1016/j.jada.2008.11.025
- 765 Thivel, D., Isacco, L., Montaurier, C., Boirie, Y., Duche, P., & Morio, B. (2012). The 24-h  
766 energy intake of obese adolescents is spontaneously reduced after intensive exercise:  
767 a randomized controlled trial in calorimetric chambers. *PLoS One*, 7(1), e29840. doi:  
768 10.1371/journal.pone.0029840

- 769 Thivel, D., Isacco, L., Rousset, S., Boirie, Y., Morio, B., & Duche, P. (2011). Intensive  
770 exercise: a remedy for childhood obesity? *Physiol Behav*, *102*(2), 132-136. doi:  
771 10.1016/j.physbeh.2010.10.011
- 772 van Loon, L. J., Saris, W. H., Verhagen, H., & Wagenmakers, A. J. (2000). Plasma insulin  
773 responses after ingestion of different amino acid or protein mixtures with  
774 carbohydrate. *Am J Clin Nutr*, *72*(1), 96-105.
- 775 Wang, Y. C., Gortmaker, S. L., Sobol, A. M., & Kuntz, K. M. (2006). Estimating the energy  
776 gap among US children: a counterfactual approach. *Pediatrics*, *118*(6), e1721-1733.  
777 doi: 10.1542/peds.2006-0682
- 778 Woods, S. C., Lutz, T. A., Geary, N., & Langhans, W. (2006). Pancreatic signals controlling  
779 food intake; insulin, glucagon and amylin. *Philos Trans R Soc Lond B Biol Sci*,  
780 *361*(1471), 1219-1235. doi: 10.1098/rstb.2006.1858
- 781 Woodward-Lopez, G., Kao, J., & Ritchie, L. (2011). To what extent have sweetened  
782 beverages contributed to the obesity epidemic? *Public Health Nutr*, *14*(3), 499-509.  
783 doi: 10.1017/s1368980010002375
- 784 Zandstra, E. H., Mathey, M. F., Graaf, C., & van Staveren, W. A. (2000). Short-term  
785 regulation of food intake in children, young adults and the elderly. *Eur J Clin Nutr*,  
786 *54*(3), 239-246.
- 787 Zemel, M. B., Shi, H., Greer, B., Dirienzo, D., & Zemel, P. C. (2000). Regulation of  
788 adiposity by dietary calcium. *FASEB J*, *14*(9), 1132-1138.
- 789 Zheng, M., Rangan, A., Olsen, N. J., Andersen, L. B., Wedderkopp, N., Kristensen, P.,  
790 Grontved, A., Ried-Larsen, M., Lempert, S. M., Allman-Farinelli, M., & Heitmann,  
791 B. L. (2015). Substituting sugar-sweetened beverages with water or milk is inversely  
792 associated with body fatness development from childhood to adolescence. *Nutrition*,  
793 *31*(1), 38-44. doi: 10.1016/j.nut.2014.04.017

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## Figure Legends

808 **Figure 1.** Mean  $\pm$  SEM concentrations of plasma GLP-1<sub>7-36</sub> (pg·mL; panel A) and plasma  
809 glucagon (pg·mL; panel B), obtained from fingertip-capillary blood samples. Graphs depicted  
810 on the left are from the chronic milk snack condition (n = 9) whereas graphs depicted on the  
811 right are from the fruit-juice condition (n = 8). For the left sided graphs, grey shaded boxes  
812 represent values obtained on the first experimental visit (day-0, baseline), whereas white  
813 shaded boxes represent values obtained on the last day (day-28, follow up) of each  
814 intervention phase. For the right sided graphs, grey shaded circles represent values obtained  
815 on the first experimental visit (day-0, baseline), whereas white shaded circles represent values  
816 obtained on the last day (day-28, follow up) of each intervention phase. To convert GLP-1<sub>7-36</sub>  
817 (pg·mL) and plasma glucagon (pg·mL) to their corresponding SI units multiply values by  
818 0.298 and 0.287, respectively. Mid-morning snack items were distributed at 90 min post-  
819 breakfast, as represented by the grey shaded area.

820 **Figure 2.** Mean  $\pm$  SEM concentrations of plasma insulin (pmol·L; Panel A), plasma leptin  
821 (pg·mL; Panel B) and capillary blood glucose (mmol·L; Panel C), obtained from fingertip-  
822 capillary blood samples. Graphs depicted on the left are from the chronic milk snack  
823 condition (n = 9) whereas graphs depicted on the right are from the fruit-juice condition (n =  
824 8). For the left sided graphs, grey shaded boxes represent values obtained on the first  
825 experimental visit (day-0, baseline), whereas white shaded boxes represent values obtained  
826 on the last day (day-28, follow up) of each intervention phase. For the right sided graphs,  
827 grey shaded circles represent values obtained on the first experimental visit (day-0, baseline),  
828 whereas white shaded circles represent values obtained on the last day (day-28, follow up) of  
829 each intervention phase. Mid-morning snack items were distributed at 90 min post-breakfast,  
830 as represented by the grey shaded area. Please note: significant observations relate to time-  
831 averaged AUC fingertip-capillary variables.

832 **Figure 3.** Mean  $\pm$  SEM *ad libitum* pasta meal (Panel A, n = 11) and free-living (Panel B, n =  
833 11) energy intake (kJ) following acute mid-morning milk snack consumption, relative to an  
834 isoenergetic and isovolumetric serving of fruit-juice. Please note: free-living energy intake  
835 was considered as calories consumed/reported for the remainder of the study as calculated by  
836 weighed food record.

837 **Figure 4.** Mean  $\pm$  SEM *ad libitum* pasta meal (Panel A, n = 11) and free-living (Panel B, n =  
838 11) energy intake (kJ) on the first (day-0, baseline) and last (day-28, endpoint) day of each  
839 intervention phase. Grey shaded boxes represent values obtained during the daily milk snack  
840 condition (n = 9), whereas grey shaded circles represent values obtained during the daily  
841 fruit-juice condition (n = 8).

**Table 1.** Nutritional composition of the snack foods for both the acute and daily experiment.

	Milk <sup>1</sup>	Fruit-Juice <sup>2</sup>
Serving Size (+ mL water)	207 (10)	217
Energy content (kJ)	427	427
Carbohydrate (g)	10.2	32.0
Fat (g)	3.7	0.0
Protein (g)	7.5	1.1

Note: <sup>1</sup>milk (< 2% fat, Tesco, UK), and <sup>2</sup>fruit-juice (Pure orange juice smooth; Tesco, UK).

**Table 2.** (A) Time-averaged AUC subjective appetite data (n = 11) following acute (A) mid-morning milk snack consumption, relative to an isoenergetic and isovolumetric serving of fruit-juice. (B) Time-averaged AUC subjective appetite values obtained during the daily experiment on the first experimental visit (day 0, baseline), and last (day 28, endpoint) days of the intervention phase. Data following daily mid-morning milk snack consumption (n = 9) is expressed on the left side and data following daily fruit-juice (n = 8) is expressed on the right. All time-averaged AUC values are dichotomised according to post-breakfast (0-90 min) and post-snack (90-180 min) postprandial periods, with Values are expressed as mean (SEM), alongside their corresponding 95% CI. \* indicates a difference at the same time point relative to the fruit-juice, whereas † indicates a difference from baseline observation at the same time during the endpoint visit.

A. Acute Experiment		Post-breakfast period (time-averaged AUC <sub>0-90 min</sub> )			Post-snack period (time-averaged AUC <sub>90-180 min</sub> )		
		Milk	Fruit-juice	95% CI	Milk	Fruit-juice	95% CI
		Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)	95% CI
	Hunger (mm)	54.8 (4.3)	57.4 (4.8)	16-7, -11.6	69.5 (4.2)	67.4 (3.1)	10.8, -15.0
	Fullness (mm)	35.0 (5.1)	31.7 (4.3)	9.8, -16.5	20.8 (2.6)	26.8 (4.0)	-11.6, -0.4*
	Prospective Food Consumption (mm)	66.0 (3.3)	63.4 (4.6)	3.7, -8.9	76.9 (3.5)	72.1 (3.2)	1.8, 7.9 *
	Satisfaction (mm)	37.4 (4.1)	32.3 (3.8)	9.2, -3.5	25.3 (3.5)	28.2 (3.4)	9.2, -3.5
B. Daily Experiment		Post-breakfast period (time-averaged AUC <sub>0-90 min</sub> )			Post-breakfast period (time-averaged AUC <sub>0-90 min</sub> )		
		Milk			Fruit-Juice		
		Day 0	Day 28	95% CI	Day 0	Day 28	95% CI
		Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)	95% CI
	Hunger (mm)	50.8 (3.3)	59.3 (4.9)	21.6, -4.5	46.9 (6.0)	46.9 (6.0)	31.3, -14.2
	Fullness (mm)	34.0 (5.1)	29.7 (5.2)	6.0, -14.7	43.6 (5.9)	36.6 (7.8)	9.8, -23.8
	Prospective Food Consumption (mm)	68.2 (2.6)	73.3 (4.3)	15.1, -4.9	60.4 (6.1)	60.9 (6.0)	13.2, -13.3
	Satisfaction (mm)	35.7 (1.8)	34.5 (4.2)	9.5, -11.9	44.2 (6.1)	37.1 (7.3)	8.0, -22.2
		Post-snack period (time-averaged AUC <sub>90-180 min</sub> )			Post-snack period (time-averaged AUC <sub>90-180 min</sub> )		
		Milk			Fruit-Juice		
		Day 0	Day 28	95% CI	Day 0	Day 28	95% CI
		Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)	95% CI
	Hunger (mm)	75.8 (2.3)	78.4 (2.8)	10.1, -4.8	64.9 (4.3)	75.1 (4.2)	25.9, -5.3
	Fullness (mm)	17.1 (2.6)	18.9 (3.8)	7.9, -4.3	30.5 (5.3)	21.9 (4.9)	5.1, -22.3
	Prospective Food Consumption (mm)	81.1 (1.8)	85.3 (1.8)	9.5, -1.2	71.5 (3.7)	78.1 (4.4)	17.1, -3.8
	Satisfaction (mm)	23.0 (3.8)	18.0 (2.1)	3.6, -13.6	32.1 (4.9)	23.5 (5.4)	2.3, -19.6