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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 ± 0.9 y; BMI: 23.3 ± 3.3 kg/m²] 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 ± 0.9 y; BMI: 21.8 ± 3.7
7 kg/m²] 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-1₇₋₃₆), 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 ± 1.1 vs.
116 16.4 ± 0.7 y), body mass (69.4 ± 18.3 vs. 68.2 ± 10.5 kg), body mass index ([BMI] 22.0 ± 5.0
117 vs. 21.6 ± 2.5 kg·m²) and habitual calcium intake (814.5 ± 118.4 vs. 836.0 ± 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 ± 0.8 y; body
126 mass: 73.4 ± 11.5 kg; stature: 1.8 ± 0.1 m; BMI: 23.3 ± 3.3 kg/m²) were recruited for the

127 acute experiment and another 19 different participants (mean \pm SD; age: 16.1 ± 0.9 y; body
128 mass: 68.8 ± 13.9 kg; stature: 1.8 ± 0.0 m; BMI: 21.8 ± 3.7 kg/m²; habitual calcium intake:
129 790 ± 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: 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 (≥ 24 -h) and
142 strenuous physical activity (≥ 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-1₇₋₃₆, 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-1₇₋₃₆ 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-1₇₋₃₆ (pg·mL), glucagon (pg·mL), leptin (pg·mL) and insulin (pmol·L)
267 were simultaneously determined in 40 μL 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-1₇₋
270 ₃₆ 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-1₇₋₃₆, 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-1₇₋₃₆, 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 β -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[®], 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 α 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-1₇₋₃₆, glucagon, insulin, leptin
347 and blood glucose concentrations were comparable between conditions. This remained during
348 the post-breakfast period (time-averaged AUC_{0-90 min}), 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_{90-180 min} 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-1₇₋₃₆, 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-1₇₋₃₆, glucagon, insulin, leptin and blood glucose
357 post-breakfast (AUC_{0-90 min}) and post-snack (AUC_{90-180 min}) 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-1₇₋₃₆, glucagon, and leptin (**Figures 1**
360 **& 2**). Time-averaged AUC_{0-90 min} 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_{90-180min} (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_{90-180 min} 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. $6111.0 \pm$
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 min}). Following
395 the acute mid-morning milk snack, time-averaged AUC_{90-180 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 AUC₉₀₋₁₈₀
398 _{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 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_{90-180 min}) 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 ± 2 mg
505 Ca), supplemental calcium (670 ± 4 mg Ca) or a dairy-based product (687 ± 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

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Figure Legends

808 **Figure 1.** Mean \pm SEM concentrations of plasma GLP-1₇₋₃₆ (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-1₇₋₃₆
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 ¹	Fruit-Juice ²
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: ¹milk (< 2% fat, Tesco, UK), and ²fruit-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 _{0-90 min})			Post-snack period (time-averaged AUC _{90-180 min})			
	Milk	Fruit-juice	95% CI	Milk	Fruit-juice	95% CI	
	Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)		
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 _{0-90 min})			Post-breakfast period (time-averaged AUC _{0-90 min})			
	Milk			Fruit-Juice			
	Day 0	Day 28	95% CI	Day 0	Day 28	95% CI	
	Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)		
	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 _{90-180 min})			Post-snack period (time-averaged AUC _{90-180 min})			
	Milk			Fruit-Juice			
Day 0	Day 28	95% CI	Day 0	Day 28	95% CI		
Mean (SEM)	Mean (SEM)		Mean (SEM)	Mean (SEM)			
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	