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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

Comprising of two experiments, this study assessed the metabolic, endocrine and appetite-related responses to acute and chronic milk consumption in adolescent males (15–18 y). Eleven adolescents [mean ± SD age: 16.5 ± 0.9 y; BMI: 23.3 ± 3.3 kg/m$^2$] participated in the acute experiment and completed two laboratory visits (milk vs. fruit-juice) in a randomized crossover design, separated by 7-d. Seventeen adolescents [age: 16.1 ± 0.9 y; BMI: 21.8 ± 3.7 kg/m$^2$] completed the chronic experiment. For the chronic experiment, a parallel design with two groups was used. Participants were randomly allocated and consumed milk (n = 9) or fruit-juice (n = 8) for 28-d, completing laboratory visits on the first (baseline, day-0) and last day (follow-up, day-28) of the intervention phase. On laboratory visits (for both experiments), measures of appetite, metabolism and endocrine responses were assessed at regular intervals. In addition, eating behavior was quantified by *ad libitum* assessment under laboratory conditions and in the free-living environment by weighed food record. Acute milk intake stimulated glucagon (P = 0.027 [16.8 pg mL; 95% CI: 2.4, 31.3]) and reduced *ad libitum* energy intake relative to fruit-juice (P = 0.048 [−651.3 kJ; 95% CI: −1294.1, −8.6]), but was comparable in the free-living environment. Chronic milk intake reduced free-living energy intake at the follow-up visit compared to baseline (P = 0.013 [-1910.9 kJ; 95% CI: −554.6, −3267.2]), whereas the opposite was apparent for fruit-juice. Relative to baseline, chronic milk intake increased the insulin response to both breakfast (P = 0.031) and mid-morning milk consumption (P = 0.050) whilst attenuating blood glucose (P = 0.025). Together, these findings suggest milk consumption impacts favorably on eating behavior in adolescent males, potentially through integrated endocrine responses.



## 1. Introduction

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

From a child and adolescent perspective, fruit-juice drinks, sugar-sweetened beverages and milks are frequently reported as common beverage snack items consumed between main meals

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(Duffey et al., 2012). Despite fruit-juice providing vitamins, minerals and antioxidants, and sugar-sweetened beverages which hold a negligible nutritive value, high rates of consumption appear to promote weight gain in children and adolescents (Dennison, 1996; Dennison, Rockwell, & Baker, 1997; Woodward-Lopez, Kao, & Ritchie, 2011). Interestingly, the opposite may stand true for milk-based beverages (Dror, 2014). Indeed, emerging evidence suggests that milk-based beverages protect against adiposity in children and adolescents (Abreu et al., 2014; Barba, Troiano, Russo, Venezia, & Siani, 2005; Moore, Singer, Qureshi, & Bradlee, 2008), and the replacement of sugar-sweetened beverages with milk or water, but not fruit-juice, is inversely associated with body fatness throughout the transition from childhood to adolescence (Zheng et al., 2015). Relative to fruit-juice drinks and sugar-sweetened beverages, milk-based beverages are recognized as a nutrient-dense foodstuff and contain a host of constituents that improve the overall nutritional quality of the child and adolescent diet (Fiorito, Mitchell, Smiciklas-Wright, & Birch, 2006). Outside of the health-related benefits, recent evidence also indicates that high rates of milk consumption are positively associated with academic performance and motivation for learning in adolescents compared to sugar-sweetened beverage intake (Kim et al., 2016).

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

According to the acute literature (Birch, McPhee, Bryant, & Johnson, 1993; Mehrabani, Salehi-Abargouei, Asemi, Feizi, & Safavi, 2014; Zandstra, Mathey, Graaf, & van Staveren, 2000), mid-morning dairy snack consumption [ice-cream (Birch et al., 1993), yogurt (Zandstra et al., 2000) and milk (Mehrabani et al., 2014)] reduces energy intake and increases energy expenditure (Apolzan et al., 2006) in children and adolescents (3- to 15-y-old). However, these studies are primarily limited to acute child investigations utilizing dissimilar preloads (differing according to volume and energetic content) and single energy intake assessment (laboratory based *ad libitum* assessment). Moreover, no quantitative measures of subjective appetite and/or appetite- and metabolism-related peptides were included which may have provided valuable insights concerning the mechanisms impacting on appetite and eating behavior, and thus remains to be examined. Without a better understanding of the mechanisms impacting on appetite and eating behavior following dairy consumption, it remains challenging to reconcile the potential effects of different dairy foods on energy regulation in children and adolescents. Consequently, this study investigated the effect of acute and chronic (28-d) mid-morning milk snack consumption on subsequent metabolic, endocrine and appetite-related responses.

## 2. Materials and methods

### 2.1. Experimental design

A randomized crossover design was implemented with two experimental conditions to investigate the acute effects of milk consumption on subsequent energy intake, circulating concentrations of glucagon-like peptide-1 (GLP-$1_{7\text{-}36}$), glucagon, insulin, leptin and blood glucose, energy expenditure and subjective appetite. Experimental visits consisted of mid-morning milk (<2% fat) and an isoenergetic and isovolumetric serving of fruit-juice, each separated by 7-days. To investigate the effects of chronic milk consumption on the abovementioned metabolic, endocrine and appetite-related responses, a parallel design with two intervention groups was used. Participants were randomly allocated to groups, and received either daily mid-morning milk (<2% fat) or an isoenergetic and isovolumetric fruit-juice for 28 days. Participants made two experimental visits to the nutrition and metabolism laboratory, which were scheduled on the first (day-0, baseline) and last (day-28, follow up) days of the intervention phase. Participants were matched according to age (16.1 ± 1.1 vs. 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 vs. 21.6 ± 2.5 kg $m^2$) and habitual calcium intake (814.5 ± 118.4 vs. 836.0 ± 274.9 mg d) intake. Habitual calcium intakes were estimated using a validated food frequency questionnaire for determining calcium and vitamin D intake in adolescents (Taylor et al., 2009). All testing was completed during school-term time.

### 2.2. Participants

Participants were recruited from a local secondary school in the North-East of England, after attendance at an initial information seminar. Adolescent males between 15 and 18 y of age were eligible to participate. Eleven male adolescents (mean ± SD; age: 16.5 ± 0.8 y; body mass: 73.4 ± 11.5 kg; stature: 1.8 ± 0.1 m; BMI: 23.3 ± 3.3 kg/$m^2$) were recruited for the acute experiment and another 19 different participants (mean ± SD; age: 16.1 ± 0.9 y; body mass: 68.8 ± 13.9 kg; stature: 1.8 ± 0.0 m; BMI: 21.8 ± 3.7 kg/$m^2$; habitual calcium intake: 790 ± 217 mg d) for the daily experiment. All participants were free of milk-related allergies, diabetes or other metabolic disorders (and medication) known to affect taste, smell and appetite.

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

### 2.3. Pre-trial standardisation

Participants were instructed to record all food and fluid consumption 24-h preceding the first visit for both the acute and chronic experiments, using a self-report, weighed food diary. Participants were also advised to refrain from caffeine and alcohol consumption (≥24-h) and strenuous physical activity (≥24-h) before each experimental visit (days-0 and -28 of the daily