- 2 An afternoon snack of berries reduces subsequent energy intake compared to an
- 3 isoenergetic confectionary snack

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- 5 Lewis J. James¹, Mark P. Funnell¹ and Samantha Milner¹
- 6 ¹School of Sport, Exercise and Health Sciences, Loughborough University,
- 7 Leicestershire, UK, LE11 3TU.

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9 Corresponding author

- 10 Lewis J. James
- 11 L.james@lboro.ac.uk
- 12 School of Sport, Exercise and Health Sciences
- 13 Loughborough University

Abstract

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Observational studies suggest that increased fruit and vegetable consumption can contribute to weight maintenance and facilitate weight loss when substituted for other energy dense foods. Therefore, the purpose of the present study was to assess the effect of berries on acute appetite and energy intake. Twelve unrestrained premenopausal women (age 21 \pm 2 y; BMI 26.6 \pm 2.6 kg·m⁻²; body fat 23 \pm 3 %) completed a familiarisation trial and two randomised experimental trials. Subjects arrived in the evening (~5pm) and consumed an isoenergetic snack (65 kcal) of mixed berries (BERRY) or confectionary sweets (CONF). Sixty min later, subjects consumed a homogenous pasta test meal until voluntary satiation, and energy intake was quantified. Subjective appetite (hunger, fullness, desire to eat and prospective food consumption) was assessed throughout trials, and for 120 min after the test meal. Energy intake was less (P<0.001) after consumption of the BERRY snack (691 ± 146 kcal) than after the CONF snack (824 ± 172 kcal); whilst water consumption was similar (P=0.925). There were no trial (P>0.095) or interaction (P>0.351) effects for any subjective appetite ratings. Time taken to eat the BERRY snack (4.05 ± 1.12) min) was greater (P<0.001) than the CONF snack (0.93 ± 0.33 min). This study demonstrates that substituting an afternoon confectionary snack with mixed berries decreased subsequent energy intake at dinner, but did not affect subjective appetite. This dietary strategy could represent a simple method for reducing daily energy intake and aiding weight management.

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Keywords: Energy Balance; Appetite; Weight Management; Fruit

Introduction

Obesity is caused by a chronic positive energy balance; a sustained daily energy intake exceeding energy expenditure, resulting in the accumulation of adipose tissue and an increased mortality risk (James, 2004; Adams et al. 2006). With the increased prevalence of obesity worldwide (James, 2004), and its associated comorbidities (Guh et al. 2009), dietary strategies targeted at suppressing appetite and facilitating weight management are needed to support a reduced overall energy balance (Rolls, 2009).

Snack foods (snacks) are a fundamental aspect of dietary habits, contributing to greater than 18% of daily energy intake and between 1 - 4 feeding episodes per day (Ovaskainen et al. 2006; Bellisle et al. 2003). The consumption of energy dense, nutrient deficient snacks has been associated with overweight and obesity in adults (Bes-Rastrollo et al. 2010) and children (Bo et al. 2014), as well as poor metabolic health (Mirmiran et al. 2014). Decreasing the energy density of the diet, specifically snacks, by replacing energy dense foods with fruit and/ or vegetables has been proposed as a dietary strategy to decrease hunger and energy intake, and consequently promote weight loss (Ello-Martin et al. 2007; Houchins et al. 2013). Evidence demonstrates that reducing the energy density of a meal (Rolls et al. 1999b; Bell et al. 1998), a snack (Farajian et al. 2010; Rolls et al. 1998), and a first-course entrée prior to a meal (Rolls et al. 2004; Blatt et al. 2012), can decrease meal energy intake, both independent of and when macronutrient composition is held constant.

Observational studies indicate that increased fruit and vegetable intake can contribute to weight maintenance (i.e. preventing weight gain) and facilitate weight loss when substituted for other energy dense foods (Boeing et al. 2012). Since snacks contribute significantly to daily energy intake, replacing energy dense snacks with fruit and/ or vegetables may promote weight loss and induce positive health benefits. Previous studies have reported that a snack of dried fruit increased satiety (Furchner-Evanson et al. 2010; Farajian et al. 2010), as well as decreased subsequent energy intake (Farajian et al. 2010) compared to other snack foods. Whilst Patel et al. (2010) reported a reduced energy intake of an *ad-libitum* snack of raisins (dried fruit) or grapes compared to other snacks; to the authors knowledge no

- research has assessed the acute appetite effects of replacing an energy dense snack with fruit.
- 71 Given the paucity of data examining the effect of fruit intake on subsequent appetite
- and energy intake, this topic warrants further investigation. Therefore, the purpose of
- the present study was to compare the appetite and energy intake effects of a snack
- 74 of mixed berries (strawberries, raspberries, blackberries and blueberries) with an
- isoenergetic confectionary snack (sweets).

76 Methods

77 Subjects

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Twelve pre-menopausal women (age 21 ± 2 y; body mass 75.6 ± 8.9 kg; height 1.69 \pm 0.08 m; BMI 26.6 \pm 2.6 kg·m⁻²; body fat 23 \pm 3 %) volunteered for this study, which was approved by the Loughborough University Ethics Approvals (Human Participants) Sub Committee (reference number: R14-P128). All subjects were healthy, non-smokers, weight stable for the past 6 months (self-reported), and not taking medications known to affect appetite. Each subject provided written informed consent, completed a medical screening questionnaire and a three-factor eating questionnaire (Stunkard & Messick, 1985) prior to commencement of the study. Subjects were not restrained, disinhibited or hunger eaters. Using previous data from our laboratory (Clayton et al. 2014), an expected between trial difference of ~420 kJ. between trial correlation of 0.5, an α of 0.05 and a β of 0.2, it was estimated that 13 subjects would be required to reject the null hypothesis (Faul et al. 2009). Therefore, 15 subjects were recruited, but 3 subjects dropped out after completing the familiarisation trial (2 due to other time constraints and 1 due to becoming pregnant). Each subject completed a preliminary trial and two experimental trials in a randomised counterbalanced order.

Pre-trial standardisation

Subjects arrived for trials 4 h after lunch, but were able to drink water *ad-libitum* until 2 h before arrival. To ensure similar metabolic conditions prior to each experimental trial, subjects recorded their dietary intake and habitual physical activity for the day of and day preceding their first experimental trial. The diet and activity patterns were replicated prior to the second experimental trial and adherence to these requirements were verbally checked. Subjects also refrained from any strenuous exercise or alcohol intake during this period. Trials were scheduled to minimise the possibility of hormone related appetite fluctuations. Three subjects were not using any form of contraceptive (n=3) and their trials took place during the early-mid follicular phase of their menstrual cycle (days 5-11). Seven subjects were using a combined contraceptive pill, and their trials took place after at least 2 days continuous pill use and after day 4 of their menstrual cycle. Two subjects had a

107 progesterone only contraceptive implant and their trials were separated by exactly 7 108 days. . 109 Preliminary trial 110 During the preliminary trial, subject's height and weight were recorded before 111 skinfold measurements were obtained from the triceps, biceps, subscapular and 112 suprailiac for the estimation of body fat percentage (Durnin & Womersley, 1974). 113 Subjects then completed an appetite questionnaire (Flint et al. 2000) and were 114 familiarised with the *ad-libitum* pasta test meal. 115 Experimental trials 116 Experimental trials commenced in the late afternoon (~5pm), with the specific time 117 standardised for each individual subject. Upon arrival, subjects voided their bladder 118 and bowels, and body mass was recorded in light clothing (Adam Equipment Co., 119 AFW-120K, UK). Thereafter, subjects completed a subjective appetite questionnaire, 120 before being provided with a snack of either mixed berries (BERRY) or confectionary 121 (CONF). The snacks were matched for energy content, with the BERRY snack 122 consisting of 40 g strawberries, 40 g raspberries, 40 g blackberries and 40 g

blueberries, and the CONF snack consisting of 19.4 g sweets (Bassetts Jelly Babies

Berry Mix, Modelez UK, Birmingham, UK) (Table 1). Each snack was accompanied

by 100 ml water. Subjects were instructed to consume the snack continuously as if it

was an afternoon snack, and the time taken for complete ingestion was recorded. All

trials took place in a dedicated feeding laboratory and subjects remained in complete

isolation throughout, except for essential interaction with the experimenter.

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Table 1. Snack energy and macronutrient composition.

	BERRY	CONF
Energy (kJ)	272	272
Energy density (kJ/g)	1.7	14.0
Protein (g)	1.5	0.7
Carbohydrate (g)	12.1	15.5
Fat (g)	0.4	0.0
Fibre (g)	3.6	0.0
Water (g)	142	3

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Sixty min after the start of the snack, subjects were presented with a homogenous pasta test meal, which they ate ad-libitum until voluntary satiation. The test meal consisted of fusilli pasta, Bolognese pasta sauce and olive oil (Tesco Stores Ltd., Cheshnut, UK); each meal received identical heating and cooling. The energy density of the meal was 5.87 ± 0.03 kJ/g and was not different between trials (P=0.596). The test meal was served to subjects in a custom built feeding booth inside an isolated feeding laboratory. Subjects were initially served a large bowl of pasta (~700 g) and a glass of water (~500 g). After 7.5 min, these were removed and replaced with a fresh bowl of pasta (~700 g) and glass of water (~500 g), and subjects continued eating until voluntary satiation. Before the meal, subjects received standardised instructions to eat until they were "comfortably full and satisfied". Subjects had 30 min in which to eat and remained in the feeding laboratory for the entire 30 min period, during which time food was continuously available inside the feeding booth. Subjects indicated satiation by leaving the feeding booth and taking a seat in the feeding laboratory. The point at which subjects left the feeding booth was recorded. All subjects left the feeding booth within the 30 min period and did not return to the feeding booth. Food and water intake were quantified by weighing bowls and glasses before and after consumption (PCB Electronic Precision Scale, Kern & Sohn GmbH, Balingen, Germany), and energy intake was determined using manufacturer values.

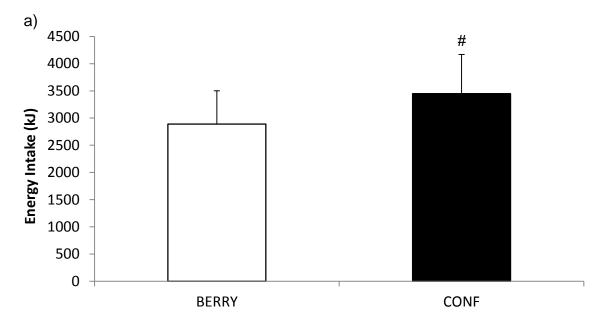
Additional appetite questionnaires were completed 15 min and 30 min after the snack, immediately before and after the pasta test meal, as well as 30 min, 60 min

and 120 min after the pasta test meal. Subjects left the laboratory after completing the post-meal questionnaire, but were instructed not to eat, drink or perform any physical activity until the final questionnaire had been completed 120 min later. For each appetite questionnaire visual analogue scales were used to rate hunger "How hungry do you feel?", fullness "How full do you feel?", desire to eat (DTE) "How strong is your desire to eat?", prospective food consumption (PFC) "How much food do you think you could eat?", and nausea "How nauseous do you feel?". Verbal anchors were placed at 0 mm and 100 mm and these were "not at all" and "extremely" for hunger, fullness, DTE and nausea and "none at all" and "a lot" for PFC. Immediately after the snack, subjects rated the pleasantness "How pleasant was the snack?", bitterness "How bitter was the snack?", and sweetness "How sweet was the snack?" of the snack on 100 mm visual analogue scales. Again, the verbal anchors "not at all" and "extremely" were placed at 0 mm and 100 mm, respectively.

Statistical analysis

Data were analysed using SPSS (version 21, SPSS Inc, Chicago, IL) and were initially checked for normality of distribution using a Shapiro-Wilk test. Appetite sensations were analysed using two-way repeated measures ANOVA. Where the assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimate. *Post-hoc* t-tests or Wilcoxon signed rank tests were used where appropriate and the family wise error rate was controlled using the Holm-Bonferroni correction. Pre-trial body mass, snack ratings, as well as energy intake, eating rate and water intake at the *ad-libitum* pasta meal were analysed using t-tests or Wilcoxon signed rank tests as appropriate. Data are presented as mean \pm SD unless otherwise stated. Data sets were accepted as being significantly different when $P \le 0.05$.

Results Pre-trial measures There was no difference between trials for pre-trial body mass (BERRY 75.12 ± 8.99) kg; CONF 75.09 \pm 9.19 kg; P=0.876), hunger (P=0.477), fullness (P=0.136), DTE (P=0.922), PFC (P=0.319) or nausea (P=0.463). Ad-libitum meal Energy intake at the ad-libitum meal was greater during CONF than BERRY (BERRY 2890 \pm 611 kJ; CONF 3449 \pm 719 kJ; P<0.001), with a mean increase of 19.5 ± 9.7 % during CONF (range 8.3 - 34.7 %; Figure 1). Water consumed with the meal was not different between trials (BERRY 362 ± 122 g; CONF 365 ± 179 g; P=0.925), although there was a tendency for total water consumption (from both food and drink) to be greater during CONF (BERRY 692 ± 128 g; CONF 765 ± 153 g; P=0.077). All subjects terminated eating within the 30 min feeding period and there was no difference between trials for time spent eating (BERRY 10.21 ± 1.76 min; CONF 11.06 \pm 2.33 min; P=0.119). There was a trend for eating rate during the ad-libitum test meal to be greater during CONF (BERRY 286 ± 60 kJ/min; CONF 333 ± 133 kJ/min), although this did not reach significance (P=0.081).



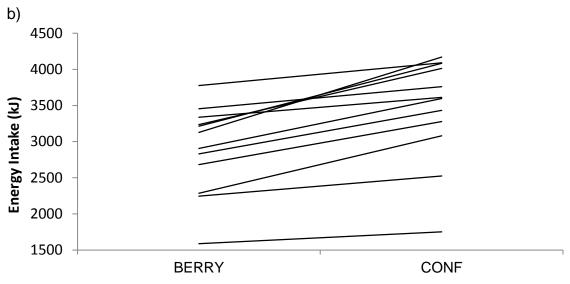


Figure 1. Mean (a) and individual (b) energy intakes (kJ) at the *ad-libitum* meal after consumption of BERRY and CONF. # Indicates significantly different from BERRY. Data are mean ± SD.

Subjective appetite ratings There were main effects of time for all subjective appetite ratings (P<0.001). There were no main effects of trial (hunger *P*=0.162; fullness *P*=0.730; DTE *P*=0.088; PFC P=0.095) or interaction effects (hunger P=0.499; fullness P=0.483; DTE P=0.540; PFC P=0.351) for any of the subjective appetite ratings (Figure 2). There was also no time (P=0.566), trial (P=0.987) or interaction (P=0.474) effect for nausea (data not shown).

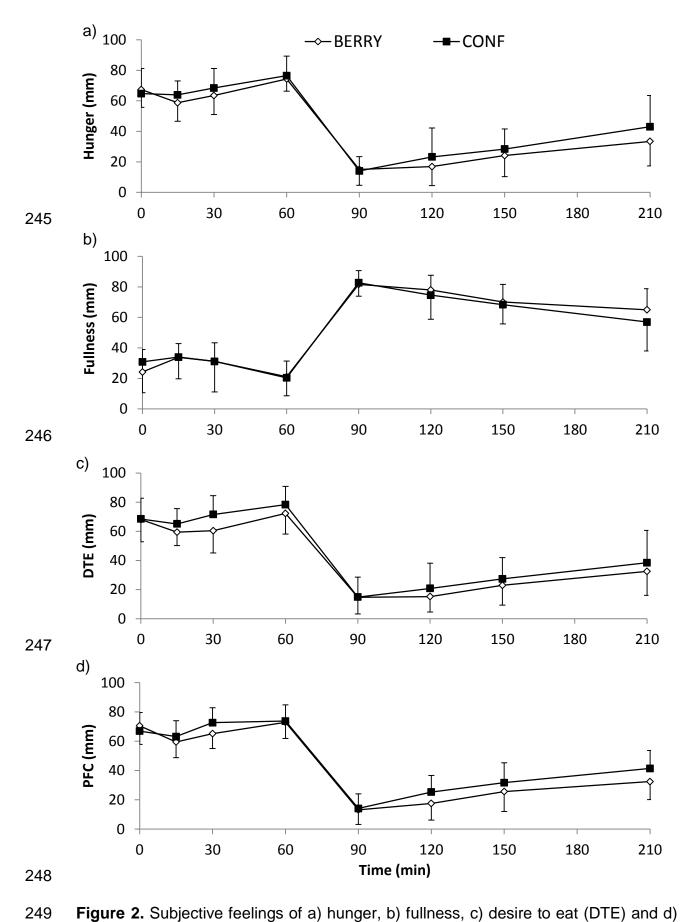


Figure 2. Subjective feelings of a) hunger, b) fullness, c) desire to eat (DTE) and d) prospective food consumption (PFC). Data are mean ± SD.

251 Snacks

The BERRY snack took longer to consume than the CONF snack (4.05 \pm 1.12 min vs. 0.93 \pm 0.33 min; *P*<0.001). The BERRY snack was rated as more pleasant and more bitter, as well as less sweet than the CONF snack (*P*<0.001; Figure 3).

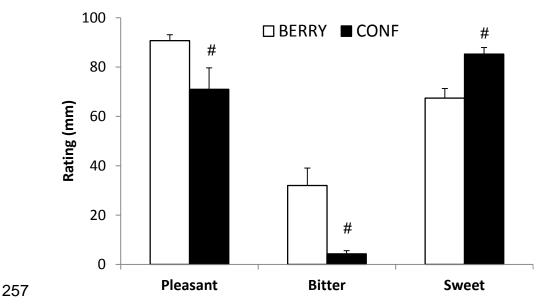


Figure 3. Subjective ratings of the snacks. # Indicates significantly different from BERRY. Data are mean ± SD.

Discussion

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The aim of the present study was to compare the appetite and subsequent energy intake effects of a snack of mixed berries with an isoenergetic confectionary snack.

The main finding was that energy intake at an *ad-libitum* test meal provided 1 h after the snack was ~20% greater after consumption of the confectionary snack than after the mixed berries snack.

To our knowledge, this is the first study to compare the acute effects of a fruit (specifically berries) snack to an energy dense confectionary snack food on subsequent appetite and energy intake. Previous investigations have assessed the effect of dried fruit on subsequent appetite and energy intake (Farajian et al. 2010; Furchner-Evanson et al. 2010) or with-in snack energy intake (Patel et al. 2013). Furchner-Evanson et al. (2010) reported that compared to low-fat cookies, an isoenergetic snack of dried plums increased satiety after ingestion, but had no effect on ad-libitum energy intake at a meal 2 h later. In a similar experiment, Farajian et al. (2010) reported increased satiety after a snack of dried prunes compared to an isoenergetic amount of bread, as well a reduction in ad-libitum food intake at a meal 3 h after the snack. In a different study design Patel et al. (2013) reported that when allowed to consume a snack ad-libitum children (4-11 years) consumed less energy when provided with an after school snack of raisins or grapes (i.e. dry and fresh fruit) than when they were provided potato chips or cookies. Taken together with the results of the present study, these studies suggest that substituting other snack foods with fruit may reduce acute energy intake from the snack or at the next eating opportunity.

Whilst the scope of the present investigation does not allow the mechanisms responsible for the observed finding to be elucidated, there are a number of potential explanations for these findings. The difference in energy density of the snack (Rolls et al. 1998), eating rate of the snacks (Zhu and Hollis, 2014) or expected satiety related to the snacks (Brunstrom, 2014) might all have contributed to the observed effects.

Since the two snacks were matched for energy content and similar in macronutrient composition, the decrease in subsequent energy intake following the mixed berries snack could have been due to the considerably lower energy density (BERRY 1.7

kJ/g; CONF 14 kJ/g) and larger volume (BERRY 160 g; CONF 19.4 g). Rolls et al. (1998) assessed the effect of decreasing the energy density and increasing the volume of milk, from 300 ml to 450 ml and 600 ml, while maintaining the energy content and macronutrient composition. Decreasing the energy density suppressed hunger and increased fullness, as well as reduced energy intake at an ad-libitum lunch 30 min after consumption of the milk. In a separate study, Rolls et al. (1999a) found that decreasing the energy density and increasing the volume of chicken casserole, by adding 356 g of water to produce chicken casserole soup, enhanced satiety and decreased energy intake at an ad-libitum lunch 5 min later. The volume of water and food in the abovementioned studies far exceed the water present in the mixed berries snack (142 g), and therefore, it seems less likely that the lower energy density and larger volume of the mixed berries snack were responsible for the decrease in energy intake at the ad-libitum meal. The mechanisms relating to a reduced energy density and increase in volume on subsequent decreases in energy intake are unknown. However, cognitive factors, such as expected satiety (Brunstrom, 2014), and sensory factors, such as oral processing time, mechanoreceptors and chemoreceptors in the oropharyngeal and gastro-intestinal tracts (Read et al. 1994), have been proposed.

Recent literature indicates that slowing ingestion rate, and subsequently increasing meal duration, can reduce energy intake (Andrade et al. 2008) and increase postprandial satiety (Kokkinos et al. 2010; Zandian et al. 2009; Azrin et al. 2008). Moreover, manipulating oral processing time, through an increase in the number of chewing cycles, has been shown to reduce food intake, by 9.5% and 14.8%, when the number of chews was increased to 150% and 200% from baseline, respectively (Zhu & Hollis, 2014). The aforementioned studies manipulated within-meal oral processing time, but the present study suggests that the oral processing time of the snacks might have impacted on eating rate during the test meal, which possibly affected ad-libitum energy intake. There was a trend (P=0.081) for eating rate to be slower during the ad-libitum meal following the mixed berries snack (286 \pm 60 kJ/min) compared to the confectionary snack (333 \pm 133 kJ/min), which could have contributed to the decrease in energy intake and warrants further investigation.

In contrast to two previous studies (Furchner-Evanson et al. 2010; Farajian et al. 2010), we did not observe any differences in post-ingestive appetite between the

snacks. Furchner-Evanson et al. (2010) reported that post-ingestive satiety was greater after a snack of dried plums compared to low-fat cookies and white bread. Subjects also reported a decreased desire to eat during the dried plum trial compared to the low-fat cookie trial. Similarly, Farajian et al. (2010) found a reduction in hunger, desire to eat, and motivation to eat, as well as increased satiety, after a snack of dried prunes compared to an isoenergetic bread snack. The dissimilar findings between previous studies (Furchner-Evanson et al. 2010; Farajian et al. 2010) and the present study could be due to the lower energy content of the snacks provided in the present study (272 kJ vs. ~1000 kJ). Despite the lower energy content, the volume (160 g) and energy (272 kJ) of the mixed berries consumed in the present study would be considered as a tangible snack, providing greater ecological validity to the present study results. Additionally, as snacks tend not to eaten to satiety (Brunstrom et al. 2008), their expected satiety and consequent effects on subjective appetite ratings may be under-estimated.

Other studies indicate that foods high in fibre content can promote satiety (French & Read, 1994) and decrease energy intake during subsequent eating opportunities (Burley et al. 1993). Proposed mechanisms include increased mastication, decreased food energy density, promotion of gastric distention, and decreased rate of gastric emptying and nutrient absorption resulting in lower postprandial glucose levels and insulin secretion (Howarth et al. 2001). There has been some suggestion that the fibre content of a snack might impact upon subsequent energy intake (Farajian et al. 2010). However, Flood-Obbagy and Rolls (2009) found no difference in *ad-libitum* energy intake 15 min after consuming isoenergetic applesauce (containing fibre), apple juice without fibre and apple juice with re-introduced fibre. The applesauce and apple juice with re-introduced fibre contained more fibre (4.8 g) than the berries in the present study (3.6 g) and the dried prunes (3.6 g) in Farajian et al. (2010). This indicates that the fibre present in the mixed berries snack in this study was unlikely to influence satiety or subsequent energy intake.

In contrast to within-meal events, it has been proposed that prior to consuming a food/ meal, an 'expected satiety' (expectation of a foods effect on fullness) is estimated from previous experience and memory of recent consumption (Brunstrom, 2014). This 'expected satiety' may largely dictate consequent meal size, and perceived hunger and fullness (Brunstrom, 2014; Brunstrom et al. 2008). In order to

energy match the conditions in the present study; 19.4 g of the confectionary snack were consumed, compared to 160 g of mixed berries. Due to the considerably lower volume of sweets used, the 'expected satiety' of the confectionary snack may have been lower than the mixed berries snack. Therefore, a lower 'expected satiety' could have led to an increased energy intake during the *ad-libitum* meal, or on the contrary, a higher 'expected satiety' of the mixed berries snack, to a lower meal energy intake. This is re-enforced by Flood-Obbagy and Rolls (2010) who found a decrease in energy intake after consuming apple segments compared to isoenergetic apple juice and applesauce. Prior to consumption, the apple segments were perceived as being more satiating than the isoenergetic serving of apple juice. For future studies it may be beneficial to quantify subjects' satiety expectations to the specific foods used in the study (Brunstrom et al. 2008).

Whilst in an acute setting replacing a confectionary snack with mixed berries might reduce subsequent energy intake, whether this results in a chronic reduction in energy intake is beyond the scope of this investigation. Future investigations should seek to examine the effect of such a dietary intervention on weight management, as well as a number of other outcomes, such as acute dietary compensation and energy expenditure. With the exception of one subject, all the subjects in this experiment were female university students aged 18-25 and thus the homogeneity of the population group likely explains the consistencyof the data. Although a greater number of similar subjects would be unlikely to alter the results, future studies should seek to examine the influence of similar snacking interventions in a larger more heterogeneous population.

In conclusion, the present study demonstrates that, although no differences for subjective appetite were present after a snack of mixed berries compared to an isoenergetic confectionary snack, *ad-libitum* energy intake at a pasta meal 1 h later was reduced by 19.5 ± 9.7 % after the mixed berries snack. Replacing an energy dense confectionary snack with a snack of mixed berries might represent a useful strategy to reduce subsequent energy intake and facilitate weight management. Future studies should seek to examine the effect of chronically replacing confectionary snacks with fruit and/ or vegetables to determine the effects on body mass and composition during a chronic intervention.

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