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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.

8

9 **Corresponding author**

10 Lewis J. James

11 L.james@lboro.ac.uk

12 School of Sport, Exercise and Health Sciences

13 Loughborough University

14 **Abstract**

15 Observational studies suggest that increased fruit and vegetable consumption can
16 contribute to weight maintenance and facilitate weight loss when substituted for other
17 energy dense foods. Therefore, the purpose of the present study was to assess the
18 effect of berries on acute appetite and energy intake. Twelve unrestrained pre-
19 menopausal women (age 21 ± 2 y; BMI 26.6 ± 2.6 kg·m⁻²; body fat 23 ± 3 %)
20 completed a familiarisation trial and two randomised experimental trials. Subjects
21 arrived in the evening (~5pm) and consumed an isoenergetic snack (65 kcal) of
22 mixed berries (BERRY) or confectionary sweets (CONF). Sixty min later, subjects
23 consumed a homogenous pasta test meal until voluntary satiation, and energy intake
24 was quantified. Subjective appetite (hunger, fullness, desire to eat and prospective
25 food consumption) was assessed throughout trials, and for 120 min after the test
26 meal. Energy intake was less ($P<0.001$) after consumption of the BERRY snack (691
27 ± 146 kcal) than after the CONF snack (824 ± 172 kcal); whilst water consumption
28 was similar ($P=0.925$). There were no trial ($P>0.095$) or interaction ($P>0.351$) effects
29 for any subjective appetite ratings. Time taken to eat the BERRY snack (4.05 ± 1.12
30 min) was greater ($P<0.001$) than the CONF snack (0.93 ± 0.33 min). This study
31 demonstrates that substituting an afternoon confectionary snack with mixed berries
32 decreased subsequent energy intake at dinner, but did not affect subjective appetite.
33 This dietary strategy could represent a simple method for reducing daily energy
34 intake and aiding weight management.

35

36 **Keywords:** Energy Balance; Appetite; Weight Management; Fruit

37 **Introduction**

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

45 Snack foods (snacks) are a fundamental aspect of dietary habits, contributing to
46 greater than 18% of daily energy intake and between 1 - 4 feeding episodes per day
47 (Ovaskainen et al. 2006; Bellisle et al. 2003). The consumption of energy dense,
48 nutrient deficient snacks has been associated with overweight and obesity in adults
49 (Bes-Rastrollo et al. 2010) and children (Bo et al. 2014), as well as poor metabolic
50 health (Mirmiran et al. 2014). Decreasing the energy density of the diet, specifically
51 snacks, by replacing energy dense foods with fruit and/ or vegetables has been
52 proposed as a dietary strategy to decrease hunger and energy intake, and
53 consequently promote weight loss (Ello-Martin et al. 2007; Houchins et al. 2013).
54 Evidence demonstrates that reducing the energy density of a meal (Rolls et al.
55 1999b; Bell et al. 1998), a snack (Farajian et al. 2010; Rolls et al. 1998), and a first-
56 course entrée prior to a meal (Rolls et al. 2004; Blatt et al. 2012), can decrease meal
57 energy intake, both independent of and when macronutrient composition is held
58 constant.

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

69 research has assessed the acute appetite effects of replacing an energy dense
70 snack with fruit.

71 Given the paucity of data examining the effect of fruit intake on subsequent appetite
72 and energy intake, this topic warrants further investigation. Therefore, the purpose of
73 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
75 isoenergetic confectionary snack (sweets).

76 **Methods**

77 *Subjects*

78 Twelve pre-menopausal women (age 21 ± 2 y; body mass 75.6 ± 8.9 kg; height 1.69
79 ± 0.08 m; BMI 26.6 ± 2.6 kg·m⁻²; body fat 23 ± 3 %) volunteered for this study, which
80 was approved by the Loughborough University Ethics Approvals (Human
81 Participants) Sub Committee (reference number: R14-P128). All subjects were
82 healthy, non-smokers, weight stable for the past 6 months (self-reported), and not
83 taking medications known to affect appetite. Each subject provided written informed
84 consent, completed a medical screening questionnaire and a three-factor eating
85 questionnaire (Stunkard & Messick, 1985) prior to commencement of the study.
86 Subjects were not restrained, disinhibited or hunger eaters. Using previous data from
87 our laboratory (Clayton et al. 2014), an expected between trial difference of ~420 kJ,
88 between trial correlation of 0.5, an α of 0.05 and a β of 0.2, it was estimated that 13
89 subjects would be required to reject the null hypothesis (Faul et al. 2009). Therefore,
90 15 subjects were recruited, but 3 subjects dropped out after completing the
91 familiarisation trial (2 due to other time constraints and 1 due to becoming pregnant).
92 Each subject completed a preliminary trial and two experimental trials in a
93 randomised counterbalanced order.

94 *Pre-trial standardisation*

95 Subjects arrived for trials 4 h after lunch, but were able to drink water *ad-libitum* until
96 2 h before arrival. To ensure similar metabolic conditions prior to each experimental
97 trial, subjects recorded their dietary intake and habitual physical activity for the day of
98 and day preceding their first experimental trial. The diet and activity patterns were
99 replicated prior to the second experimental trial and adherence to these
100 requirements were verbally checked. Subjects also refrained from any strenuous
101 exercise or alcohol intake during this period. Trials were scheduled to minimise the
102 possibility of hormone related appetite fluctuations. Three subjects were not using
103 any form of contraceptive (n=3) and their trials took place during the early-mid
104 follicular phase of their menstrual cycle (days 5-11). Seven subjects were using a
105 combined contraceptive pill, and their trials took place after at least 2 days
106 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
123 blueberries, and the CONF snack consisting of 19.4 g sweets (Bassetts Jelly Babies
124 Berry Mix, Modelez UK, Birmingham, UK) (Table 1). Each snack was accompanied
125 by 100 ml water. Subjects were instructed to consume the snack continuously as if it
126 was an afternoon snack, and the time taken for complete ingestion was recorded. All
127 trials took place in a dedicated feeding laboratory and subjects remained in complete
128 isolation throughout, except for essential interaction with the experimenter.

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135 **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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137 Sixty min after the start of the snack, subjects were presented with a homogenous
 138 pasta test meal, which they ate *ad-libitum* until voluntary satiation. The test meal
 139 consisted of fusilli pasta, Bolognese pasta sauce and olive oil (Tesco Stores Ltd.,
 140 Cheshnut, UK); each meal received identical heating and cooling. The energy
 141 density of the meal was 5.87 ± 0.03 kJ/g and was not different between trials
 142 ($P=0.596$). The test meal was served to subjects in a custom built feeding booth
 143 inside an isolated feeding laboratory. Subjects were initially served a large bowl of
 144 pasta (~700 g) and a glass of water (~500 g). After 7.5 min, these were removed and
 145 replaced with a fresh bowl of pasta (~700 g) and glass of water (~500 g), and
 146 subjects continued eating until voluntary satiation. Before the meal, subjects
 147 received standardised instructions to eat until they were “comfortably full and
 148 satisfied”. Subjects had 30 min in which to eat and remained in the feeding
 149 laboratory for the entire 30 min period, during which time food was continuously
 150 available inside the feeding booth. Subjects indicated satiation by leaving the feeding
 151 booth and taking a seat in the feeding laboratory. The point at which subjects left the
 152 feeding booth was recorded. All subjects left the feeding booth within the 30 min
 153 period and did not return to the feeding booth. Food and water intake were quantified
 154 by weighing bowls and glasses before and after consumption (PCB Electronic
 155 Precision Scale, Kern & Sohn GmbH, Balingen, Germany), and energy intake was
 156 determined using manufacturer values.

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

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

172 *Statistical analysis*

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

184 **Results**

185 *Pre-trial measures*

186 There was no difference between trials for pre-trial body mass (BERRY 75.12 ± 8.99
187 kg; CONF 75.09 ± 9.19 kg; $P=0.876$), hunger ($P=0.477$), fullness ($P=0.136$), DTE
188 ($P=0.922$), PFC ($P=0.319$) or nausea ($P=0.463$).

189 *Ad-libitum meal*

190 Energy intake at the *ad-libitum* meal was greater during CONF than BERRY
191 (BERRY 2890 ± 611 kJ; CONF 3449 ± 719 kJ; $P<0.001$), with a mean increase of
192 19.5 ± 9.7 % during CONF (range 8.3 - 34.7 %; Figure 1). Water consumed with the
193 meal was not different between trials (BERRY 362 ± 122 g; CONF 365 ± 179 g;
194 $P=0.925$), although there was a tendency for total water consumption (from both food
195 and drink) to be greater during CONF (BERRY 692 ± 128 g; CONF 765 ± 153 g;
196 $P=0.077$). All subjects terminated eating within the 30 min feeding period and there
197 was no difference between trials for time spent eating (BERRY 10.21 ± 1.76 min;
198 CONF 11.06 ± 2.33 min; $P=0.119$). There was a trend for eating rate during the *ad-*
199 *libitum* test meal to be greater during CONF (BERRY 286 ± 60 kJ/min; CONF $333 \pm$
200 133 kJ/min), although this did not reach significance ($P=0.081$).

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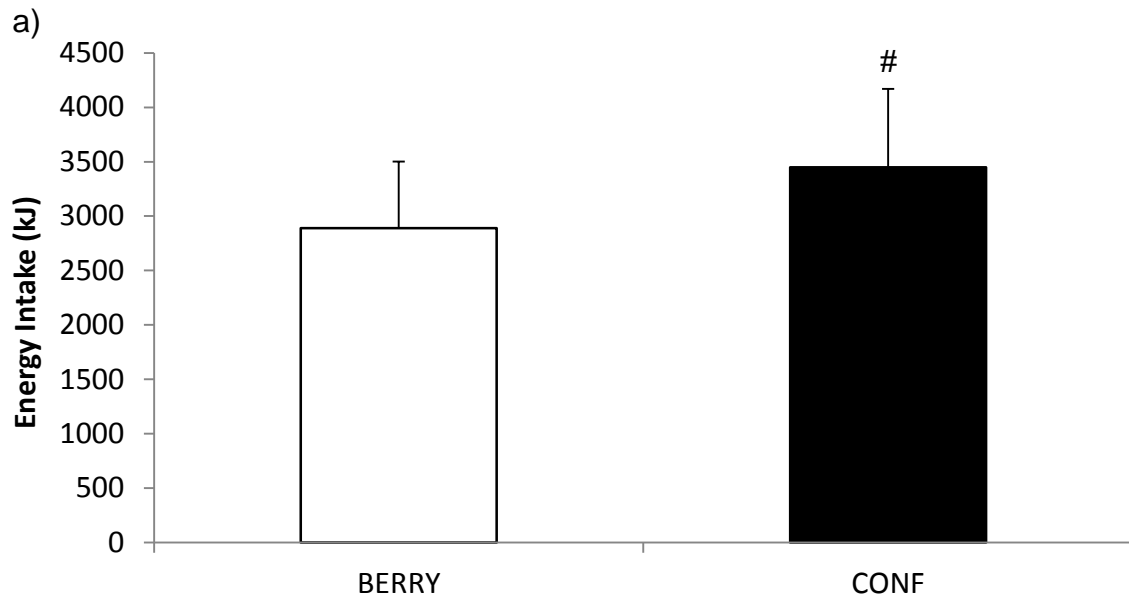
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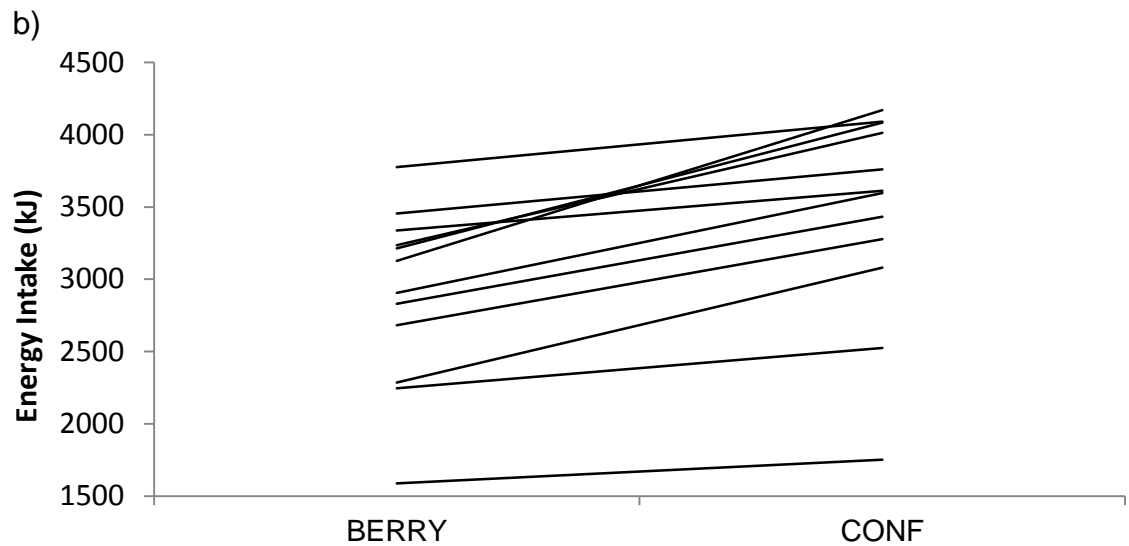
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213 **Figure 1.** Mean (a) and individual (b) energy intakes (kJ) at the *ad-libitum* meal after
 214 consumption of BERRY and CONF. # Indicates significantly different from BERRY.
 215 Data are mean \pm SD.

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221 *Subjective appetite ratings*

222 There were main effects of time for all subjective appetite ratings ($P < 0.001$). There
223 were no main effects of trial (hunger $P = 0.162$; fullness $P = 0.730$; DTE $P = 0.088$; PFC
224 $P = 0.095$) or interaction effects (hunger $P = 0.499$; fullness $P = 0.483$; DTE $P = 0.540$;
225 PFC $P = 0.351$) for any of the subjective appetite ratings (Figure 2). There was also
226 no time ($P = 0.566$), trial ($P = 0.987$) or interaction ($P = 0.474$) effect for nausea (data not
227 shown).

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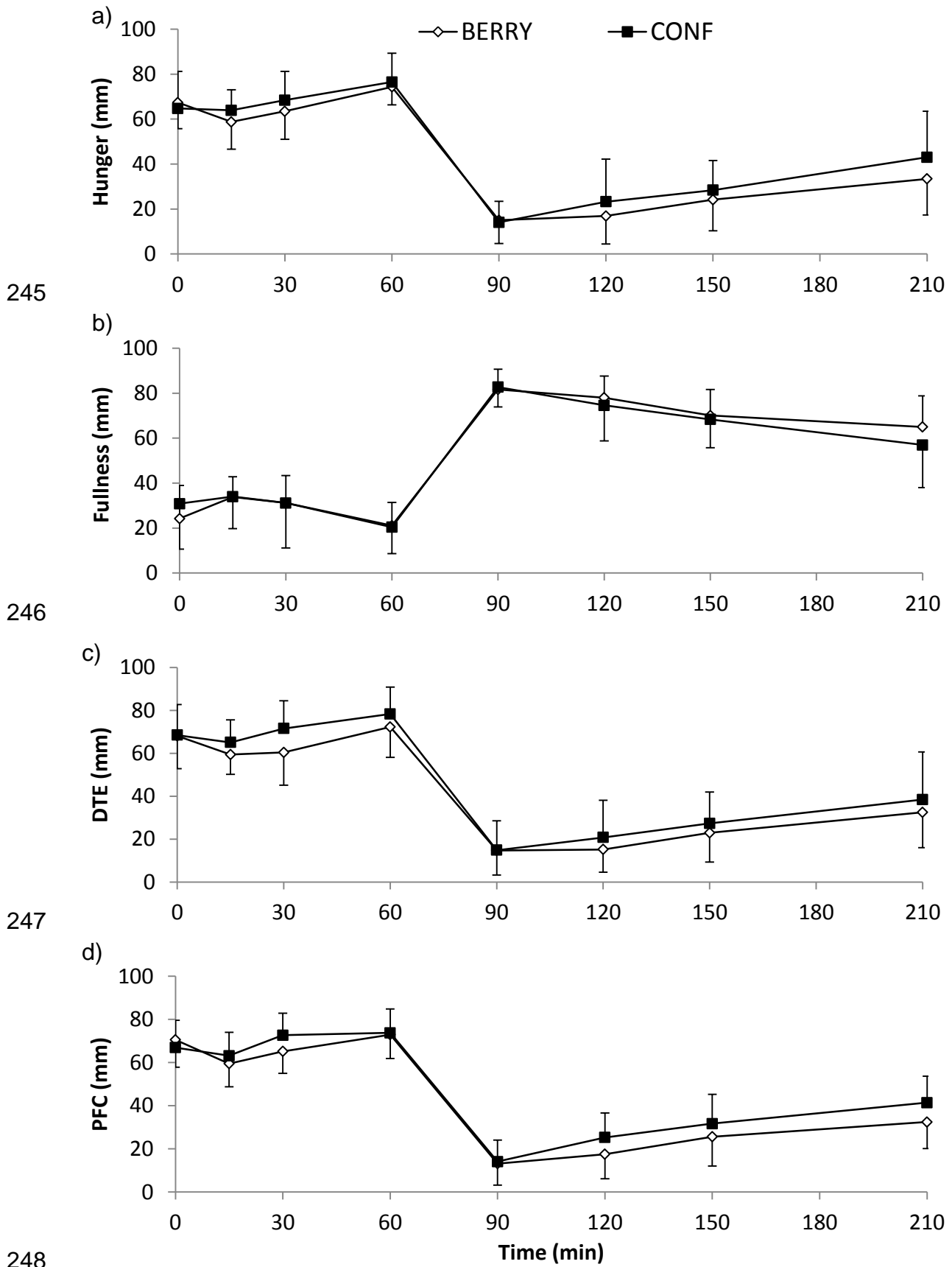
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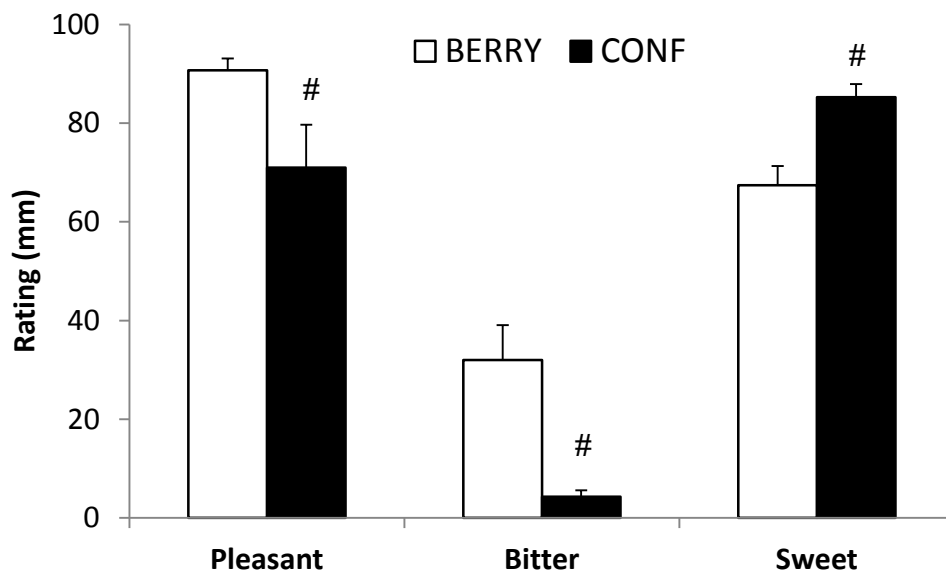
249 **Figure 2.** Subjective feelings of a) hunger, b) fullness, c) desire to eat (DTE) and d)
 250 prospective food consumption (PFC). Data are mean \pm SD.

251 Snacks

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

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258 **Figure 3.** Subjective ratings of the snacks. # Indicates significantly different from
259 BERRY. Data are mean \pm SD.

260 Discussion

261 The aim of the present study was to compare the appetite and subsequent energy
262 intake effects of a snack of mixed berries with an isoenergetic confectionary snack.
263 The main finding was that energy intake at an *ad-libitum* test meal provided 1 h after
264 the snack was ~20% greater after consumption of the confectionary snack than after
265 the mixed berries snack.

266 To our knowledge, this is the first study to compare the acute effects of a fruit
267 (specifically berries) snack to an energy dense confectionary snack food on
268 subsequent appetite and energy intake. Previous investigations have assessed the
269 effect of dried fruit on subsequent appetite and energy intake (Farajian et al. 2010;
270 Furchner-Evanson et al. 2010) or with-in snack energy intake (Patel et al. 2013).
271 Furchner-Evanson et al. (2010) reported that compared to low-fat cookies, an
272 isoenergetic snack of dried plums increased satiety after ingestion, but had no effect
273 on *ad-libitum* energy intake at a meal 2 h later. In a similar experiment, Farajian et al.
274 (2010) reported increased satiety after a snack of dried prunes compared to an
275 isoenergetic amount of bread, as well a reduction in *ad-libitum* food intake at a meal
276 3 h after the snack. In a different study design Patel et al. (2013) reported that when
277 allowed to consume a snack *ad-libitum* children (4-11 years) consumed less energy
278 when provided with an after school snack of raisins or grapes (i.e. dry and fresh fruit)
279 than when they were provided potato chips or cookies. Taken together with the
280 results of the present study, these studies suggest that substituting other snack
281 foods with fruit may reduce acute energy intake from the snack or at the next eating
282 opportunity.

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

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

292 kJ/g; CONF 14 kJ/g) and larger volume (BERRY 160 g; CONF 19.4 g). Rolls et al.
293 (1998) assessed the effect of decreasing the energy density and increasing the
294 volume of milk, from 300 ml to 450 ml and 600 ml, while maintaining the energy
295 content and macronutrient composition. Decreasing the energy density suppressed
296 hunger and increased fullness, as well as reduced energy intake at an *ad-libitum*
297 lunch 30 min after consumption of the milk. In a separate study, Rolls et al. (1999a)
298 found that decreasing the energy density and increasing the volume of chicken
299 casserole, by adding 356 g of water to produce chicken casserole soup, enhanced
300 satiety and decreased energy intake at an *ad-libitum* lunch 5 min later. The volume
301 of water and food in the abovementioned studies far exceed the water present in the
302 mixed berries snack (142 g), and therefore, it seems less likely that the lower energy
303 density and larger volume of the mixed berries snack were responsible for the
304 decrease in energy intake at the *ad-libitum* meal. The mechanisms relating to a
305 reduced energy density and increase in volume on subsequent decreases in energy
306 intake are unknown. However, cognitive factors, such as expected satiety
307 (Brunstrom, 2014), and sensory factors, such as oral processing time,
308 mechanoreceptors and chemoreceptors in the oropharyngeal and gastro-intestinal
309 tracts (Read et al. 1994), have been proposed.

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

323 In contrast to two previous studies (Furchner-Evanson et al. 2010; Farajian et al.
324 2010), we did not observe any differences in post-ingestive appetite between the

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

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

353 In contrast to within-meal events, it has been proposed that prior to consuming a
354 food/ meal, an 'expected satiety' (expectation of a foods effect on fullness) is
355 estimated from previous experience and memory of recent consumption (Brunstrom,
356 2014). This 'expected satiety' may largely dictate consequent meal size, and
357 perceived hunger and fullness (Brunstrom, 2014; Brunstrom et al. 2008). In order to

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

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

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

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396

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