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We are what we (think we) eat: The effect of expected satiety on subsequent calorie consumption

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Key words: Expected satiety; Hunger; Fullness; Caloric intake; Cognition; Total Ghrelin

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Abstract

Varying expected satiety (ES) for equi-calorie portions of different foods can affect subsequent feelings of hunger and fullness and alter consumption. To our knowledge, no study has manipulated ES for an equal portion of the same solid food, appetite has not been measured >3 hours and studies have not consistently measure later consumption. It is also unclear whether changes in hunger, fullness or later consumption are related to a physiological response. The aims of this study were to use the same solid food, to measure participants' response over a 4-hour inter-meal period, to measure later consumption and to assess whether any effect of ES was related to a physiological (i.e. total ghrelin) response. Using a within-subjects design, 26 healthy participants had their ES for omelettes manipulated experimentally, believing that a 3-egg omelette contained either 2 (small condition) or 4 (large condition) eggs. When ES was higher (large condition) participants ate significantly fewer calories at a lunchtime test meal (mean difference = 69kcal [\pm 95% CI 4 - 136]) and consumed significantly fewer calories throughout the day (mean difference = 167kcal [\pm 95% CI 26 - 309]). As expected, there was a main effect of time on hunger and fullness, but no main effect of 'portion size' ($p > .05$). There was also a significant interaction between time and portion size for hunger. There was no evidence for any significant differences being the result of changes in total ghrelin. Overall, the data suggest that ES for a solid food can be manipulated and that, when given at breakfast, having a higher ES for a meal reduces lunchtime and whole day caloric consumption.

1. Introduction

One way to reduce levels of overweight and obesity is to encourage people to actively reduce caloric intake. However, dieters report high levels of hunger when energy intake is lowered (Shumaker, Ockene, & Riekert, 2009), people generally consider foods marketed as 'healthy' to be less filling and consume them in larger amounts (Provencher, Polivy & Herman, 2009; Suher, Raghunathan & Hoyer, 2015) and when people actively decide to restrict calorie intake it can lead to disinhibition and overeating (Polivy & Herman, 1985). Rather than focusing on dieting, an alternative may be to utilise the influence of expectations. In particular, if people's expected satiety (ES) for a food can be increased, they may then select a smaller portion of food (as they will not expect to need as much to stave off hunger) or they may select the same portion but feel greater satiety (leading to lower consumption and/or snacking at a subsequent eating opportunity) (Forde, Almiron-Roig, & Brunstrom, 2015). As such, it may be possible to encourage people to eat less without overt 'healthy' messages, while still maintaining satiety, and without requiring a person to actively restrict consumption in a way that can prompt subsequent overeating.

In a study by Brunstrom et al. (2011), one group ('large') was shown a greater amount of constituent ingredients than the other group ('small') but everyone in the study was given an equi-calorie smoothie made from the same ingredients. Those in the 'large' group reported greater ES and went on to report higher levels of fullness and lower levels of hunger 3 hours post-consumption. In a similar study, Crum et al. (2011) used product labels to describe the same milkshake as either a 620-calorie 'indulgent' shake or as a 140-calorie 'sensible' shake. In a repeated measures design, participants consumed both milkshakes (one week apart) and provided three blood samples for total ghrelin levels: one at the start of the procedure, one after participants had viewed and rated the milkshake label for 40 minutes, and one after consuming and rating the taste of the milkshake. While hunger reports did not significantly differ, total ghrelin levels increased more when reading the 'indulgent' label, suggesting an increase in some aspect of appetite, and had a steeper decline after consuming the 'indulgent' shake, suggesting reduced hunger, in comparison to the 'sensible' shake. Finally, Hogenkamp et al. (2013) gave participants either high kcal (HC) or low kcal (LC) information along with an LC or HC yogurt preload in a within participants, cross-over design. Total ghrelin was measured at baseline, as well as at 20, 40 and 60 minutes. The study found that participants consumed less food at a later ad-lib eating opportunity when given a LC preload with HC information, in comparison to a LC pre-load with LC information. However, there was no evidence of an anticipatory hormone response (also see Hoffman et al., 2018 that showed no total ghrelin effect when manipulating satiety expectations).

Other research in this area has shown that increasing the viscosity of a drink can increase ES (McCrickerd et al., 2012) and that a thicker yoghurt pre-load results in less consumption at a later test lunch (Chambers, Ells, & Yeomans, 2013; for yogurts see also Yeomans & Chambers, 2011). Similar work has also utilised soup (Brunstrom et al., 2012), drinks (Bertenshaw et al., 2013; McCrickerd,

Chambers, & Yeomans, 2014a) and milkshakes (Mattes & Rothacker, 2001) with thicker, creamier and more viscous versions of equicaloric products increasing participants' satiety expectations. Simply increasing the weight of the bowl containing the food has also had a small but significant effect on increasing ES (Piqueras-Fiszman & Spence, 2012). A recent study also showed that giving participants a placebo pill for 'enhanced appetite' led to significantly less hunger and increased satiety in comparison to controls (Hoffman et al., 2018).

To date, much of the work in this area has relied on the manipulation of liquids. However, meals are usually consumed in a solid form and, even when given in a similar macronutrient composition and equivalent energy density (ED), liquids consumed as a pre-load can result in significantly less energy intake at the next eating opportunity, differing reports of hunger and fullness and differing hormone responses, when compared to the response induced by consumption of solid food (Leidy, Apolzan, Mattes, & Campbell, 2010). Furthermore, while the viscosity of the liquid is influential in the evaluation of its satiating qualities (Hogenkamp, Stafleu, Mars & de Graaf, 2012) and can alter later consumption (McCrickerd, Chambers, & Yeomans 2014b), beverages are generally considered as thirst quenching, whereas solid foods are considered satiety inducing (Hogenkamp, Mars, Stafleu & de Graaf, 2012). Therefore, it is currently unclear whether findings relating to expected satiety based on liquids would translate to solid foods.

In order to examine the influence of differences in expectations towards an equicaloric portion of a solid food, a similar design to that used by Brunstrom et al. (2011; also see Ratliff et al., 2010) was utilised. In a within-participants design, we manipulated participants' ES for the same omelette breakfast, by showing participants different amounts of ingredients (eggs and cheese) that were purportedly used to make the omelettes. We hypothesised that when participants are shown the large portion of eggs/cheese ('large' condition) they would have a significantly higher ES than when they are shown a small portion of ingredients ('small' condition). In line with previous research (Brunstrom et al., 2011), we also hypothesised that when participants consumed the omelette in the 'large' condition, they would go on to report significantly lower levels of hunger and higher levels of fullness over the inter-meal period.

Ascertaining whether or not ES for a solid food can be manipulated and the effect this could have on hunger and fullness will extend current understanding. However, in relation to reducing energy intake, it is only helpful if reported increases in ES result in a reduction of calories selected at the meal for which ES is increased, reduced consumption at the next meal, or if there is an impact on energy consumed over the course of the day. If participants report different expectations for the same food over two visits, but then go on to consume the same number of calories over the course of the respective days, the applications for altering expectations for solid foods are limited. As such, the study reported in this paper gave participants the test food as a breakfast meal and measured appetite reports over 4

hours, a timescale that is generally representative of the period between ‘breakfast’ and ‘lunch’ (e.g. 8am-12pm). We then provided participants with lunch in order to measure subsequent food intake. Furthermore, food weighing scales and a paper/pen food diary were given to participants so that they could record their consumption throughout the rest of the day. We hypothesised that when participants were in the ‘large’ omelette condition they would go on to consume fewer calories at lunch than when in the ‘small’ condition. Furthermore, we hypothesised that participants would consume fewer calories over the entire day (breakfast, lunch and self-reported evening consumption) when they believed that they consumed a 4-egg omelette for breakfast.

Finally, we measured participants’ orexigenic hormone (total ghrelin) levels before (baseline) and immediately after (~20 minutes) consuming the omelettes (as well as after 30, 60, 120, 180 and 240 minutes, in line with appetite reports). Ghrelin is secreted by the stomach, is dependent on the person’s nutritional state (pre-prandial increases and post-prandial decreases (Ariyasu et al., 2001; Tschöp et al., 2001; Gibbons et al., 2013)), and is thought to be an appetite-stimulatory signal (see Müller et al., 2015, for a review). As well as the Crum et al. (2011) study showing a difference in total ghrelin response for the same milkshake, ghrelin is of interest in relation to expectations as the pre-prandial increase in ghrelin correlates with reported hunger and the initiation of meals in the absence of time and food-related cues (Cummings Frayo, Marmonier, Aubert & Chapelot, 2004), levels change when equicaloric amounts of food with made with different macronutrients are consumed (Ratliff et al., 2010; Gibbons et al.), and levels are similar when food is consumed vs. sham feeding (Arosio et al., 2004). If total ghrelin is related to expectations, then we hypothesised that when participants were in the ‘large’ condition they would have a significantly more rapid decline in total ghrelin concentrations post consumption in comparison to when they consumed the omelette in the ‘small’ condition (as in Crum et al.). Having a measure of total ghrelin will contribute to our understanding of whether physiological responses may account for any differences in subsequent intake or appetite reports when expectations for an equicaloric solid food portion is altered.

2. Method

2.1. Overview

Participants visited the human nutrition unit (HNU) at the Rowett Institute for breakfast on two occasions, 1 week apart. On arrival, written informed consent was taken and participants were fitted with a flexible cannula inserted into their arm and a baseline blood sample was taken (overnight fasted). Baseline measures of appetite (hunger and fullness) were also provided (visual analogue scales) and their height and weight were measured. Participants were then taken to a dining room where they could be seated alone. Participants were shown either a ‘small’ portion or a ‘large’ portion of ingredients prior to each of the two breakfasts. As with previous research (Brunstrom et al., 2011), participants were told that they were being shown the ingredients to check for potential allergens.

Once participants confirmed that the ingredients were suitable for any dietary requirements, the ingredients were taken away to the kitchen under the pretence that they would be used to make their omelette. Participants were then brought the cooked omelette after a few minutes (to mimic a congruent preparation time). The omelettes on both visits were made from the same set of ingredients. Participants first tasted the omelette and then reported their expected satiety (Brunstrom et al., 2011), before consuming the breakfast in full. Post consumption appetite reports and corresponding blood samples were taken immediately after consumption and then for a further 4 hours (30mins, 60, 120, 180, 240). Participants were then given a pasta lunch. Prior to leaving the HNU, a food diary and food weighing scales were provided for participants to record their food and drink consumption for the remainder of the day. Ethical approval for the study was obtained from the Rowett Ethics Review Panel.

2.2. *Participants*

Participants were students at the University of Aberdeen and members of the public. Prior to arrival for Visit 1, participants were allocated alternately to the small-portion and the large-portion condition. However, if more than one person was tested on the same day, they were both allocated to the same initial condition in order to better maintain the manipulation (though any pairs were asked not to discuss the study during their participation). As any subsequent measure was dependent on a difference in ES, this was used to calculate power. The difference in ES reported in Brunstrom et al. (2011; using the same manipulation in a between participants design) had an effect size of approximately 0.76 (Cohen's *d*). Using this approximate effect size, $\alpha = .05$, and power set at .8, G*Power (Faul, Erdfelder, Lang, & Buchner, 2007) gave an estimated $N=13$. Given that this study was aiming to use solid food and not a drink, and that two visits were required so attrition might occur, we recruited a total of 26 participants (18 female). Of these, 16 participants received the 'small' information during Visit 1 and 10 received the 'large' information during Visit 1. Participants were aged between 18 and 42 years (mean = 21, S.D. = 4.9), and had a mean BMI 24.1 (3.6); 2 were underweight, 16 healthy, 5 overweight and 3 were categorised as obese. All participants received £30 for their time and any expenses accrued in taking part.

2.3. *Materials*

2.3.1. *Visual Analogue Scales (VAS)*

All VAS measures were completed as paper and pen reports. Participants' hunger was measured using a visual analogue scale anchored 0 ('Very hungry') - 100 ('Not at all') and fullness anchored 0 ('Very full') - 100 ('Not at all') with the question "How HUNGRY/FULL (as appropriate) do you feel RIGHT NOW?" (as in Brunstrom et al., 2011). Liking for the omelette and pasta were anchored 0 - (dislike very much) 100 (like very much) with the question "How much do you like the Omelette/Pasta (as appropriate)?".

2.3.2. *Expected Satiety measure*

Expected satiety for the omelettes was measured using a computer-based ‘method of adjustment’ developed by Brunstrom and colleagues (Brunstrom, Collingwood, & Rogers, 2010; Brunstrom & Rogers, 2009) and the same measure was used in a previous study (Brunstrom et al., 2011). Participants were asked to first taste the omelette, imagine eating the whole meal, and then to think about how long they would expect it to stave off hunger. Participants then completed four computer-based trials, each consisting of a comparison food (pasta, pizza, chips, curry and rice) presented in the centre of a 20-inch EliteDisplay E201 monitor. Participants could change the amount of food displayed on the screen by pressing the left (to decrease) or right (to increase) arrow keys and it was their task to select an amount of food in each of these trials that, if it was given instead of the omelette, would stave off hunger for a similar period of time, i.e. that they believe to be equally satiating. Participants pressed the ‘enter’ key when they were happy with a given selection and the next food appeared. When completing this task, participants were specifically asked to ‘ignore how much you do or do not like the foods on the screen and do not consider whether you would want to eat them right now’. This was to encourage them to focus solely on the satiety that they believed the foods would deliver. Further, it was felt that non-breakfast foods would prevent participants from just selecting a ‘default’ amount based on experience (e.g. 2 pieces of toast) rather than focusing on ES. The tool is programmed to present the foods in a random order each time it is used, to display a random amount at the start of each trial and represents a range of between 50 kcal to 1250 kcal, on a logarithmic scale.

2.3.3. *Omelette (breakfast)*

Participants were shown 4 eggs (Tesco brand (Hertfordshire, UK), medium, free range) with 60g of cheese (Tesco brand, mature, coloured cheddar cheese) in the ‘large’ condition and 2 eggs with 30g of cheese in the ‘small’ condition. The cooked omelettes were made from 3 eggs and 45g of cheese and contained approximately 460kcal. As this was a within-participants design, we tried to slightly alter the appearance of the omelettes across the two conditions in order to support the narrative that the omelettes differed in relation to the amount of ingredients. To do this, when in the ‘small’ condition the omelette was cooked in a pan with a circumference of 50.24cm and when in the large condition it was cooked in a pan with a 62.80cm circumference. The effect was that the ‘small’ omelette covered slightly less of the plate when it was subsequently served to the participant in the dining room.

Although ‘medium’ eggs were used throughout, there is a natural variance in the weight. However, differences are small, and the weight of the raw eggs and the cooked omelettes were comparable across the two conditions (see Table 1). Measures of liking were taken immediately after consumption in both conditions (see Table 1) and familiarity for omelettes (‘Never’, ‘Less than once per year’, ‘Once a year’, ‘Monthly’ or ‘Every week’, coded: 1 (low) - 5 (high)) was measured at Visit 1 (mean (SD) = 3.9 (1.1)).

Table 1. Details of the omelette ingredients and participants reported liking of the breakfast and lunch, separated by condition.

Parameter	Condition - Mean (SD)	
	Small portion	Large portion
Weight of raw eggs (g)	155 (1)	156 (1)
Cooked omelette (g)	191 (1)	193 (1)
Omelette liking (100-mm scale) ^a	80 (4)	78 (4)
Pasta liking (100-mm scale) ^a	86 (3)	82 (3)

^a 0 = dislike very much; 100 = like very much

2.3.4. Pasta lunch

A 930g portion of pasta (Tesco brand, Penne Pasta Quills - 400g cooked) and sauce (Lloyd Grossman Tomato and Basil Pasta Sauce - 530g) was presented in a large bowl for participants to serve themselves on to a separate plate, using a serving spoon. The whole serving consisted of approximately 960 kcal. Measures of liking were taken immediately after consumption in both conditions (see Table 1).

2.3.5. Food diary

On both test days, participants were given food diaries and kitchen scales to record their food and drink intake upon leaving the HNU, until they went to sleep that evening. Volunteers were asked, where possible, to use the portable scales (Salter electronic kitchen scales) to weigh the foods and any leftovers and to record all food items eaten and all fluids consumed. The accuracy of the scales was checked (using known weights) before use and all were within a suitable range (<3g variance per 1000g). As well as being given verbal instructions, participants were given full written guidance on how to complete the diary and weigh the food/drink. The data from the food diaries were analysed using WinDiets Nutritional Analysis Software (Version 1.0, The Robert Gordon University, Aberdeen, UK).

2.4. Procedure

Participants were told that the study was taking place in order to assess the effects of a ‘high fat, high protein breakfast on feelings of hunger, fullness and people’s physiological response’ and that they would be given different omelette breakfasts over two visits. Participants who contacted the researcher were given a full information sheet and the chance to ask any questions, prior to agreeing to take part. Those people who were willing to be part of the study subsequently visited the HNU in the morning (between 07:00-09:00) having been asked to fast from at least 22:00 the evening before (participants were asked to avoid all food and calorie-containing drinks). While arrival time for visit 1 varied for each person, arrival for visit 2 was arranged to match the time of their first visit.

Baseline measures of hunger and fullness were taken, participants reported the time they had consumed their last meal and what they had eaten (Note: in a reminder email for Visit 2, participants were told what they had reported as their last meal the evening prior to Visit 1 and they were asked, if possible, to eat a similar amount of food at a similar time the evening before the their second visit). Height and weight were measured (shoes and outer jacket removed) once these initial responses were recorded, followed by insertion of a cannula with a 3-way valve into the arm and two baseline blood samples taken (further details given in 'Biochemical Analysis' section below).

After cannulation, participants were taken to a private room containing a table, chair, cutlery, as well as space for them to consume their breakfast and to have a computer/monitor display. Here they were shown one of two sets of ingredients that were purportedly to be used to make their omelette. The ingredients were shown to the participant under the guise of a check for potential allergens. Once participants confirmed that the ingredients shown to them would not cause any allergic response, they were asked to wait a moment while the ingredients were passed to the kitchen so that the omelette could be cooked. The time of presentation was noted by the researcher.

While the omelette was being prepared, participants had the computer task (ES measure) explained to them and any questions regarding the task were answered. Once the omelette was cooked it was presented to the participants and their ES was recorded. Water was also provided for the participants. All participants consumed the omelette in its entirety on both visits.

Once the omelette was consumed, participants reported their hunger and fullness, their liking for the omelette and how often ('Never', 'Less than once per year', 'Once a year', 'Monthly', and 'Every week') they consumed omelettes. Participants were then immediately returned to the medical area for their post consumption blood samples which were taken 20 minutes from the point at which the omelette ingredients were presented to the participant. This was achieved for the entire sample, thus, while eating time was not explicitly measured, the time taken to cook the omelette, complete the ES measure, consume the omelette, complete the post consumption measures of appetite and liking and to be escorted back to the medical area for the second set of blood samples, was < 20mins. Finally, participants were shown to a communal waiting area where they remained between the subsequent measures/samples. This area offered them a more comfortable environment to wait during the 4-hour testing period with sofas, a TV, books and internet access. Further measures of hunger and fullness, along with corresponding blood samples, were taken 30, 60, 120, 180 and 240 minutes later. At the first visit, during one of the hour-long waiting periods, the use of the food diary and scales were explained.

Finally, 4 hours post consumption, participants completed their final pre-lunch hunger and fullness ratings, the final blood samples were taken, and the cannula was removed. At this point participants were returned to the same dining room that was used in the morning and they were given a

pasta and tomato sauce lunch. Participants were told to 'eat as much or as little as you like, until you are pleasantly full'. After lunch, participants reported their hunger, fullness, liking of the pasta and their familiarity with pasta. The test was repeated after a 1-week washout period and participants were shown the alternative set of ingredients at Visit 2. Measures of height, weight and familiarity were not taken on Visit 2.

As the protocol required a food diary to be completed for the afternoon/evening of the second visit, debriefs were carried out later (usually within 1 week of the second visit). At the debrief participants were asked what they thought the study was about. While the exact details varied, all participants reported a belief that the study was in line with the narrative originally given by the researcher ('the effects of different amounts of fat and protein and the effect this has on hunger/fullness/physiological response'). The true aims of the study were revealed, participants were debriefed, and any remaining questions were answered. Finally, participants were thanked for their participation and paid £30 for their time.

2.5. Blood sampling and analysis

Blood was collected into EDTA-containing monovettes for the measurement of total ghrelin and samples were immediately treated with a 120µl mixture of the serine protease inhibitors 4-(2-aminoethyl) benzenesulfonylfluoride hydrochloride (Roche Diagnostics Ltd, West Sussex, UK) and protease inhibitor cocktail (Sigma-Aldrich, Poole, UK). For the measurement of glucose, triglycerides (TAG), non-esterified free fatty acids (NEFA), low-density lipoprotein cholesterol (LDL), high-density lipoprotein cholesterol (HDL) and total cholesterol, blood was collected into lithium heparin monovettes. Plasma was obtained by centrifugation at 3000 x g for 15 minutes at 4°C. Plasma was stored in 500µl aliquots at -70°C for later analysis. The plasma analysis was carried out at the Rowett Institute, Technical Services department using the Konelab analyser (Thermo Fischer Scientific, Waltham, MA, USA). The human ghrelin RIA kit from Millipore (#GHRT -89HK: Millipore, Billerica, MA, USA) method was as described in the kit protocol.

Total ghrelin concentrations were measured by human RIA (GHRT-89HK; Millipore UK Ltd, Watford, UK) with inter-assay variability of 8.7% and intra-assay variability of 6.9%. Glucose, TAG, NEFA, LDL, HDL and total cholesterol concentrations were determined using a discrete automated clinical analyser (Kone Oyj, Espoo, Finland) using commercial kits (Labmedics, Manchester, UK). The cannulation process failed on at least one visit for two of the participants, leaving 24 participants with all biochemical measures. All methods were carried out following appropriate Standard Operating Procedures (SOPs) by trained personnel.

2.6. Data analysis

Prior to the main analysis, paired samples t-tests were used to compare all baseline measures. For the main analysis, participants' measures of ES for the 'small' (see two eggs) and 'large' (see four eggs) conditions were also compared using a paired samples t-test. Reports of hunger and fullness were converted into 'change scores' by subtracting baseline measures from those recorded post-consumption (as in Brunstrom et al., 2011). These scores were then analysed using a 2 ('small'/'large' portion) x 6 (time: post-consumption, 30, 60, 120, 180, 240 mins) analysis of variance (ANOVA). As the assumption of sphericity was violated, a Huynh-Feldt correction was applied. An interaction between portion information and time in relation to hunger reports was followed up with t-tests using Bonferroni corrections.

Total ghrelin responses were analysed using a 2 ('small'/'large' portion) x 7 (time: baseline, post-consumption, 30, 60, 120, 180, 240 min) ANOVA. Again, a Huynh-Feldt correction was applied. Finally, lunchtime consumption (pasta), post-lunch intake (food diaries) and each day's total intake (sum of omelette, pasta and food diaries) were calculated (kcal) and each analysed using a paired samples t-tests. Given the previous evidence described and directional hypotheses outlined, one-tailed tests were used. All analyses were completed using SPSS, version 24 (SPSS, IBM).

3. Results

3.1. Baseline measures and participant characteristics

The weight of the raw ingredients used to make the omelettes, the weight of the cooked omelettes and the reports of liking for the omelettes and pasta, across the two visits (see Table 1, above), as well as fasted hunger and fullness reports, and the lipid profile, across the two visits (see Table 2) revealed no significant differences (all $p > .05$).

Table 2. Mean (SD) baseline measures. Separate values are provided for each condition.

<u>Parameter</u>	<u>Condition</u>	
	'Small' portion (2 eggs, 30g cheese)	'Large' portion (4 eggs, 60g cheese)
Hunger (100-mm scale) ¹	29 (25)	28 (22)
Fullness (100-mm scale) ¹	82 (20)	81 (21)
Glucose (mmol/L) ²	5.03 (0.3)	4.98 (0.4)
Triglycerides (mg/dL) ²	0.87 (0.3)	0.89 (0.3)
LDL-C (mg/dL) ²	2.29 (0.8)	2.19 (0.8)
HDL-C (mg/dL) ²	1.48 (0.5)	1.49 (0.5)
Total Cholesterol (mg/dL) ²	4.32 (1)	4.25 (1.1)
Nonesterified fatty acids (NEFA) (mg/dL) ²	0.65 (0.3)	0.62 (0.3)

¹N = 26; ²N = 24. LDL-C indicates low-density lipoprotein cholesterol; HDL-C indicates high-density lipoprotein cholesterol.

3.2. Expected satiety measure:

Figure 1 shows participants' mean [\pm 95% CI] expected satiety when either the small or large amount of ingredients had been shown. In the 'large' condition the omelette was expected to deliver approximately 122kcal more satiety than the 'small' condition (36%). This reflects a large effect ($d = 0.94$) and is statistically significant ($t(25) = 5.05$, $p < .001$, one tailed).

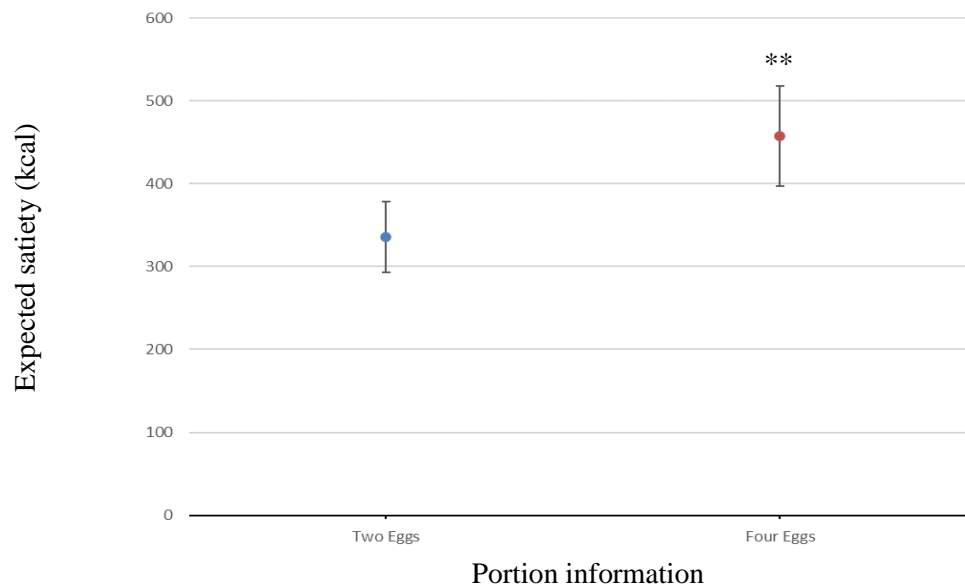


Fig. 1. Mean [\pm 95% CI] expected satiety (kcal) associated with ‘small’ (two eggs) and ‘large’ (four egg) displays (N=26). Note: asterisk depicts significance at $p < .001$, one-tailed.

3.3. Hunger and fullness:

The mean (\pm SEM) hunger change-scores are shown in figure 2. A repeated measures ANOVA showed that, as would be expected, there was an overall main effect of time on hunger ($F(1, 1.855) = 107.26, p < .001, \eta_p^2 = .811$). There was no main effect of portion information (small vs. large) on overall hunger reports ($F(1, 1) = 2.06, p = .164, \eta_p^2 = .076$) (marginal means for overall hunger in the small condition, $M = 29 [18 - 39]$ and large condition ($M = 36 [25 - 47]$) but the interaction between the two variables (portion information*time) was significant ($F(1, 4.192) = 3.06, p = .018, \eta_p^2 = .109$). Post-hoc t-tests were used to investigate this significant interaction. These indicated the greatest differences were at 120 mins ($t(25) = -2.380, p = 0.0125$, one-tailed) and 180 mins ($t(25) = -1.880, p = 0.036$, one-tailed) but neither was statistically significant after applying the Bonferroni correction for multiple comparisons (corrected $\alpha = .008$).

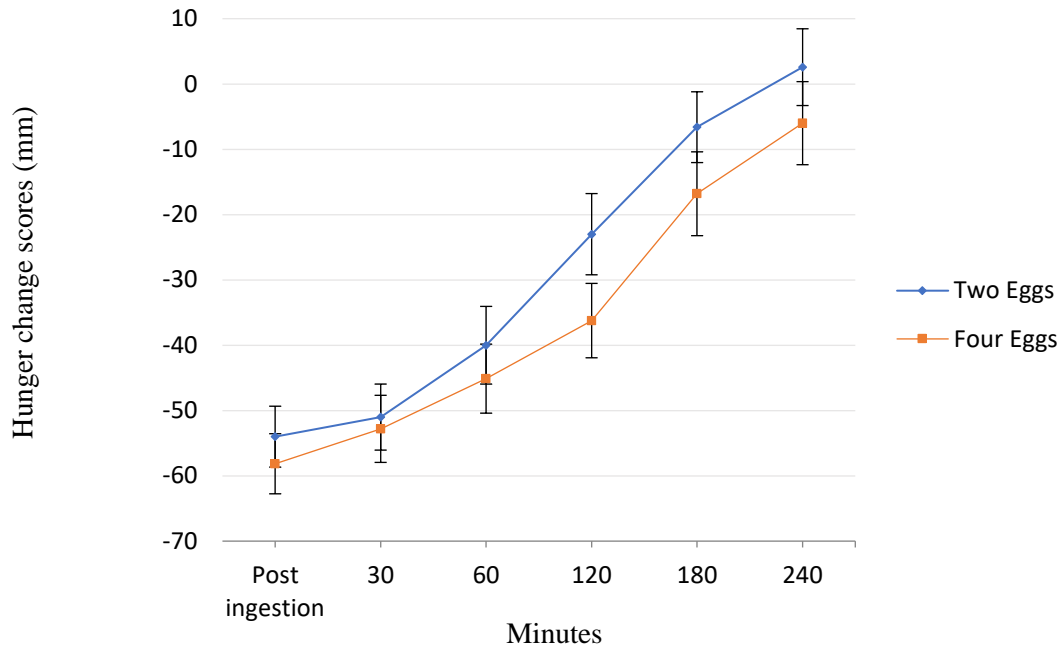


Fig. 2. Mean (\pm SEM) change in reported hunger in the small (see two) and the large (see four) conditions (N=26).

The mean (\pm SEM) fullness change-scores are shown in figure 3. A repeated measures ANOVA showed that there was an overall main effect of time on reported fullness ($F(1, 2.231) = 111.2$, $p < .001$, $\eta_p^2 = .816$). There was no main effect of portion information (small vs. large) on overall fullness reports ($F(1, 1) = 1.248$, $p = .275$, $\eta_p^2 = .048$) (marginal means for overall fullness in the small condition, $M = 36$ [27 - 44] and large condition $M = 39$ [30 - 49]) and the interaction between the two variables (portion information*time) did not reach significance ($F(1, 3.495) = 1.17$, $p = .33$, $\eta_p^2 = .045$).

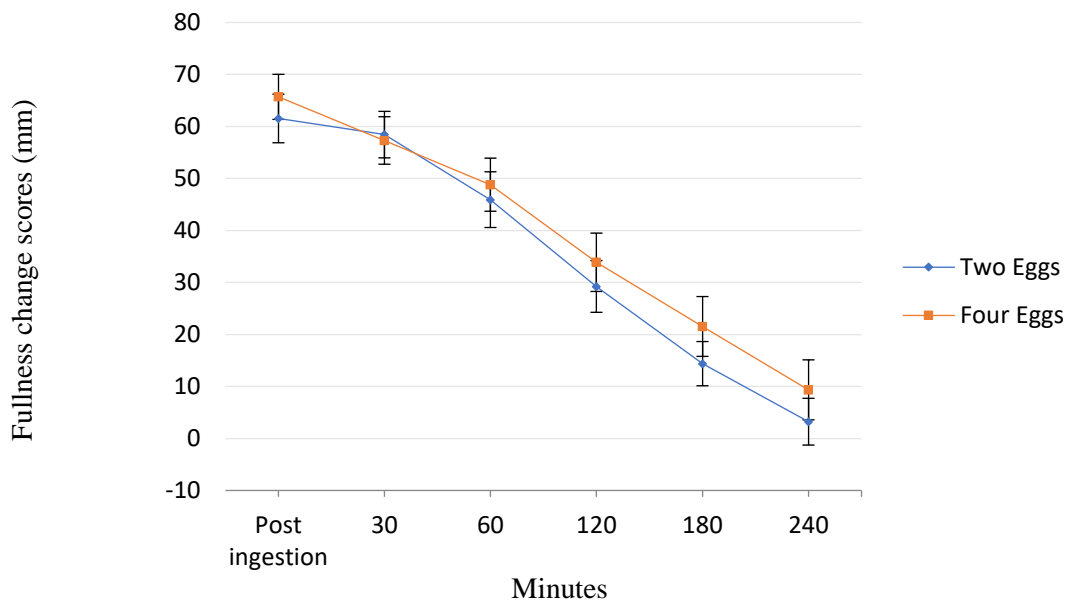


Fig. 3. Mean (\pm SEM) change in reported fullness in the small (see two) and the large (see four) conditions (N=26)

3.4. Pasta, Food Diaries and Day's Total Intake

When participants thought they had consumed a 4-egg omelette (large condition) they ate approximately 70 fewer calories at lunch than when they thought that they had consumed a 2-egg omelette (small condition) (a 12% difference) ($M_{\text{large}} = 530$ [\pm 95% CI 428-632]; $M_{\text{small}} = 599$ [\pm 95% CI = 496-703]). This reflects a small ($d = 0.29$) but statistically significant ($t(25) = 2.187$, $p = .019$, one-tailed) effect. Participants' food diaries showed that, when participants were in the large condition, they went on to consume 1022 kcal [\pm 95% CI = 826 - 1217] and when they were in the small condition they went on to eat 1119 kcal [\pm 95% CI = 932 - 1306]. This difference in later consumption did not reach statistical significance ($t(25) = 1.172$, $p = .126$, one-tailed). As a final measure of consumption, the whole day's calorie intake was calculated as a composite score of the omelette, pasta lunch and food diaries to see if overall intake differed by portion condition. As can be seen in figure 4, these data show that, on average, participants consumed 167 fewer calories [\pm CI 26 - 309] when they thought they had consumed a four egg omelette (a 9% difference). This reflects a small-medium effect ($d = 0.33$) and is statistically significant ($t(25) = 2.12$, $p = .02$, one-tailed).

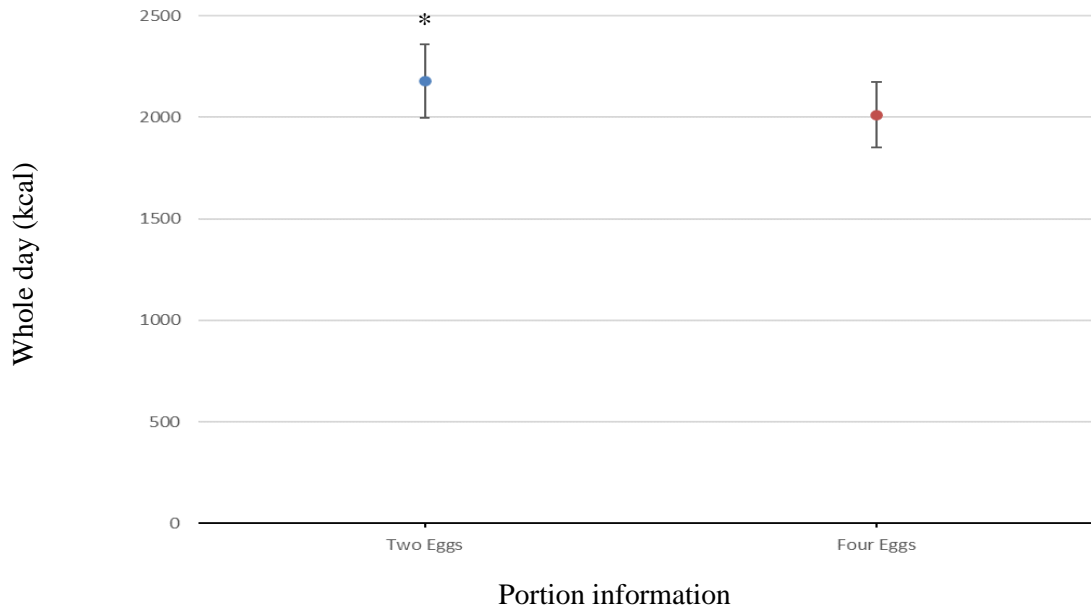


Fig. 4. Mean [\pm 95% CI] kcal for combined (omelette, pasta, food diaries) consumption after the 'small' (see two) and the 'large' (see four) conditions (N=26). Note: asterisk depicts significance at $p < .05$, one-tailed.

3.5. Total ghrelin response

The mean (\pm SEM) total ghrelin (pM) measures across the test period (baseline - 4 hours) are shown in figure 5. A repeated measures ANOVA showed that there was an overall main effect of time on total ghrelin response ($F(2.84, 65.39) = 3.04, p = .038, \eta_p^2 = .117$). There was no main effect of portion information (small vs. large) on total ghrelin response ($F(1, 23) = .283, p = .6, \eta_p^2 = .012$) (marginal means for overall total ghrelin in the small condition, $M = 307.8 [262.3 - 353.4]$ and large condition $M = 314.2 [271.5 - 356.9]$) and the interaction between the two variables (portion information*time) did not reach significance ($F(2.57, 59.02) = .501, p = .365, \eta_p^2 = .044$).

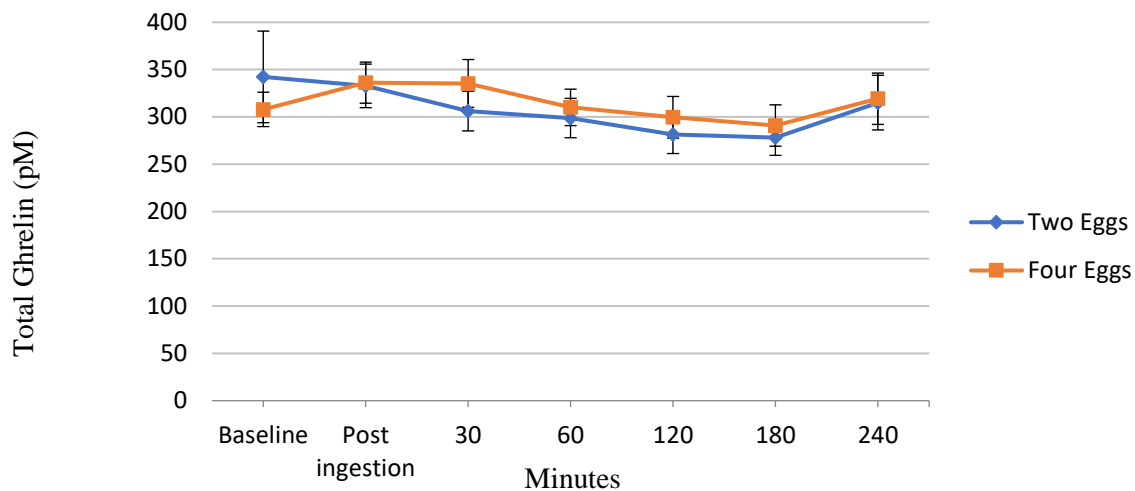


Fig. 5. Mean (\pm SEM) total ghrelin (pM) in the ‘small’ (see two) and the ‘large’ (see four) conditions (N=24)

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4. Discussion

This study reports some novel findings. Firstly, our hypothesis that participants' ES for a solid food could be manipulated was supported. This extends previous work that demonstrated the same effect using smoothies (Brunstrom et al., 2011). Our second hypothesis, based on the Brunstrom et al. (2011) study, was that participants' hunger and fullness reports would differ over the inter-meal period because of the difference in ES. Our data provide some support for this hypothesis in relation to hunger (though not fullness). We found that there was a significant interaction between portion size information and time on self-reported measures. While post-hoc tests did not definitively identify what is driving this interaction, both our data and prior research/theory suggest that this could be due to differences in perceived hunger around the mid-point (120 minutes and, to a lesser degree, 180 minutes) of the inter-meal period.

These findings fit well with a prior study by Brunstrom et al. (2012). In an independent measures design, four groups of participants were shown either 500ml or 300ml of soup and then went on to consume a congruent amount or had the amounts altered ('see 300ml consume 500ml' or 'see 500ml and consume 300ml'). The data showed that participants' subsequent reports of hunger were initially driven by the amount that they had consumed. However, after 2 hours, hunger reports were related to the perceived rather than actual amount of soup consumed. In line with this, our findings show that, despite anticipatory differences in ES, initial hunger reports related to the physiological consequences of eating a 3-egg omelette and this was the overriding driver of appetite reports (see Blundell, Rogers & Hill, 1987; Brunstrom et al., 2012). However, at around the mid-point (2-3 hour measures), the interaction and post hocs suggest that initial expectations became more influential.

Finally, at the 4 hour point, appetite reports across the two visits were, again, driven by the fact that participants had consumed the same 3-egg omelette on both occasions. However, the study was powered for a difference in ES and it is important to highlight that the measures of hunger did trend in the hypothesised direction across the 4 hours of measures (see fig. 2). As such, it may be that this measure was underpowered and ES playing a greater role across the inter-meal period cannot be ruled out.

Our third hypothesis was that participants would consume fewer calories when in the large than compared to the small condition. Our data support this hypothesis showing that when they thought that they had eaten a 4-egg omelette, participants consumed less pasta at lunch. Consumption after lunch until the end of the day (as measured by weighed food diaries) did not reach statistical significance. However, the overall comparison (day's total intake) was significantly different (9.1%) when participants thought that they had eaten a 4-egg omelette in comparison to when they thought they had eaten the 2-egg omelette (an mean decrease of 167 calories).

Such a difference is potentially important. Dynamic weight loss models (see Thomas et al., 2013) are based on the first law of thermodynamics incorporating body composition, age, height, gender result in a curvilinear pattern of weight loss over time. As such, when looked at from an individual level, estimates of calorie restriction range from 300-1500 kcal/day (Blundell, 2011). Therefore, a reduction of ~167kcal, if shown to be consistent in further studies and across different meal types, as a result of doing nothing more than eating a food that a person considers to be a higher ES, could contribute significantly.

Research with liquid and semi solids foods has consistently shown that increasing ES affects subsequent appetite ratings and/or consumption (Brunstrom et al., 2011; Chambers et al., 2013; Hogenkamp et al., 2013; McCrickerd et al., 2012; Yeomans & Chambers, 2011). This study provides some additional support in relation to solid foods using a similar methodology. However, it is unclear whether these differences are the result of memory (Higgs, 2002; Higgs, Williamson, & Attwood, 2008; Robinson et al., 2013) or due to a conditioned, physiological response (Crum et al., 2011; Cummings et al., 2004; Feillet, 2010; Hogenkamp et al., 2013; Ott et al., 2011, 2012; Schüssler et al., 2012). Cognitive processes such as memory for consumption influence both short term (e.g. pre-load/immediate test meal) and longer term (e.g. 2hr delay before lunch) food intake (Robinson et al., 2013). Therefore, when participants are asked to report their hunger and fullness for a period following a meal, it may be that they recall episodic memory encoded at the time of consuming that meal and this influences their reports/later consumption (Brunstrom, 2014).

Our study measured participants' total ghrelin response over the 4-hour test period. We hypothesised that when in the 'large' condition participants would show a significantly greater decline in total ghrelin concentrations post consumption, in comparison to when they consume the omelette in the 'small' condition, as in Crum et al. (2011). Our results do not support this hypothesis and no

difference in total ghrelin was shown. This is in line with Hogenkamp et al. (2013) where participants who were given a low kcal (LC) yogurt preload with high kcal (HC) information consumed less food at a later ad-lib eating opportunity, in comparison to a LC pre-load with LC information, but showed no anticipatory hormone response. As in our study, they concluded that post-prandial levels of total ghrelin were related to the actual pre-load intake rather than the information provided.

There are differences in the exact timings for when the blood samples were taken in Hogenkamp et al. (2013), Crum et al. (2011) and the current study which could account for differences in the findings in relation to total ghrelin (see Hogenkamp et al.). A tentative alternate explanation is that it could be that a relatively large difference in expectations is required to produce a response in the form of a total ghrelin change. Participants in our study were led to believe that their consumption over the two visits would differ by 100% (2 or 4 eggs), in the Hogenkamp et al. study it was 195% difference. However, in Crum et al. the purported difference was 343% over the two eating episodes, by far the largest. Finally, it could be that descriptors are important and the response is more related to psychological craving. A study by Veldhuizen, Nachtigal, Flammer, de Araujo and Small (2013) used verbal descriptors (“healthy” vs. “treat”) and found that midbrain and hypothalamus responses to a low-calorie fruity drink more closely resembled the response to a milkshake when they described as a “treat” rather than “healthy”. This is in line with Crum et al. who used the terms “sensible” and “indulgent”. As such, it may be labelling that is important to brain-gut responses, rather than differences in expected energy per se. Further research could investigate these possibilities.

Ghrelin is a 28-amino acid peptide and exists as two isoforms: acyl ghrelin and des-acyl ghrelin. It is catalysed into its biologically ‘active’ forms (acyl ghrelin) by *O*-acyltransferase (GOAT) (Steinert et al., 2017). Modification of ghrelin is vital for it to bind with its receptor (growth hormone secretagogue type 1a (GHS-R1a)), which, when activated, increases energy intake (Kojima et al., 1999; Wren et al., 2000). Des-acyl ghrelin does not bind to GHS-R1a and may act independently from the actions of ghrelin (Fernandez et al. 2016). It is important to highlight that the present study, and those like it (Ratliff et al., 2010; Crum et al., 2011; Hoffman et al., 2018), measured total ghrelin, which consists of both acyl and des-acyl ghrelin. While circulating levels of total ghrelin do show a pre-prandial rise and post-prandial fall (Cummings et al., 2001), caution is warranted when comparing studies that have measured total ghrelin with those that have measured acyl and/or des-acyl ghrelin. Researchers exploring the effects of ES manipulation on appetite regulation should consider distinguishing between the different forms of ghrelin, to further enhance the validity of their findings.

One further point worth considering here relates to the overall aim of increasing ES. A *change* in the ES for a given substance vs. some ‘standard’ has been demonstrated when eating (a snack, as part of a ‘tasting session’, as a ‘pre-load’) or evaluating (texture, viscosity, visual appearance) various foods/drinks (see Forde et al., 2015, for an overview). However, fewer studies have given these as

actual test meals to examine the later effects on appetite and consumption. Where the latter occurs, it is important to try and differentiate between those studies that have compared an *increase* in ES vs. what might be considered an otherwise ‘normal’ sized meal, and those that have compared a *decrease* in ES in comparison to a ‘normal’ meal for that eating occasion. Both create a relative difference in ES, but potentially have different applications.

For example, in the Brunstrom et al. (2011) study participants were given a fruit smoothie (drink). The ES for the ‘small’ portion was measured at around 250 calories and for the ‘large’ around 330 calories. This may be indicative of a ‘low’ ES (the ‘small’ condition) vs. something closer to ‘normal’ ES (the ‘large’ condition). While it creates a disparity for the purposes of the study investigation, the ‘large’ would not be considered a large lunch, per se (Public Health England recommends an approximate 600kcal lunch intake (NHS, 2018)). In the current study participants were given an omelette (solid food). The ES for the ‘small’ portion was measured at around 335kcal and for the ‘large’ around 457kcal. This is more indicative of a ‘normal’ ES (the ‘small’ condition) vs. a ‘high’ ES (the ‘large’ condition) for breakfast (data from the National Diet and Nutrition Survey 2008–2014, suggests breakfast for 19-64-year olds is, on average, 341kcal (Gaal, Kerr, Ward, McNulty & Livingstone, 2018)) and, as such, the ‘large’ was likely considered a large breakfast. With much of the work on ES using semi liquids/liquids (which will be more limited in terms of the overall ES that they can confer), it is important to differentiate between those studies that decrease ES increase and those that increase ES, for a given eating occasion, as the two have diametric applications (decreases in ES can be used to encourage an increase in kcal intake and an increase in ES can be used to encourage a decrease in intake).

Further research has already been identified above, but some additional suggestions are warranted. Firstly, while the findings presented are promising, the effect on later consumption has only been shown over one day. When using drinks, the effect of increasing ES through modification of sensory aspects diminished over repeated exposures and had less impact on later consumption (Yeomans, McCrickerd, Brunstrom & Chambers, 2014). As such, it would be important to replicate our effect on calorie intake over a longer period. Secondly, this study utilised a high fat, high protein meal at breakfast. Additional research should look to expand this to other macronutrients and at other mealtimes. Finally, future studies should aim to get a baseline measure of appetite reports and later consumption based on participants’ habitual responses to their breakfast meal. This would allow for a comparison of how changes in ES impact on these measures.

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