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In search of flavour-nutrient learning: A study of the Samburu pastoralists of North-Central Kenya

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Abstract

Much of our dietary behaviour is learned. In particular, one suggestion is that 'flavour-nutrient learning' (F-NL) influences both choice and intake of food. F-NL occurs when an association forms between the orosensory properties of a food and its postingestive effects. Unfortunately, this process has been difficult to evaluate because F-NL is rarely observed in controlled studies of adult humans. One possibility is that we are disposed to F-NL. However, learning is compromised by exposure to a complex Western diet that includes a wide range of energy-dense foods. To test this idea we explored evidence for F-NL in a sample of semi-nomadic pastoralists who eat a very limited diet, and who are lean and food stressed. Our Samburu participants (*N*= 68) consumed a sensory-matched portion (400g) of either a novel low (0.72 kcal/g) or higher (1.57 kcal/g) energy-dense semi-solid food on two training days, and an intermediate version on day 3. Before and after each meal we measured appetite and assessed expected satiation and liking for the test food. We found no evidence of F-NL. Nevertheless, self-reported measures were very consistent and, as anticipated, expected satiation increased as the test food became familiar (expected-satiation drift). Surprisingly, we observed insensitivity to the effects of test-meal energy density on measures of post-meal appetite. To explore this further we repeated a single training day using participants (*N*= 52) from the UK. Unlike in the Samburu, the higher energy-dense meal caused greater suppression of appetite. These observations expose interesting cross-cultural differences in sensitivity to the energy content of food. More generally, our work illustrates how measures can be translated to assess different populations, highlighting the potential for further comparisons of this kind.

Key words: flavor-nutrient learning, cross cultural, Samburu, expected satiation, energy compensation, appetite

Highlights

Common measures of appetite and satiety can be used in cross-cultural studies

Little evidence for flavour-nutrient learning in Samburu pastoralists

Cross-cultural differences are observed in responsiveness to energy content of food

'Expected-satiation drift' observed in pastoralists from North-Central Kenya

Introduction

Adult humans have a remarkable capacity to describe subtle differences in the acceptability of particular dishes and food items on a menu. By contrast, human infants are born with clear hedonic reactions to basic tastes (bitter, sweet, and sour) (Ganchrow, Steiner, & Daher, 1983) and to very specific flavours encountered in utero (Mennella & Beauchamp, 1996). This observation suggests that much of our dietary behaviour is modified and learned over time. One hypothesis is that beliefs about foods are shaped by a process known as ‘flavour-nutrient learning’ (F-NL). F-NL is thought to occur when an association forms between the orosensory characteristics of a food (a conditioned stimulus [CS]) and the detection of its nutritive value (an unconditioned stimulus [US]), after it has been consumed. When a food has a high nutritive value its sensory characteristics are remembered and the underlying association leads to an increase in preference. Non-human omnivores are very good at ‘flavour-nutrient learning’ (F-NL) (Sclafani, 1997, 2004). However, it remains unclear whether F-NL plays a significant role in shaping human dietary behaviour.

Unfortunately, advance in this area has been disappointing. In part, this reflects the fact that we still know very little about the expression of F-NL in humans (for further discussion see (Brunstrom, 2005, 2007)). A major hurdle has been a persistent difficulty observing reliable examples of F-NL under controlled conditions (Brunstrom, 2004; Yeomans, 2012). Only nine studies have demonstrated changes in flavour preference that are consistent with F-NL (for a comprehensive review see (Yeomans, 2012)). A further five studies have failed to observe learning. We also suspect these null results are unrepresentative owing to publication bias. Several suggestions have been mooted to improve upon previous protocols (Yeomans, 2012). However, relatively little attention has been paid to the nature of the participants. One possibility is that restrained eaters show impaired learning (Brunstrom, Downes, & Higgs, 2001; Brunstrom & Mitchell, 2007). It has also been suggested that children show better learning than adults, because they lack latent inhibition (Brunstrom, 2005; Lublow & Moore, 1959).

One reason why F-NL might be so clearly evident in non-human animals is that subjects are tested having been exposed previously to a very monotonous diet (lab chow) (Pérez, Fanizza, & Sclafani, 1999). This may actually help to facilitate the process of acquiring flavour-nutrient associations. A few studies have explored the effects of exposure to dietary variety on F-NL in rodents. Some support this hypothesis (Boakes, Rossi-A, & Garcia-Hoz, 1987; Warwick & Schiffman, 1991) and one does not (Pérez et al., 1999). Either way, we suggest that extrapolation of these findings to humans may be innapropriate for two reasons. First, the degree of dietary complexity that can be introduced is small relative to the diversity of experience in many human cultures. Second, it unclear whether the effects of dietary complexity reflect an impairment in F-NL or the absence of an abnormal heightened ability to learn that is observed in animals that are fed a highly monotonous diet.

Following the above, we reason that F-NL may be intact in humans. However, a Western diet might compromise the process. Modern self-serve supermarkets stock 50,000 or more items (Institute, 2014), reflecting the enormous variety of foods and flavours to which many of us are exposed. Humans could conceivably have limited capacity to learn multiple flavour-nutrient associations which is quickly exceeded by this relatively unnatural stimulus variety. Once an upper limit has been reached then learning is impaired.

Across cultures dietary variability is strongly associated with per-capita consumption and with energy availability (Ruel, 2003). One population, the Samburu, typifies one end of this distribution. The dietary habits and culture of the Samburu have been studied over many years (Holtzman, 2009). However, their capacity to learn flavour-nutrient associations has not been explored previously. Indeed, to our knowledge no study has considered cross-cultural differences in sensitivity to F-NL. The Samburu are an indigenous population who live in remote areas of North-Central Kenya. They are semi-nomadic pastoralists who tend to consume a very simple diet comprising primarily meat, milk, maize, and sometimes blood from their livestock (Holtzman, 2009). We reasoned that if F-NL is compromised by a complex diet then we might observe evidence for learning in a sample of Samburu who encounter only a limited range of foods.

In studies of human dietary learning changes in preference for a novel flavour tend to be assessed using visual-analogue ratings. These measures are anchored with end points such as ‘extremely liked’ or ‘very pleasant.’ A concern is that these expressions may be translated and interpreted very differently across cultures. In response, we incorporated pictorial representations into several of our measures. For example, we used a series of stylised happy and sad faces to assess changes in preference for our test food. Previously, we have also used various computer-based tasks to elicit information from respondents based on responses to pictures of foods served in different portions (Brunstrom, Shakeshaft, & Scott-Samuel, 2008). This approach ensures that the stimuli are tightly controlled across trials and across participants. In particular, we have used food images to assess the ‘expected satiety’ and the ‘expected satiation’ of different foods (Brunstrom, 2011, 2014; Brunstrom, Collingwood, & Rogers, 2010). In some of these tasks, participants are required to pick a particular food image that corresponds with the amount that they would need to stave off hunger between meals (expected satiety) or to pick an amount that would leave them feeling full at lunchtime (expected satiation). In the present study we incorporated similar measures to assess changes in beliefs after exposure to a novel low or higher energy-dense test food. In several studies we have shown that estimates of expected satiation tend to ‘drift’ in a predictable fashion with experience. Specifically, with increasing familiarity, foods are expected to deliver greater satiation (Brunstrom, Shakeshaft, & Alexander, 2010; Hardman, McCrickerd, & Brunstrom, 2011; Irvine, Brunstrom, Gee, & Rogers, 2013). This effect is highly reliable in samples drawn from the UK. To determine whether it generalises to other cultures we explored evidence in the Samburu. Evidence of this kind is also helpful, because it implies a valid translation and interpretation of measures across cultures.

More generally, this project represented an initial attempt at an interdisciplinary collaboration that fuses the comparative perspective of cultural anthropology and experimental psychology. Anthropologists tend to place greater emphasis on the cultural specificity and malleability of eating behaviour and experiences. This is perhaps most famously exemplified in Mintz’s anthropological/historical study of sugar (Mintz, 1985), arguing that—despite a basic human, or even primate attraction to sweetness—the explosive growth of sugar consumption in Europe during the 17th through 19th century could only be explained with attention to the specific historical and cultural conditions that shaped sugar’s meanings and uses. Other anthropologists have taken this culturally specific approach much further suggesting, for instance, that even what are regarded as basic human sensory experiences of taste are highly culturally mediated, since even what sensory scientists and physiologists construe as “basic tastes” may be culturally constructed out of a far greater array of sensory possibilities than is encompassed within the four or five tastes that are acknowledged in Western science (Howes 1991, 2003; Sutton 2010; Trubek 2008), or because even these basic tastes may be experienced in ways that are radically at odds with how biologically oriented science assumes them to be (Mol 2012). Such arguments, while highly thought-provoking and grounded in sound descriptive data, do not, however, meaningfully address the empirically based, hypothesis-driven questions raised by experimental psychologists with an interest in understanding core mechanisms that shape human eating behaviours and reactions to food. This project, then, holds promise to build synergistically on the strength of each discipline, providing greater breadth to psychological approaches that focus almost exclusively on quite culturally similar Western populations while bringing greater empirical rigor and deeper explanatory power and meaning to the diversity of eating experiences found in anthropological approaches.

Study 1

Methods

Overview

Participants were each tested over three separate test sessions. On day one and two they consumed a fixed portion of a novel test food. Half of the participants received a low energy-dense version and the other half received a high energy-dense version. In the final test session all participants received an intermediate energy-dense version. Before consuming each meal we measured beliefs about the test meal. This included an assessment of expected satiation and a measure of liking and ranked preference. We also assessed appetite before the test meal and for three hours after it had been consumed.

Participants

Participants were recruited in the Samburu District of North Central Kenya. Most were illiterate. Therefore, consent was elicited by way of verbal confirmation. All were informed that the purpose of the study was to understand how Samburu respond to novel foods. Our sample was self-selecting and participants were recruited into our study without screening. All participants were offered the equivalent in Kenyan currency of $2 (USD) per day in remuneration for their assistance. Locally this amounts to a typical wage for a single day of manual labour. Seventy participants were recruited by word of mouth (34 males and 36 females). Ethical approval was granted by the Western Michigan University Human Subjects Institutional Review Board.

Novel test food

The higher energy-dense version of the test food was formulated by combining instant ClearJel® (a modified corn starch derivative) with powdered milk, sucrose, maltodextrin and water. ClearJel® was used because it thickens the mixture and has good stability at room temperature. In combination, this produces a novel food that is viscous and which can be prepared and served without the need for refrigeration. The low energy-dense version looked and tasted very similar. A reduction in energy density was achieved by reducing the sucrose content and by removing maltodextrin. Sweetness was then restored by adding Splenda, a sucralose-based low-energy sweetener (manufactured by Tate and Lyle). The intermediate energy-dense version was formulated by mixing equal measures (by weight) of the low and high energy-dense version. Table 1 shows the specific amounts of each ingredient (per 1000 ml) in the three formulations.

Measures

Appetite: Appetite was assessed in two ways. First, we used a set of silhouette pictures previously developed by Faith *et al.* (Faith, Francis, Sherry, Scanlon, & Birch, 2002; Faith, Kermanshah, & Kissileff, 2002) to assess fullness in preschool-age children. Briefly, five different male silhouettes were presented on a single card (210 mm x 297 mm). From left to right, each silhouette depicted an incremental increase in the amount of food in the stomach. In our version the participants were instructed to pick the silhouette that corresponded to their current level of fullness. Responses were coded from 1 to 5 with '5' representing maximum fullness. In a second task we obtained measures of the maximum amount of food that could be consumed at that moment. Separate measures were taken for boiled eggs (peeled), red kidney beans, and boiled potatoes. For each food, the participants were shown a picture book depicting a set of food portions that ranged from 20 kcal to 800 kcal in 20 kcal increments. All colour images were 230 mm x 200 mm and the foods were presented on an identical dinner plate. Picture numbers (1 to 40) of maximum selected portions were recorded and subsequently converted to a portion size (kcal). The books were presented to the participants in a random order. This approach is based on software that has been used widely in the lab of two of the authors (Brunstrom & Rogers, 2009) and elsewhere (Farah, Brunstrom, & Gill, 2012; Ferriday & Brunstrom, 2008; Hogenkamp et al., 2013).

Expected satiation: As with appetite, the expected satiation of the test food was assessed using a set of silhouette figures and photographic picture books. In both tasks a portion of test food was placed in front of the participant. Using the silhouette pictures the participants were instructed to select a silhouette that corresponded with their anticipated fullness after consuming the test food. After completing this task they were shown the three picture books in a random order. In turn they were instructed to pick the amount of food that would produce the same fullness as the test food. Again, picture numbers were recorded and then converted to portion sizes (kcal).

Preference and liking: Preference was measured using a ranking task. Participants were shown the test food along with separate colour photographs of a 400-kcal portion of boiled eggs, kidney beans, and boiled potatoes. Participants were instructed to arrange the four foods in order of preference, 1= worst and 4= best. Liking was assessed using a scale that has been used previously with children. The scale was anchored on the right with a stylised happy face (upturned mouth) and on the left with an otherwise identical unhappy face (downturned mouth). The scale was subdivided into 10 equal units with vertical markers labelled 0, 10, 20, 30, …100. The participants were instructed to sample the test food and then to use this scale to indicate their liking for its taste.

Procedure

Participants were tested around lunchtime over three consecutive days. As is standard practice, they were required to abstain from eating for three hours prior to each test session. On day one all participants provided informed consent. Measures of appetite were then obtained and the participants were shown a portion of the test meal. To achieve equal numbers, the participants were allocated to receive the high or low energy-dense version, alternately, on arrival. Participants then completed the expected satiation, liking, and preference tasks. They were then instructed to consume the test food in its entirety. After this meal a second set of appetite measures was taken and participants were instructed to abstain from eating or drinking for 90 minutes. Further sets of appetite ratings were taken at 30-minute intervals over this period. The second and third test sessions were identical to the first. However, in the third session all participants received the intermediate test meal.

At the end of the final session the age of the participants was recorded and they provided a measure of their height (mm) and weight (kg). At this point their data were made anonymous. All procedures were explained to participants in their vernacular (Samburu) by a local Samburu-speaking research assistant who was also fluent in English. One of the authors (Holtzman) has extensive training and experience studying Samburu culture. He is fluent in Kiswahili and proficient in Samburu, and supervised the administration of all measures and the recording of all responses.

Data analysis

Two participants failed to complete the study, leaving 36 females (mean age = 37.7 [*SD*= 15.8] years) and 32 males (mean age = 47.6 [*SD* = 18.3]) included in the final analysis.

ANOVA was used to explore the effects of test-meal energy content on appetite. For both measures (silhouette and maximum portion selection) we calculated ‘difference scores’ based on post-meal values after subtracting corresponding pre-meal values at baseline. ‘Energy density’ (high/low) was treated as a between-subjects factor and both ‘day’ (1-3) and ‘time’ (0, 30, 60 and 90 min) were treated as within-subject factors. To analyse our maximum portion-selection data we included ‘food type’ (potatoes, kidney beans, and boiled eggs) as a within-subjects factor.

Arguably, the final test day should be scrutinized independently for effects of prior exposure on post-meal appetite. Since all participants received an identical test meal to consume, the effects of previous allocation to either the high or low energy-dense condition can be taken as evidence that learning has occurred. Therefore, we also conducted separate ANOVAs on appetite difference-scores taken on this day.

Seven participants were excluded from our analysis (high energy-dense condition $n= 2$) of data from the silhouette task because they had one or more missing datum. These omissions are attributed to transcription problems or experimenter error. Similarly, in a small number of cases we failed to record a response in our maximum portion-selection task ($n= 30$, 0.012% of responses). Three participants had several missing values and were removed from our analysis on this basis (higher energy-dense condition $n= 2$). In the remaining data we failed to record a single response to one of the three picture foods on five separate occasions (0.002% of responses). In these cases we substituted missing data with the mean of the participant’s responses to the other two picture foods at that time point.

To explore effects of prior exposure to the high or low energy-dense test food on our measures of preference and liking, we submitted our data to separate mixed-model ANOVAs, with 'energy density' (low/high) as a between-subject factor and 'day' (1-3) as a within-subjects factor. To assess our two measures of expected satiation we used the same ANOVA to explore responses in the silhouette selection task and a modified version for our portion-selection data. In this modified version we also included 'food type' (potatoes, kidney beans, and boiled eggs) as a within-subjects factor. For each of these measures, in cases where we failed to record a response(s) in a test session, we removed participants from our analysis. Three participants were withdrawn from our analysis of the two expected-satiation measures and three from the measure of liking. All analyses were conducted using Minitab 16.2.4.

Results

Participant characteristics

Participants in the high and low energy-dense group did not differ significantly in their gender (χ^2 = 0.003, *df* = 1, *p*= .95), age (*t*= 1.47, *df*= 66, *p*= .14) or BMI (*t*= 0.54, *df*= 66, *p*= .59). For associated counts and means (+/- *SD*) see Table 2.

Appetite measures

Analysis of portion-selection difference-scores across the three days revealed a main effect of time (F[3,189]=164, p<.001). Fullness was at its maximum immediately after consuming the test meal and it returned to a level higher than baseline 90 minutes after the end of the test meal (mean change in selected portion (+/- *SD*); 0 min= 59 kcal +/- 94, 30 min= 25 kcal +/- 99, 60 min= -13 kcal +/- 103, 90 min= 64 kcal +/- 119). Our analysis also revealed a main effect of food type ($F[2, 126]= 16.2$, $p< .001$), reflecting a small difference (relative to baseline) in the amount of each food that was selected to achieve satiation (egg = -28.9 kcal; kidney beans = 5.5 kcal; potatoes = 28.8 kcal).

All interaction terms that included energy density failed to reach significance (all $p> .05$). This failure to observe effects of energy is illustrated in Figure 1 (panels a, b, and c). Mean (+/- *SEM*) portion-selection difference scores are shown across conditions and values are provided for each post-meal interval (0-90 min) on separate days. It would also appear that participants experienced a rapid recovery of their fullness to pre-meal levels. Across test days, after 60 minutes, the participants reported being as full or in some cases even less full than they had felt prior to consuming the test meal.

Our analysis also revealed a significant interaction between day and time ($F(6,378)= 9.14$, $p< .001$) that was not predicted from the outset. Inspection of Figure 1 (panel c) shows that this is likely to reflect a more pronounced hunger rebound on day three. Two other interaction terms were also significant, both reflecting effects of food type (1. Day x Food Type, $F(4, 252)= 125.5$, $p= .016$; 2. Time x Food Type, $F(6, 378)= 6.9$, $p<.001$). These are difficult to interpret and were not predicted from the outset. Therefore, they were not explored in detail.

Analysis of difference scores from the silhouette task also revealed a main effect of time, $F[3,177]= 281.1$, $p< .001$. Immediately after consuming the test meal the participants experienced the greatest increase in fullness (mean fullness difference score = 1.29, *SD* +/-

0.69). Figure 1 (panels d, e, and f) shows that mean (+/- *SEM*) portion-difference scores decreased over time and that at 90 minutes the scores were slightly higher than they had been before eating at baseline (mean +/- SD; 0 min= 1.3 +/- 0.70, 30 min= 0.85 +/- 0.75, 60 min= 0.36 +/- 0.86), 90 min= -0.08 +/ 0.84). All other main effects and interaction terms failed to reach significance. Again, post-meal fullness (portion selection) was largely unaffected by the energy density of the test meal.

One exception is a difference that was observed on day three (see Figure 1, panel f). Our separate analysis of responses from only this final test session revealed a main effect of energy density on fullness difference scores (silhouette task), $F[1, 177]= 5.2$, $p= .026$. However, this effect is counterintuitive. Participants who had previously been exposed to the low energy-dense test food reported a relatively greater increase in fullness after consuming the intermediate energy-dense test food. By contrast, our analysis of selection difference scores on day three (Figure 1, panel c) revealed no such effects of energy density ($p> .05$).

Expected satiation

Our analysis of responses from the portion-selection task failed to reveal significant main effects of day or energy density, and the interaction between day and energy density was also non-significant (all $p> .05$). However, we did observe a main effect of day, $F(2,126)= 7.9$, $p= 0.001$. Consistent with evidence for expected-satiation drift, over time, the novel test food increased in expected satiation as it became more familiar. On average, it was expected to deliver the same satiation as 146.3 kcal (*SD* +/- 61.4) of the matched foods (collapsed across food type). By day two and three this value increased to 157.6 kcal (*SD*

+/- 68.2) and 171.1 kcal (*SD* +/- 65.8) kcal, respectively. We also found a main effect of food type, $F(2,126)= 42.9$, $p< .001$. Consistent with responses in our appetite task, the foods differed in the amount (kcal) that was selected to match the expected satiation of the test food (mean values +/- *SD*, egg= 187.6 kcal +/- 67.9; beans= 149.5 kcal +/- 67.5; potato = 137.8 kcal +/- 50.3).

Analysis of responses in our silhouette fullness task failed to reveal a significant main effect of day, and the main effect of energy density and its interaction with day were both non-significant (all p>.05). This failure to observe clear effects of energy density on expected satiation is illustrated in Figure 2. Panel a shows mean (+/- *SEM*) portion selections collapsed across food type. Panel b shows mean (+/- *SEM*) fullness scores from the silhouette task. Separate values are provided for each test day. On day three, participants who previously experienced the high energy-dense test meal tended to regard it as having higher expected satiation. However, we note that in real terms these differences are very small and subsequent *post-hoc* analyses failed to identify significant differences in either task (p> .05 for both the portion selection task and the silhouette task).

Liking and preference

We assessed the affective quality of the test food on each test day. Liking was assessed using a simple 100-point line rating and preference was assessed by recording the ranked position of the test food relative to pictures of egg, kidney beans, and boiled potatoes. Immediate inspection of the data indicated that the test food was highly liked. Figure 3 shows mean values (+/- *SEM*) over the three test days. Respectively, panels a and b show liking ratings and average ranked values (1= ranked highest and 4= ranked lowest). Across the test days the test food was consistently rated between above 80 on our 100-point scale. In the ranking task the test food tended to be ranked around the second position, indicating that it was well liked and consistently more acceptable than at least one of the other comparison foods. Our analysis of the liking ratings revealed only one main effect. Over time the test food tended to be rated slightly higher, $F(2,129) = 9.96$, $p < .001$ (means +/- *SD*; day 1= 84.0 +/- 15.33; day 2 = 84.1 +/- 16.4; day 3 = 91.9 +/- 12.4). Our analysis of preference ranks revealed no significant main effects or interaction terms. Importantly, across both measures, we found no evidence that responses were mediated by exposure to the high or low energy-dense version of the test food.

Interim discussion

In this study we explored evidence for F-NL in an adult sample that had not been exposed to a wide variety of different foods, as is typical in a Western diet. We found very little evidence that learning took place, suggesting that dietary variety is not responsible for previous failures to demonstrate learning in humans.

In relation to this interpretation, a potential concern is that our participants failed to follow instructions or otherwise misunderstood the various measures that were used to show that learning had occurred. It remains difficult to rule out this possibility with certainty. Nevertheless, aspects of the data suggest this was not the case. For example, the pattern of post-meal fullness was broadly as expected. Greatest fullness was reported immediately after the test meal and this attenuated over time. In addition, we have evidence of considerable sensitivity in one of our measures of expected satiation. Across test days, participants selected increasingly larger portions of potatoes, kidney beans, and boiled eggs to match the expected satiation of the test food. This expected-satiation drift is consistent with recent evidence that expected satiation increases after a novel food become familiar (Brunstrom, Shakeshaft, et al., 2010; Brunstrom et al., 2008; Hardman et al., 2011; Irvine et al., 2013). The underlying cause remains unclear (for a related discussion see (Brunstrom, Shakeshaft, et al., 2010)). Nevertheless, this work confirms the robust nature of this phenomenon and shows that it is preserved across cultures with very different dietary customs.

Several observations were unexpected and merit consideration. First, we were surprised to see how much our novel test food was liked. Based on our own informal observations we expected the food to be regarded as merely acceptable. Instead, it was rated around 85 points on a 100-point scale and it was ranked above other otherwise familiar foods (roughly midpoint) in our preference-ranking task. This observation raises questions about whether a Western sample would show the same high level of acceptability and the extent to which this played a role in the outcome of the study.

Second, and very unexpectedly, we observed that the Samburu participants returned to their pre-meal levels of hunger and fullness within 60 minutes of consuming the test meal. We found this very surprising given the size of the meal consumed (~400g) and its energy content (641 kcal in the high energy-dense condition). This raised questions about cross-cultural differences in the profile of the satiety response to our test food. To our knowledge comparisons of this kind have not been undertaken previously. Finally, we found it striking that our Samburu participants showed a complete lack of sensitivity to the effects of our energy manipulation. Across test sessions and measures, we found very little evidence that post-meal appetite was influenced by the energy content of the test meal. Intuitively, we expected the converse – that in a food-stressed population we would see heightened sensitivity to differences in the energy content of a meal. It is often concluded that insensitivity to the energy content of food is a potential cause of overconsumption and obesity (Birch & Fisher, 1998; Campbell, Hashim, & Van Itallie, 1971; Cecil et al., 2005; Cornier, Grunwald, Johnson, & Bessesen, 2004; Davidson & Swithers, 2004; Johnson & Birch, 1992, 1994; Jones & Mattes, 2014; Kral et al., 2012). A demonstration of insensitivity in an ostensibly lean population is important because it would challenge this widely held view.

To explore these observations further we decided to run a similar study with a University sample in the United Kingdom. This study was abbreviated to a single session, focusing specifically on the sensitivity (or lack thereof) to energy density in the test food and on return of appetite after consuming it. We presented participants with the same high and low energy-dense versions of the test food that we used in Samburu (same formulations and volume) and repeated a single training session (day 1) from Study 1. This enabled us to compare measures of liking, expected satiation, and appetite, both across high and low energy-dense conditions, and with corresponding data collected in Samburu.

Study 2

Methods

Participants

Participants (23 males and 37 females) were recruited from the staff and student population of the University of Bristol, UK. Each participant was offered £15 (UK pounds) in remuneration for their assistance. Ethics approval was granted by the University of Bristol Faculty of Science Research Ethics Committee. Participants provided informed and signed consent prior to participation.

Measures and test food

High and low energy-dense versions of the test food were identical to those in Study 1 (see Table 1). We also used identical measures of appetite (silhouette and portion-selection), expected satiation (silhouette and portion-selection), and liking (ranking and rating).

Procedure

Participants were tested on a weekday at 11.30 or 13.30. Prior to arrival, they were asked to refrain from eating for three hours. A measure of height and weight was taken and participants were required to confirm verbally that they had abstained from eating for three hours. Participants then completed baseline measures of appetite. On arrival, the participants were allocated alternately to receive the low or the high energy-dense test food. All other details of the procedure were identical to the first training session in Study 1. Briefly, participants tasted the test food and completed the expected satiation, liking, and preference tasks. They then consumed the test food and sets of appetite ratings were taken every 30 minutes until 90 minutes had elapsed.

Data analysis

Two female participants reported feeling ‘sick’ and withdrew from the study. The remaining participants comprised 35 females (mean age = 23.8 [*SD*= 9.4] years) and 23 males (mean age = 22.3 [*SD* = 6.9]).

ANOVA was used to explore the effects of test-meal energy content on appetite. As in Study 1, for both measures (silhouette and maximum portion selection), we calculated ‘difference scores’ based on post-meal values after subtracting corresponding pre-meal values at baseline. ‘Energy density’ (high/low) was treated as a between-subjects factor and ‘time’ (0, 30, 60 and 90 min) was treated as a within-subject factor. To analyse our maximum portion-selection data we also included ‘food type’ (potatoes, kidney beans, and boiled eggs) as a within-subjects factor. For each measure, separately, we used independent *t*-tests to explore differences in the expected satiation of and liking for the low and high energy-dense test food. No participants had missing data.

Finally, to establish evidence for cross-cultural differences, we repeated these analyses and included ‘sample’ as an additional between-subject factor. To facilitate a meaningful comparison between the Samburu and the UK, in each case, we included and compared data from the first test day on Study 1. All analyses were conducted using Minitab 16.2.4.

Results

Participant characteristics

Participants in the high and low energy-dense conditions did not differ significantly in their gender ($\chi^2 = 1.80$, *df* = 1, *p*= .18), age (*t*= 0.23, *df*= 56, *p*= .23) or BMI (*t*= 1.00, *df*= 56, *p*= .32). For associated counts and means (+/- *SD*) see Table 3.

Appetite measures

Analysis of difference scores from the silhouette task revealed a main effect of time, *F*[3,168]= 33.22, *p*< .001. Immediately after consuming the test meal the participants experienced the greatest increase in fullness (mean fullness difference score = 1.29, *SD* +/- 0.69). In this sample, the energy content of the test food had a significant effect on appetite. Relative to baseline, participants who received the high energy-dense test meal were more likely to select silhouette images depicting fullness, *F*[1,168]= 5.92, *p*= .018. This tendency was evident at all post-meal intervals (see Figure 4, panel a). Our analysis of cross cultural differences (comparing Study 1 with Study 2) revealed a significant interaction between energy density and sample, *F*[3,363]= 5.30, *p*= .023. To illustrate relative differences in sensitivity to the energy density of the test meal we have included mean silhouette differences scores from both studies in Figure 4.

Our analysis of portion-selection difference scores failed to find a significant effect of energy density. However, prospective appetite scores did change in the inter-meal interval (*F*[3,168]= 26.10, *p*<.001. As in Study 1, appetite was diminished immediately after consuming the test meal. However, in this case it failed to restore to baseline levels, even after 90 minutes (mean change in selected portion +/- *SD*; 0 min= 179 kcal +/- 157, 30 min= 149 kcal +/- 173, 60 min= -118 kcal +/- 164, 90 min= 100 kcal +/- 167). Our analysis of cross cultural differences revealed a highly significant main effect of sample, *F*[1,714]= 44.66, $p<.001$. Relative to the Samburu sample (Study 1) our UK sample experienced a more marked reduction in appetite that was sustained for 90 minutes after the test meal. This difference is represented graphically in Figure 4, panel b. *Post hoc*, we were interested to explore baseline differences in portion selection across studies. Separately, for the three types of food (potatoes, beans, and egg), we compared means using independent *t* tests. In each case, we found a significant difference; potatoes, $t(124)= 2.99$, $p=.003$; beans, $t(124)= 3.06$, $p=.003$; egg, $t(124)= 7.9$, $p<0.001$. Averaged across test foods, the UK sample selected larger portions (UK mean = 280 kcal, *SD*= 151.0; Samburu mean = 169.1, *SD*= 61.7), indicating that they had a greater appetite at baseline.

Expected satiation

We found no significant difference between the expected satiation of the test food in participants who received the high or low energy-dense versions ($p= 0.15$). However, we did observe a main effect of food type, *F*[2, 112]= 37.48, $p< 0.001$. Consistent with Study 1, a larger portion of egg was selected to match the expected satiation of the test food (mean values +/- *SD*, egg= 396.9 kcal +/- 141.6; beans= 293.4 kcal +/- 155.2; potato = 264.5 kcal +/- 160.5). When we compared results across studies, we found a highly significant effect of sample *F*[1, 244]= 95.17, $p< 0.001$. Across food types, the Samburu sample matched significantly smaller portions to the test meal, indicating that they expected it to deliver less satiation (mean values +/- *SD*, Samburu= 147.3 kcal +/- 63.6; UK= 318.3 kcal +/- 162.1). In other words, relative to the Samburu, the UK sample expected the test food to deliver roughly twice as much satiety (when compared with egg, potato and beans).

Liking and preference

As in Study 1, we assessed the affective quality of the test food using a preference scale and a ranking task (ranking relative to pictures of egg, kidney beans, and boiled potatoes). Across conditions the difference in rated preference failed to reach significance, $t(54)=0.98$, $p= .33$ (means +/- *SD*; low energy-dense condition = 45.1 +/- 17.0; high energy-dense condition = 40.3 +/- 19.6). Similarly, we found no significant difference in the ranked position (4 = highest, 1 = lowest) of the test food, $t(54)=0.16$, $p= .87$, (means +/- *SD*; low energy-dense condition = 1.55 +/- 0.87; high energy-dense condition = 1.59 +/- 0.73). However, when we compared the UK sample with the Samburu sample (data from day 1) we observed a very clear difference in liking and preference. The Samburu rated the test food as more liked than the UK sample, $F(1,122)=186.4$, $p< .001$, and ranked it much higher, $F(1,123)= 67.9$, $p< .001$. Figure 5 shows associated mean values (+/- *SEM*).

General discussion

For the most part, empirical studies of human dietary behaviour have tended to focus on measures taken from people who eat a Western diet. This probably reflects the geographic location of laboratories with interests in these measures and the recruitment of participants from local populations. Humans have the morphology of a hunter gatherer and our genotype has changed very little since the introduction of agriculture. Therefore, the extent to which ‘normal’ dietary behaviour is ever observed is open to debate. By Western standards the Samburu are food stressed and tend to be very lean (Holtzman, 2009). But perhaps more importantly, their diet and cultural norms around food are very unlike those associated with a Western diet. Therefore, studying the Samburu is helpful because it offers an opportunity to test accepted 'facts' about human dietary behaviour. If behaviours are observed that are inconsistent with these facts then this would imply that they are culturally specific rather than universal, as assumed previously. In particular, this reasoning might be helpful in the study of obesity. Implicitly or explicitly, the behaviour of lean people is often interpreted as being 'normal' (Schachter, 1968). However, a concern is that observations of normal behaviour might otherwise reflect specific strategies that offer protection from an obesogenic, Western, diet (*e.g.*, self-imposed food restriction). In response to this concern, we suggest that cross-cultural comparisons may be helpful because they can be used to evaluate and challenge principles that are otherwise regarded as 'universal' determinants of human dietary behaviour. It is in this context that we consider the main outcomes of our work. These are reviewed in the sections that follow.

Flavour-nutrient conditioning

In related studies participants are sometimes offered a fixed portion of the test meal in the final test session (Birch, McPhee, Steinberg, & Sullivan, 1990). Learning is expressed in an analysis of subsequent *ad libitum* food intake – a conditioned decrease of intake is evidenced in participants who received a high energy-dense test meal during training. Here, we chose to provide a fixed portion of the test food and looked for evidence of learning in measures of post-meal appetite over a 90-minute period. This decision was motivated by the opportunity to obtain a sensitive measure of appetite using our image-based psychophysics and a concern that our sample might eat extremely large *ad libitum* meals, which might mask evidence for learning.

Our sample was drawn from a population that consumes a relatively restricted range of foods. Nevertheless, none of our outcomes provided evidence for F-NL, suggesting that learning is not suppressed by exposure to a Western diet. Instead, our findings add to a broader and emerging consensus that this form of associative conditioning is difficult to demonstrate in humans (Yeomans, 2012). This leads to one of two possibilities. First, humans do indeed use flavour-nutrient associations to modify their dietary behaviour and our paradigms and measures are poorly suited to detect learning. In this regard, we note recent conflicting evidence incorporating measures obtained using fMRI (de Araujo, Lin, Veldhuizen, & Small, 2013). Alternatively, F-NL may not be the primary process by which preferences are acquired in humans. Historically, hunter gatherers coexisted in groups of up to a hundred members. In this context, observational learning might be more important than F-NL because it enables the learner to draw on the collective wisdom of a group rather than having to replicate and rely on learning at an individual level. Consistent with this idea, peer modelling is found to have a robust effect on preferences in humans (Birch, 1980) and the potency of this process appears to be moderated by the level of social connection with the observer. For example, social facilitation from parents appears to be especially important (Harper & Sanders, 1975) as is the effect of congruence in age and gender across the observer and the observed (Shutts, Banaji, & Spelke, 2010). One possibility is that F-NL merely complements this process - by shifting preferences gradually over long periods – its role is exposed in highly controlled experimental conditions. However, outside the laboratory, and alongside collective observational learning, its normal role is to ‘nudge’ behavioural responses to foods over longer periods. For now, we recognise the speculative nature of this proposal. Nevertheless, it would appear to account for a broad range of observations in this field, including various failures to demonstrate F-NL in humans (the present example included) and more robust evidence for effects of peer modelling in the acceptance of new foods, especially early in childhood.

Evidence for learned tolerance?

Before we rule out the possibility of F-NL, one feature of our data merits further consideration. In Study 1 we measured appetite for 90 minutes after our participants consumed the test meal. In our silhouette task they reported the same fullness during training (day one and day two) irrespective of whether they received the high or low energy-dense meal. However, on day three we observed a significant difference. Despite the fact that all participants received an intermediate version, those who had previously consumed the high energy-dense version reported feeling less full than those who previously consumed the low energy-dense version (see Figure 1, panels e - g). On face value this would seem at odds with evidence for F-NL. This is because previous studies have shown the converse - that repeated exposure to a novel energy-dense test food increases its post-meal satiety effect rather than reduces it. Specifically, when issued an intermediate energy-dense test food, participants tend to go on to consume more if they have been previously exposed to a low energy-dense version than to a high energy-dense version (Birch et al., 1990; Booth, Lee, & McAleavey, 1976). Nonetheless, there is another form of learning that is rarely discussed in this field but which is consistent with our findings.

Previously, satiety has been characterised as a form of learned tolerance (Woods, 1991). Eating provides energy to the body. However, the process of metabolising food is also disruptive because it challenges homeostatic processes that regulate our internal milieu. Humans learn to associate drug-related cues with the perturbation in homeostatic systems that caused drug ingestion causes. This is useful because it enables them to counter homeostatic disruption by recruiting anticipatory physiological responses that minimise disruption, before it occurs. In the same way Woods has suggested that we learn to anticipate the effects of a meal and initiate a preparatory defence in advance of eating. It is well established that sight and smell of food can come to elicit a modest pre-prandial increase in insulin that forms part of a preparatory defence against an increase in blood fuels in a dose-dependent manner. Importantly, this process not only protects the body but it enables it to tolerate the consumption of larger portions. This learned tolerance might be expressed as a *reduction* rather than an increase in satiety.

In relation to our data from Study 1, one interpretation is that participants who received the high energy-dense test food acquired a learned tolerance. In the final test session, this learning was exposed when an intermediate energy-dense food was consumed and the post-ingestive consequences (the unconditioned stimulus) no longer followed as anticipated. In other words, the body readied itself for calories that it did not receive. The manifestation of this learned tolerance is an increased capacity to consume an energy-dense meal (reduced satiety) which, when replaced with an intermediate energy-dense version, left our participants feeling less full than those who had been exposed to a low energy-dense meal. In future, this idea merits consideration because it has the potential to explain previous failures to identify evidence for learned controls of meal size. More generally, very little is known about the learned tolerance of meals in humans. One possibility is that this process accounts for a relative insensitivity to the effect of energy density on satiety (Kral, Roe, & Rolls, 2004). However, rather than demonstrating unresponsiveness, our account implies a highly sensitive process that adapts and optimises a satiety-response to food – with the net effect that high and low energy-dense foods produce broadly similar satiety (gram for gram). A strong test of this hypothesis would be to measure the satiety response to a familiar high energy-dense food that is reformulated (unexpectedly) to have a low energy density. Consistent with evidence for learned tolerance, we would expect a blunted satiety response to the test food relative to other familiar foods that also have the same low energy density.

Cross-cultural differences in sensitivity to energy density

A striking and unpredicted outcome was a cross-cultural difference in sensitivity to the energy-density of our novel test food. The high energy-dense version produced relatively greater fullness than the low energy-dense version. However, this effect was present only in our UK sample and not in our Samburu sample. We suspect this difference is unlikely to result from a failure to translate assessments of fullness. In our Samburu sample the pattern of responding was broadly similar to that observed in the UK. In both groups, as anticipated, fullness increased immediately after eating and this attenuated gradually over time.

One possibility is that there are general cross-cultural differences in the expression of satiety and its effect on behaviour. In related studies (unpublished) we have observed a very consistent pattern in the Samburu – a remarkable capacity to consume extremely large meals when these are offered *ad libitum*. Perhaps as expected, very large meals tend to be followed by a period of rest or even sleep. This is because eating is associated with a period of somnolence that is probably mediated by changes in melatonin and orexins (Burdakov et al., 2006). Eating single large meals rather than multiple smaller meals is also associated with an acute cognitive impairment that is expressed across a range of tasks (Hewlett, Smith, & Lucas, 2009). Our UK sample comprised primarily staff and students at a university. One possibility is that they were especially sensitive to the negative consequences of this 'post-lunch dip' and that this heightened their awareness and expression of self-reported fullness. Culturally, we suspect that our Samburu sample were more accommodating of the soporific effects of eating around lunchtime and, for this reason, they showed a relative lack of sensitivity to the energy density of the test meal. In a more recent study (unpublished) we offered a group of Samburu a very large meal to consume. In relation to this idea, it may be relevant that one participant joked "The problem is that you've given us a very big meal but you haven't given us as place to sleep!" We also note the striking difference between the effect of the test meal on fullness in our UK and Samburu samples. As shown in Figure 4, self-reported fullness was much higher in the UK sample. Consequently, the Samburu experienced a rapid return to baseline (pre meal) fullness within an hour and, after consuming the test food, reported having a capacity to consume roughly three times more food than the UK sample. Again, this observation is highly consistent with the hypothesis that a cultural difference exists in the capacity to consume large meals. In the case of the Samburu, this may reflect a greater learned capacity to tolerate and therefore capitalise on large portions on occasions when they are available. In future it would be interesting to repeat this manipulation and to explore the effects of eating high and low energy-dense foods on self-report measures of alertness and objective measures of cognitive performance. More generally, studies of this kind might incorporate a comparison with other cultures, including those that are accustomed to taking siestas after a midday meal.

A related possibility is that our Samburu sample failed to discriminate between the high and the low energy-dense test food because the absolute difference in energy content was relatively small. As noted above, on many occasions we observed our Samburu sample consuming very large meals (perhaps 2-3 times the size expected in a UK sample). For example, in an unrelated study, we measured *ad libitum* lunchtime intake of a meal of beans and maize (unpublished data) in 24 participants. Irrespective of gender, roughly 80% consumed between 800g and 1500g (in some cases even more). By contrast, in Study 1, our test food was approximately 400g and the high and low energy-dense versions contained 290 kcal and 641 kcal, respectively. As a ratio, this difference is large. However, in relation to a much larger meal, the absolute difference in energy intake may be marginal. In other words, perhaps paradoxically, the Samburu show a relative lack of sensitivity to the effects of energy density for two reasons. First, they are relatively less concerned about the negative postingestive effects of a consuming large meal – when an opportunity arises then they are more willing to trade a large meal against the torpor that it might generate. Second, the absolute difference in the energy content of a relatively small meal is of less relevance to a Samburu sample than to a UK sample. This is because unless a meal is so large that it challenges physical capacity, its energy-density is largely irrelevant. In a food-stressed environment it makes little sense to reject an opportunity to eat even if the food has a low energy-density. Further, it follows that the selective preference for a high energy-dense foods in a Western diet results not only from its general availability but also its accessibility in very large portions. Consistent with this idea, in children, increasing the serving size of an energy-dense entrée is associated with a marked decrease in *ad libitum* intake of lower energy-dense foods, including fruits and vegetables (Savage, Fisher, Marini, & Birch, 2012). Presumably, competition between low and high energy-dense foods tends to increase when their combined volume exceeds a physical upper limit. When this happens, the value of a low energy-dense food becomes downgraded because it compromises the capacity to consume foods that confer greater biological value. Again, these ideas also overlap with questions around 'satiety tolerance' and potential cross-cultural differences in their expression as a response to maximise food intake (irrespective of energy density). Hopefully, they also serve here as useful pointers to interesting cross-cultural comparisons that might extend the work we present here.

Differences in liking and sweetness-nutrient consistency

A comparison of liking scores across studies would seem to indicate that the Samburu liked the test food much more than the UK sample. Indeed, the difference was marked. The Samburu rated the test food towards the upper extreme of the visual-analogue scale whereas the UK sample rated it just under halfway. One possibility is that this reflects conceptual differences in the way that the scale is used and, in particular, the possibility that the Samburu were generally more positive because they value all foods more highly. However, a potential problem with this idea is that the Samburu also ranked the test food much more highly than the UK sample relative to three familiar foods (potatoes, eggs, and beans). In light of this, it may be relevant that the test foods were sweetened with sucrose and sucralose. In Samburu culture sucrose is often regarded as a luxury commodity and it is common to add it in large quantities to tea, especially at breakfast (Holtzman, 2009). One possibility is that liking for the test food reflects a generalisation based on relative differences in preference for sweetness. The potential unhealthy effects of sugar consumption has received a great deal of attention recently (Lustig, Schmidt, & Brindis, 2012). However, concerns have also been raised about the use of low-energy sweeteners. In particular, one hypothesis is that they compromise the ability to use sweetness to anticipate the energy content of food and to moderate intake on this basis. Consistent with this view, animals that are reared experiencing non-predictive sweet-calorie experiences show poor compensation for calories in sweet-tasting foods and they experience a rapid gain in bodyweight (Davidson & Swithers, 2004; Swithers, Baker, & Davidson, 2009). In relation to this observation it is worth noting that a cross-cultural comparison with the Samburu may offer a key opportunity to explore the same process in humans. Our Samburu sample consumed sucrose regularly, yet they had never encountered a low-energy sweetener, either as a raw ingredient or as a sweetening agent in a beverage. Therefore, they serve as an interesting ‘control’ against which to compare samples drawn from the UK and elsewhere, where exposure to low-energy sweeteners is extremely common. If the relationship between sweetness and the energetic content of food is intact in the Samburu then this might also explain their lack of sensitivity to the energy density of the test meal. As noted above, children appear to be sensitive to manipulations to the energy density of foods and lose this ability as they get older (Johnson, McPhee, & Birch, 1991). Presumably, this is because they rely increasingly on prior experience - sensory and other cues are used to predict the nutrient effects of foods in advance of their absorption. Consistent with predicted effects of flavour-nutrient inconsistency, the satiety response of our UK sample might have been governed solely by postingestive nutrient sensing. In the absence of consistent sweetness-nutrient pairings, sweetness was ignored (the UK sample were ‘childlike’). By contrast, sweetness may be a potent cue for calories in the Samburu (supported by their liking) and this may have overshadowed the immediate postingestive effects of our energy density manipulation. One way to begin to test this hypothesis is to compare the satiety responses of a Samburu and UK-based sample after exposure to novel and familiar bland and sweetened foods and beverages.

Concluding remarks

This work represents a novel fusion of cultural anthropology and experimental psychology to address fundamental questions about human dietary behaviour. Perhaps the most important outcome is that we have demonstrated that research of this kind is practical and that measures and techniques that are commonplace on university campuses can be adapted and translated for use in this cross-cultural context. Again, we believe this is critical, because it offers an opportunity to identify universal principles and to dissociate these from culturally-specific determinants of human dietary behaviour. This has direct relevance to a broad range of questions, including those relating to overeating, dietary control, and obesity. Already, our approach has generated a set of new and in some cases unexpected observations. However, it has also helped to inspire further questions that now form the basis for a programme of ongoing collaborative research.

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Figure captions

Figure 1. Measures of appetite relative to baseline (pre meal). Separate means (+/- *SEM*) are provided for days 1-3 and at 0, 30, 60 and 90 minutes after consuming the test meal. Portion-selection difference scores are shown in panels a, b and c. Fullness-difference (silhouette-selection) scores are shown in panels d, e, and f. Positive values indicate that the test meal increased fullness relative to pre-meal levels of fullness. Respectively, open and closed symbols represent participants in the low energy-dense and the high energy-dense conditions.

Figure 2. Measures of expected satiation – Panel a shows mean (+/- *SEM*) portions (collapsed across food type) selected (kcal) to match the satiation expected from the test food. Panel b shows the mean (+/- *SEM*) image number selected in the silhouette fullness tasks. In both cases, higher numbers indicate increased expected satiation from the test food. Respectively, open and closed symbols represent responses from participants in the low energy-dense and the high energy-dense conditions. Separate values are provided for test days 1-3.

Figure 3. Panel a shows mean (+/- *SEM*) liking ratings for the test food. Panel b shows the mean (+/- *SEM*) ranked position of the test food relative to three other familiar foods, 4= highest ranked and 1= lowest ranked. Respectively, open and closed symbols represent responses from participants in the low energy-dense and the high energy-dense conditions. Separate values are provided for days 1-3.

Figure 4. Mean (+/- *SEM*) post-meal appetite relative to baseline (pre meal) at 0 minutes, 30 minutes, 60 minutes and 90 minutes. Fullness-difference (silhouette-selection) scores are shown in panel a. Positive values indicate that the test meal increased fullness relative to pre-meal levels of fullness. Differences in portion-selection are shown in panel b. Open and closed symbols represent participants in the low and the high energy-dense conditions, respectively. For comparison, values from the Samburu on day 1 of training (Study 1) are included and connected with dashed lines.

Figure 5. Mean (+/- *SEM*) liking (panel a) and ranked preference (panel b) for the novel test meal. Separate values are provided for participants in the low energy-dense (LED) and the high energy-dense (HED) conditions. Data from the UK sample (Study 2) are indicated with solid symbols. Data from the Samburu sample (Study 1, day 1) are indicated with open symbols.

Tables

Table 1. Ingredients required to produce 1000 ml of the test food. Separate values are provided for versions that have low, high, and intermediate energy density.

	Low	High	Intermediate
Instant ClearJel® , 3.6 kcal/g	86 g	86 g	86 g
Low fat powdered milk 3.5kcal/g	35 g	35g	35 g
Sucrose, 4 kcal/g	69 g	173 g	121 g
Maltodextrin, 4 kcal/g	0 g	173 g	86.5 g
Sucrolose, 4 kcal/g	28 g	0 g	14 g
Water, 0 kcal/g	914 g	690 g	802 g
Energy	820 kcal	1816 kcal	1318 kcal
Total weight	1131 g	1155 g	1143 g
Serve weight	400 g	408 g	404 g
Serve energy	290 kcal	641 kcal	465 kcal

Table 2. Participant characteristics in Study 1. Separate frequencies and means (+/- *SD*) are provided for participants who received the low and the high energy-dense (ED) test meal.

	Low ED	High ED	Total
Males (n)	18	14	32
Females (n)	20	16	36
Height (m)	1.66 (0.67)	1.65 (0.67)	1.66 (0.07)
Weight (kg)	53.0 (6.0)	51.9 (6.7)	52.5 (6.3)
BMI	19.2 (2.0)	19.0 (2.2)	19.1 (2.1)
Age (years)	39.6 (18.1)	45.0 (16.6)	42.4 (17.6)

Table 3. Participant characteristics in Experiment 2. Separate frequencies and means (+/- *SDs*) are provided for participants who received the low and the high energy-dense (ED) test meal.

	Low ED	High ED	Total
Males (n)	14	9	23
Females (n)	15	20	35
Height (m)	1.74 (0.09)	1.73 (0.10)	1.73 (0.09)
Weight (kg)	71.4 (13.6)	68.6 (11.2)	70.0 (12.4)
BMI	23.5 (3.2)	22.7 (2.2)	23.1 (2.7)
Age (years)	23.5 (7.4)	23.0 (9.5)	23.3 (8.5)