



## Research report

Sensitivity of *ad libitum* meals to detect changes in hunger. Restricted-item or multi-item testmeals in the design of preload appetite studies<sup>☆</sup>Katy R. Wiessing<sup>a</sup>, Liping Xin<sup>a</sup>, Anne-Thea McGill<sup>a,b</sup>, Stephanie C. Budgett<sup>c</sup>, Caroline M. Strik<sup>a</sup>, Sally D. Poppitt<sup>a,d,e,\*</sup><sup>a</sup> Human Nutrition Unit, University of Auckland, 18 Carrick Place, Mount Eden, Auckland, New Zealand<sup>b</sup> School of Population Health, University of Auckland, Auckland, New Zealand<sup>c</sup> Department of Statistics, University of Auckland, Auckland, New Zealand<sup>d</sup> School of Biological Sciences, University of Auckland, Auckland, New Zealand<sup>e</sup> Department of Medicine, University of Auckland, Auckland, New Zealand

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## ABSTRACT

The aims of the study were to determine whether restricted single-item or multi-item testmeals are better able to detect prior changes in hunger and fullness when assessing *ad libitum* eating behaviour. Thirty male participants were given a low- ( $L_E$ , 0.5 MJ) or high-energy ( $H_E$ , 4.0 MJ) breakfast preload designed to induce or suppress hunger, followed 3 h later by a restricted-item ( $R_I$ ) or multi-item ( $M_I$ ) testmeal. The  $R_I$  testmeal comprised pasta + meat sauce, whilst the  $M_I$  testmeal comprised pasta + meat sauce plus bread, chicken, ham, cheese, salad, cake and fruit. The four conditions were (i)  $L_E/R_I$ ; (ii)  $L_E/M_I$ ; (iii)  $H_E/R_I$ ; (iv)  $H_E/M_I$ . Visual analogue scales (VAS) were used to rate appetite sensations and EI was measured at the lunch testmeal. As expected, increasing the energy content of the preload significantly altered VAS ratings and decreased EI at the testmeal. Following both  $L_E$  and  $H_E$  breakfasts, EI was lower at the  $R_I$  ( $L_E = 4566$  kJ,  $H_E = 3583$  kJ) compared with the  $M_I$  ( $L_E = 6142$  kJ,  $H_E = 5149$  kJ) testmeal. However, the compensatory decrease in EI in response to the  $H_E$  breakfast was not significantly greater at the  $R_I$  testmeal ( $R_I$ :  $-983$  kJ, 28.1% compensation;  $M_I$ :  $-993$  kJ, 28.4% compensation). In preload studies measuring EI, increasing the variety of an *ad lib* testmeal may not decrease the sensitivity to detect changes in hunger and fullness.

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## Introduction

When investigating appetite regulation, the most common methodology employed is the single day preload study, where short-term postprandial effects of an intervention are assessed. These studies measure the effects of a fixed nutrient, food or meal (termed the 'preload') on postprandial appetite-related ratings such as hunger and fullness, and also measure *ad libitum* food intake from one or more subsequent meals (termed the outcome 'testmeal') from which the participant eats freely (Bertenshaw, Lluch, & Yeomans, 2008; Blundell et al., 2010; Burns et al., 2000; Chan et al., 2012; Flood & Rolls, 2007; Lawton, Delargy, Brockman, Smith, & Blundell, 2000; Strik et al., 2010). In these postprandial studies, if the food or nutrient preload alters appetite sensations it is hypothesised that this in turn will change eating behaviour

and that such a change can be quantified by measuring *ad lib* energy and nutrient intake at the outcome testmeal. Yet, despite the importance of this *ad lib* testmeal in preload studies and the wide use of this method, there is little consensus as to the content and/or composition of the testmeal. Of particular interest to us was understanding whether the sensitivity of the testmeal to detect changes induced by a prior preload is altered by the composition of the meal, particularly when the number of items and choice at the meal is increased. It has long been established that increasing the variety within a meal increases the energy consumed at that meal (Raynor & Epstein, 2001; Rolls et al., 1981) possibly through disruption of habituation (Brondel, Lauraine, VanWymelbeke, Romer, & Schaal, 2009), but whether this decreases the sensitivity of the testmeal as a tool by which to detect changes in hunger has not been established.

There are theoretical advantages and disadvantages to both single-item restricted and multi-item buffet testmeals. The single item *ad lib* testmeal allows little or no choice, typically has a lower palatability rating which may further decline as multiple treatments are completed, leads to rapid onset of sensory specific satiety (SSS) (Rolls, Rowe, & Rolls, 1982), and is likely to suppress

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intake relative to a multi-item meal. Concern about the multi-item meal however includes the fact that it is at variance with the usual eating pattern of most individuals (Blundell et al., 2010), it may encourage overconsumption (Norton, Anderson, & Hetherington, 2006; Rolls et al., 1981) through delay of satiation and meal termination (Hetherington, Foster, Newman, Anderson, & Norton, 2006) which may in turn swamp the relatively small effects on appetite which are induced by a preload, and so decrease the sensitivity of postprandial studies to detect changes in eating behaviour. It has been convincingly argued (Blundell et al., 2010) that single course, restricted testmeals are most appropriate for the assessment of food and energy rather than nutrient intakes, and that nutrient intake is better addressed by presenting a buffet style testmeal which allows the participant to make additional food choices (preferences, dislikes, avoidance, nutrient choices) and for these choices to also be measured. Since energy intake (EI) is commonly the primary measurement goal in preload studies, we wanted to determine whether the choice of lunch meal style altered the sensitivity of these testmeals to quantify changes in (EI) induced by a prior preload, and whether restricted-item ( $R_I$ ) or multi-item ( $M_I$ ) meals can best detect small changes in EI.

Hence in this study we aimed to measure the effect of changing the size and variety of a lunch testmeal on *ad lib* EI following two different breakfast preloads: a low-energy ( $L_E$ ) hunger-inducing breakfast preload and a high-energy ( $H_E$ ) hunger-suppressing breakfast preload. We hypothesised that a greater choice of food items at an *ad lib* buffet testmeal would decrease the ability to discriminate between the changes in hunger induced by the two breakfast meals.

## Methods

### Participants

Thirty healthy male participants were recruited from the wider Auckland area through poster, newspaper and electronic advertisement, and all completed the four study days in random order. The mean age was 22 (5 sd) years and mean BMI was 23 (2 sd)  $\text{kg/m}^2$ . Participants came to the appetite research centre at the Auckland Human Nutrition Unit (HNU) as previously described (Lithander et al., 2008; Strik et al., 2010) for screening tests. Body weight, height, waist circumference and blood pressure were measured. Exclusion criteria included self-reported history of overweight or obesity or eating disorders, current dieter or cigarette smoker, as well as hypertension, cardiovascular disease, diabetes mellitus, and any other significant metabolic, endocrine or gastrointestinal disease. None of the participants were taking medications known to affect appetite or weight regulation. Ethical approval for this study was obtained from the Health and Disabilities Ethics (Northern X) Committee, Auckland, New Zealand and written consent to participate was obtained from each of the study volunteers. This study was registered with the Australia New Zealand Clinical Trials Registry (ACTRN1261000971033).

### Study design

This was a four arm, counter-balanced, cross-over study where the effects of a  $L_E$  (0.5 MJ, hunger-inducing) and a  $H_E$  (4.0 MJ, hunger-suppressing) breakfast on visual analogue scales (VAS) and *ad lib* EI at an  $R_I$  or  $M_I$  lunch testmeal was assessed. Hence participants were allocated to receive each of the four treatment arms in randomised order: (i)  $L_E/R_I$ ; (ii)  $L_E/M_I$ ; (iii)  $H_E/R_I$ ; (iv)  $H_E/M_I$ . Each treatment was given on a separate day, and between each study day participants returned home for a minimum 2 day washout period where they were free to resume their usual diet and exercise patterns. Participants were asked to abstain from alcohol,

significant changes in their habitual diet, and strenuous physical activity for 24 h prior to the study-day. In order to encourage compliance on the pre-treatment day participants were asked to record whether they had missed meals, had fewer or greater snacks than usual, whether they had consumed alcohol, and estimate their exercise patterns throughout the day. When patterns of food or exercise were reported to differ from estimated habitual levels on the pre-study day, participants were asked to postpone and reschedule their study visit.

### Procedures

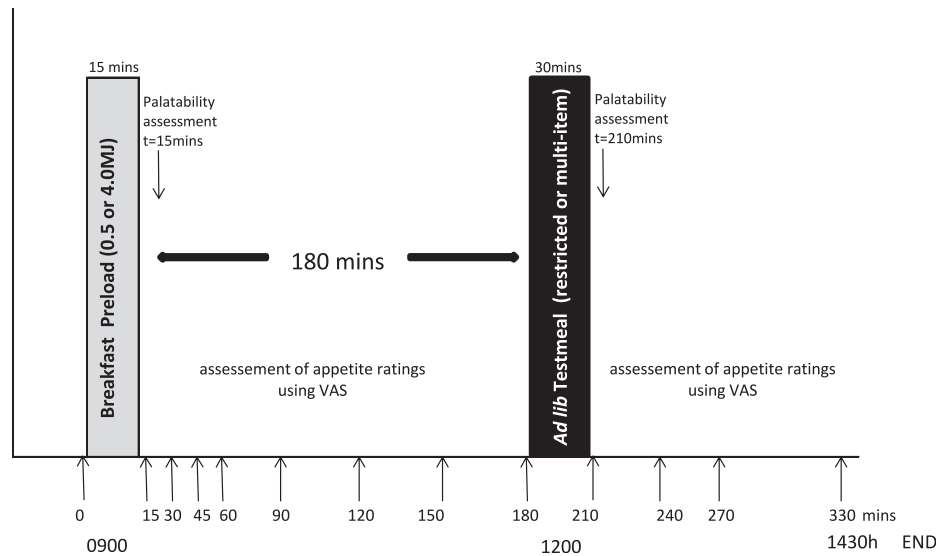
The protocol used in this study was based upon the recent European consensus document which outlines recommendations for postprandial studies assessing appetitive ratings and eating behaviour (Blundell et al., 2010). On each study day participants were asked to fast from 8 pm the previous evening and avoid morning exercise. Upon arrival at the HNU, body weight was measured (Seca, Model 708, Germany), waist circumference recorded (to the nearest 0.1 cm midway between the last rib and the crest of the ileum at the natural point of waist narrowing using a non stretch tape measure) whilst lightly clad, and any adverse events recorded. Height was measured on a single occasion at the screening visit (Seca, Model 222, Germany). The daily study protocol showing the timing of the breakfast and the *ad lib* lunch testmeal is shown in Fig. 1. At 0855 h baseline VAS rating feelings of hunger, fullness, satisfaction and current thoughts of food (TOF) were completed (Flint, Raben, Blundell, & Astrup, 2000). Thirst and nausea were also assessed using VAS. The  $L_E$  or  $H_E$  breakfasts were served at 0900 h with a glass of water (200 mL) and participants were asked to consume each meal in full but at their own pace within 15 min. No further foods were allowed during the morning. A glass of water (200 mL) was given at 1030 h. VAS ratings were measured throughout the morning and for 2 h after completion of the *ad lib* testmeal. At 1200 h, 180 min after the breakfast, either the  $R_I$  or  $M_I$  *ad lib* testmeal was served individually in separate dining rooms. Participants were asked to eat until they felt comfortably full. No distractions such as newspapers, laptop computers or mobile phones were allowed during the 30 min lunch period. Participants remained at the HNU throughout each study day and were allowed to read, use laptop computers or undertake other similar sedentary activities but were not allowed to sleep at any time during the study day. Activity was monitored with the intent of ensuring a highly repeatable, sedentary day on each of the four occasions.

### Breakfast preloads

The two breakfast meals were (i)  $L_E$  (0.5 MJ) which comprised a hazelnut and vanilla yoghurt served with a glass of water, and (ii)  $H_E$  (4.0 MJ) which comprised muesli, milk, toast, jam, butter and a banana served with a glass of water. All foods were weighed to the nearest gram and all items were required to be eaten in full. The energy and macronutrient composition of each of the two breakfast preloads was calculated using the dietary program Food-Works™ (Professional Edition, Version 5, 1998–2007, Xyris Software, Australia, Table 1).

### Visual analogue scales (VAS)

Participants rated their feelings of hunger, fullness, satisfaction and TOF using VAS. The questions asked were “How hungry do you feel?”, “How full do you feel?”, “How satisfied do you feel?” and “How much do you think you can eat now?” Ratings were recorded by placing a vertical line onto 100 mm scales, anchored at either end by statements; “I am not hungry at all/I am not full at



**Fig. 1.** Daily protocol for the study. Participants were given either a 0.5 or 4.0 MJ breakfast preload at 0900 h and a restricted single-item or buffet multi-item testmeal at 1200 h, from which they ate until comfortably full.

**Table 1**  
Energy and macronutrient composition of the two breakfast preloads.

	Low energy preload ( $L_E$ )	High energy preload ( $H_E$ )
Weight (g)	105	721
Energy (kJ)	500	4000
Fat (g)	4	26
Fat (% en)	30	24
Protein (g)	4	29
Protein (% en)	14	12
Carbohydrate (g)	16	146
Carbohydrate (% en)	55	60

% en, % of energy.

all/I am completely empty/nothing at all” on the left and “I am as hungry as I have ever been/I am totally full/I cannot eat another bite/a large amount” on the right. VAS were completed when the participants were fasted prior to the breakfast meals and then at 15, 30, 45, 60, 90, 120, 150, 180 [*ad lib* testmeal], 210, 240, 270 and 330 min after the breakfast was served. Palatability of the breakfast preloads and the testmeal was measured immediately following each respective meal (breakfast,  $t = 15$  mins; lunch,  $t = 210$  min). Participants rated the pleasantness, visual appeal, smell, taste, aftertaste and overall palatability of the meals on separate 100-mm VAS. These questions were anchored on the left by the statements “not at all pleasant (pleasantness)/bad (visual appeal, smell, taste, palatability)/none (aftertaste)” and on the right by the statements “as pleasant as I have ever tasted (pleasantness)/good (visual appeal, smell, taste, palatability)/much (aftertaste)”.

#### *Ad lib testmeal*

The *ad lib* lunch time testmeal comprised either an RI or MI buffet. The  $R_I$  testmeal comprised pasta + meat sauce, whilst  $M_I$  comprised pasta + meat sauce plus sliced bread, chicken, ham, cheese, salad items, cake and tinned peaches. Both meals were served with 1.5 L of bottled water. The participants were advised that they had 30 min for the testmeal, could eat as much or as little as they chose, and should eat until they felt comfortably full. The items presented at the lunch meals with details of serving weight, energy and macronutrient content are shown in Table 2. All discrete items (bread, chicken, ham, cheese, cake, peaches) were presented as small bite

size portions, and all items were served in moderate excess with the intent that participants would not consume the entirety of any single item. All testmeal items were weighed before and after the meal to the nearest 1.0 g (Sartorius AG, Goettingen, Germany), and energy and macronutrient content of the foods consumed were calculated using the dietary program Foodworks. The compensatory decrease in EI at the testmeal (kJ) induced by the greater energy content of the prior  $H_E$  preload (+3500 kJ) was calculated relative to this fixed energy preload and expressed as a percentage, as follows: % compensation =  $[(EI_{L_E \text{ breakfast}} - EI_{H_E \text{ breakfast}}) / 3500] * 100$ . This was calculated for the  $R_I$  and  $M_I$  testmeals separately.

#### *Statistical analyses*

VAS data assessing postprandial feelings of hunger, fullness and other appetite related sensations throughout each study visit were analysed using repeated measures Linear Mixed Model ANOVA (SAS: PROC MIXED, SAS version 9.2, SAS Institute Inc., Cary, NC, USA, 2002–2008). The participant, dietary preload, study period and study day were included in the procedure, in addition to the treatment/time interaction which addressed whether the trajectory over time during the study period differed between the breakfast conditions (diet \* time). Analyses were conducted on untransformed data and the initial baseline measure for each VAS rating was used as co-variate. Incremental area under the curve (iAUC) for repeat measures VAS data was calculated as net change from baseline between 0 and 180 min (iAUC<sub>0–180</sub>) for all parameters (Graphpad Prism version 5.03, La Jolla, CA, USA, 2009). VAS data assessing the palatability of the four breakfast meals at a single time point, and the energy and macronutrient intake data from the *ad lib* testmeals, was also analysed using SAS:PROC MIXED. Where the ANOVA was significant, Tukey's *post hoc* analysis was used for comparisons between conditions. Statistical significance was based on 95% limits ( $P < 0.05$ ).

#### **Results**

Twenty-nine male participants completed all four arms of the study. One participant withdrew. There was no provision of food or supervised exercise restriction on the day prior to each study visit, however estimates of habitual dietary habits (alcohol intake,

**Table 2**  
Energy content and macronutrient composition of foods offered at the *ad lib* testmeals.

Food item	Portion size (g)	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)
<i>Restricted-item (R<sub>I</sub>) lunch</i>					
Meat sauce	1403	5164	90	60	76
Pasta, boiled	1020	5550	45	8	263
Water	1500	0	0	0	0
<i>Multi-item (M<sub>I</sub>) lunch</i>					
Meat sauce	1403	5164	90	60	76
Pasta, boiled	1020	5550	45	8	263
Bread, multigrain	120	1164	11	3	51
Bread, white	120	1260	10	2	58
Chicken, smoked, slices	100	370	19	3	1
Ham, slices	75	329	12	2	5
Cheese, strong cheddar	95	1672	22	35	0
Cheese, mild cheddar	100	1650	23	34	0
Capsicum (red, yellow)	100	146	2	0	6
Cucumber, sliced	70	29	0	0	1
Cake, Madeira	145	2393	8	23	80
Fruit loaf	200	2860	10	23	108
Peaches, tinned	542	976	3	1	52
Margarine	250	6050	0	163	0
Mayonnaise	295	8732	3	233	4
Water	1500	0	0	0	0

CHO, carbohydrate.

number of snacks, missed meals) and physical activity level (time spent standing, sitting, watching TV/computer activities, or engaged in mild-moderate and vigorous-strenuous activity) did not differ between the study treatments ( $P > 0.05$ ).

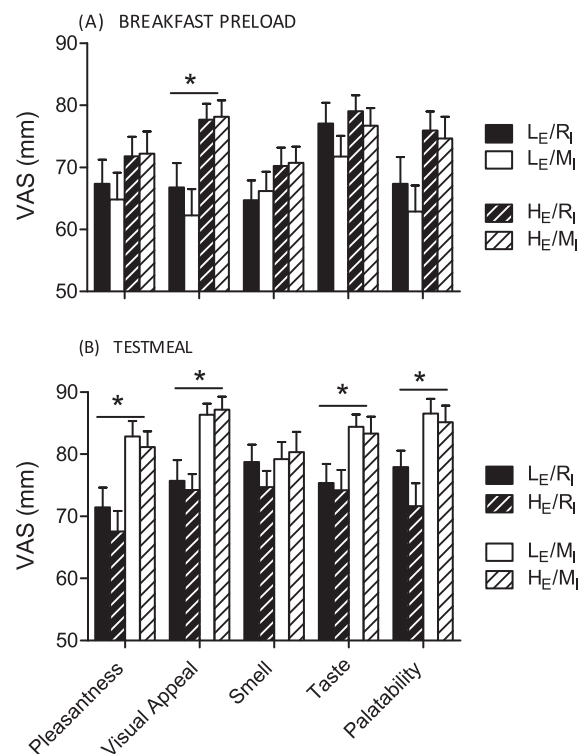
#### Visual analogue scales

##### Palatability

VAS-assessed pleasantness, visual appeal, smell, taste, and overall palatability of the breakfast meals are shown in Fig. 2A, where the two left hand bars for each parameter represent the L<sub>E</sub> breakfasts and the two right hand bars the H<sub>E</sub> breakfasts. There was a trend towards higher ratings following the 4.0 MJ H<sub>E</sub> breakfast for all of the assessed parameters, of which visual appeal was significantly higher ( $P < 0.05$ ). VAS-assessed pleasantness, visual appeal, smell, taste, and overall palatability of the *ad lib* testmeals are shown in Fig. 2B, where the two left hand bars for each parameter represent the R<sub>I</sub> testmeal and the two right hand bars the M<sub>I</sub> testmeal. As expected, pleasantness, visual appeal, taste and palatability were all significantly greater following presentation of the M<sub>I</sub> buffet-style meal (all,  $P < 0.05$ ).

##### Hunger, fullness, satisfaction, TOF

As expected, increasing the energy content of the breakfasts from 0.5 to 4.0 MJ significantly suppressed feelings of hunger and TOF (both, treatment\*time,  $P < 0.01$ ), and significantly enhanced feelings of fullness and satisfaction (both, treatment\*time,  $P < 0.01$ ) over the 3 h following the meal (Fig. 3). Figure 3a–d shows the data presented as change from baseline for VAS-rated hunger, fullness, current TOF and satisfaction during the 3 h following each of the test breakfasts, and also after presentation of the *ad lib* testmeal (at 180 min). The right hand panels (Fig. 3e–h) show the iAUC during the 180 min inter-meal interval between the preload and the testmeal. Baseline measures for all parameters were assessed following an overnight fast and were not significantly different between study days (all,  $P > 0.05$ ) suggesting a similar level of hunger and fullness at the start of each experimental day. As intended during the design of this protocol, there was very little suppression of hunger (Fig. 3e) or enhancement of fullness (Fig. 3f) by the 0.5 MJ L<sub>E</sub> breakfast, as clearly shown in the right hand panels of iAUC. VAS reproducibility was excellent in this trial

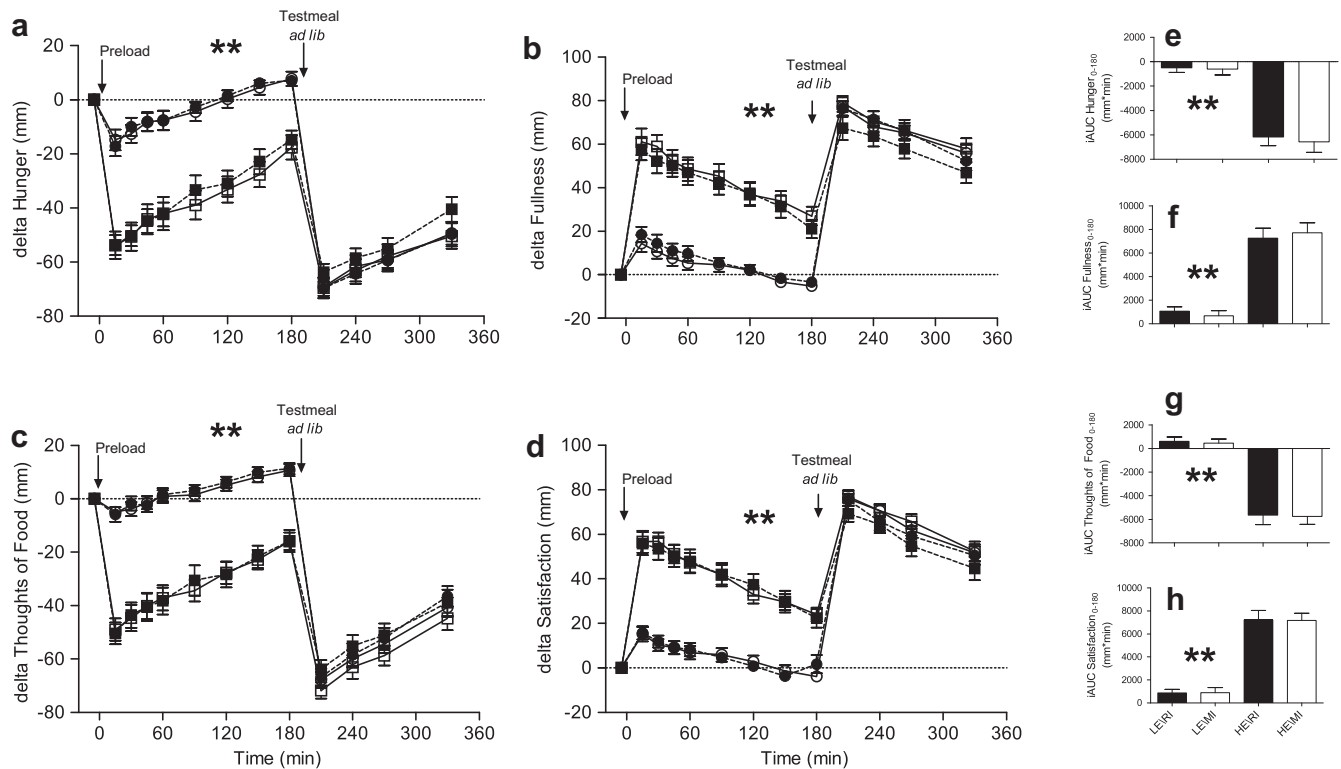


**Fig. 2.** Mean (sem) visual analogue scale (VAS) showing scores for pleasantness, visual appeal, smell, and palatability of the meals. (A) low energy (L<sub>E</sub>, open bars) and high energy (H<sub>E</sub>, hashed bars) breakfast meals (\* $P < 0.05$ , L<sub>E</sub> vs. H<sub>E</sub>); (B) restricted-item (R<sub>I</sub>, black bars) and multi-item (M<sub>I</sub>, white bars) *ad lib* testmeal where the M<sub>I</sub> buffet lunch was rated significantly more pleasant, with better visual appeal, taste and palatability (\* $P < 0.05$ , R<sub>I</sub> vs. M<sub>I</sub>).

as shown by the similarity in VAS parameters when both the two identical L<sub>E</sub> breakfasts (R<sub>I</sub> vs. M<sub>I</sub>,  $P > 0.05$ ) and the two identical H<sub>E</sub> breakfasts (R<sub>I</sub> vs. M<sub>I</sub>,  $P > 0.05$ ) were consumed (Fig. 3a–d).

##### Energy intake at *ad lib* testmeal

EI at the *ad lib* testmeal on each of the four study days is shown in Fig. 4c (right hand panel). As expected, the additional choice of



**Fig. 3.** Mean (sem) visual analogue scale (VAS) showing scores for hunger, fullness, thoughts of food and satisfaction throughout the day as change from baseline. As expected, the 4.0 MJ  $H_E$  breakfast preloads (open and closed squares) suppressed (a) hunger and (c) thoughts of food, and enhanced (b) fullness and (d) satisfaction over the 3 h following the meal. The testmeal was given immediately after VAS assessment at 180 min. Incremental area under the curve between baseline and 180 min (iAUC<sub>0-180</sub>) is shown in the far right panels (e–h).  $L_E/R_I$  (●);  $L_E/M_I$  (○);  $H_E/R_I$  (■),  $H_E/M_I$  (□).  $L_E$ , Low energy breakfast preload;  $R_I$ , restricted-item testmeal;  $H_E$ , High energy breakfast preload;  $M_I$ , Multi-item buffet testmeal. \*\* $P < 0.01$ , treatment\* $\times$ time interaction,  $L_E$  vs.  $H_E$ .

food items presented at the  $M_I$  meal (open bars) led to a higher EI compared to the  $R_I$  meal (shaded bars) following both the  $L_E$  and  $H_E$  breakfasts ( $P < 0.01$ ). Mean intakes were 4075 and 5646 kJ at the  $R_I$  and  $M_I$  testmeals respectively. Increased intake in response to the increased variety and palatability of the meal was observed. Also as expected, increasing the energy content of the breakfast preload from 0.5 ( $L_E$ ) to 4.0 MJ ( $H_E$ ) (Fig. 4a) significantly decreased EI at the *ad lib* testmeal, irrespective of the meal composition Fig. 4b ( $R_I$ :  $L_E$  4566 kJ,  $H_E$  3583 kJ;  $M_I$ :  $L_E$  6142 kJ,  $H_E$  5149 kJ; both  $P < 0.01$ ). However, the compensatory decrease in EI in response to the  $H_E$  breakfast was not significantly greater when the single item  $R_I$  testmeal was given (Fig. 4c ( $R_I$ :  $\Delta = -983$  kJ, 28.1%) than when the  $M_I$  buffet meal was given ( $M_I$ :  $\Delta = -993$  kJ, 28.4%;  $\Delta R_I$  vs.  $\Delta M_I$ ,  $P > 0.05$ ).

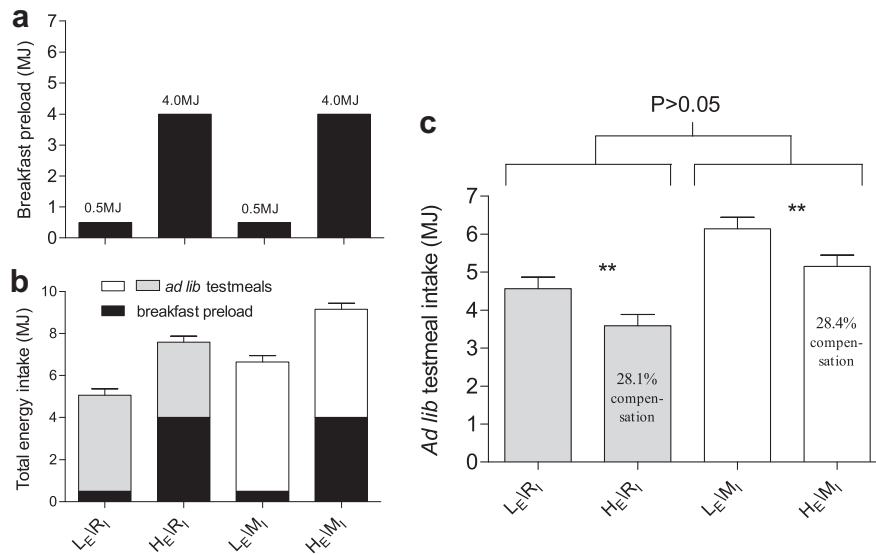
## Discussion

In this study we have shown that presenting a multi-item, palatable, buffet-style testmeal does not significantly decrease the sensitivity of the meal to detect prior changes in hunger and fullness induced through manipulation of a fixed preload, where the parameter of interest is *ad lib* EI. It was notable that the compensatory response to the breakfast preloads occurred despite the predictable energy overconsumption induced by the multi-item buffet. The increase in variety and consequent palatability of the multi-item buffet meal greatly increased *ad lib* EI (+39%) over the 30 min lunch period, yet when comparing the compensatory response to the 0.5 and 4 MJ  $L_E$  and  $H_E$  breakfasts, there was little difference between the single and multi course meals.

Ensuring that the *ad lib* outcome meal is sensitive to manipulations made within the fixed preload test meal is essential in all postprandial appetite studies. As noted by Blundell and colleagues

in their recent methodological review (Blundell et al., 2010), the apparent simplicity of the preload design and its subsequent wide use has led to a large and widely varying literature with limited consensus. The simplicity of the design hides a myriad of decisions that must be made during protocol development, and the need for good execution. Short-term, single day preload studies have become a cornerstone for appetite research driven primarily by their ease of completion and relatively low cost compared with longer-term assessments of food intake or weight loss, which are commonly conducted over days or weeks and require considerably greater resources. As a result, food companies wanting to validate satiety claims for individual foods or nutrients have been turning to the postprandial preload design (Bertenshaw et al., 2008; Burns et al., 2001; Dove et al., 2009; Harper, James, Flint, & Astrup, 2007; Lithander et al., 2008; Poppitt et al., 2011; St-Onge et al., 2004). Clear guidelines are warranted and whilst this has been addressed by some early publications (Stubbs, Johnstone, O'Reilly, & Poppitt, 1998) and further reviewed and updated in a recent ILSI document (Blundell et al., 2010), there remain areas of debate.

In order to generate robust data from preload studies there are a number of important criteria that must be met. Firstly, food intake should be measured within a controlled laboratory setting. Whilst conducting appetite studies in a natural 'free-living' setting, more pertinent to normal feeding behaviour would of course be preferable, the methodological problems associated with this inevitably limit the conclusions that can be drawn (Stubbs, Johnstone, O'Reilly, & Poppitt, 1998). Many studies present food intake data which is, at least in part, obtained from dietary reporting, and this should be regarded with considerable caution since both the poor quality and misleading nature of such data has been known for many years (Subar et al., 2003). Since eating is a behavioural activity, it is important to also realise that it can be readily influenced by social



**Fig. 4.** Mean energy intake showing (a) the fixed preloads at breakfast, (b) total energy intake from the preloads plus the *ad lib* testmeal (mean, sem), and (c) partial compensation for the  $H_E$  preload at the testmeal (mean, sem). The  $H_E$  preload suppressed intake at both the  $R_i$  (28.1% compensation) and  $M_i$  (28.4% compensation) testmeals, and there was no evidence that the  $R_i$  testmeal was more sensitive to the breakfast manipulation ( $P > 0.05$ ).  $L_E$ , Low energy breakfast preload;  $R_i$ , Restricted-item testmeal;  $H_E$ , High energy breakfast preload;  $M_i$ , Multi-item, buffet testmeal. \*\* $P < 0.01$ ,  $L_E$  vs.  $H_E$ .

environment and that careful experimental control is warranted for these studies. Collecting intake data from ‘free-living’ individuals who may be eating alone, with friends, with strangers, at home, in a restaurant, in a fast food outlet, whilst undertaking physical work, whilst relaxing, whilst watching TV, are examples of only a few of the myriad of possibilities that may be encountered if EI is assessed outside the laboratory. Allowing participants to leave the research unit during the inter-meal interval between preload and *ad lib* outcome meal should also be avoided since compliance to the protocol (i.e. no other foods or beverages) cannot be confirmed. Since preload studies are commonly small sample studies, it is also preferable to conduct a within-subject repeated measures design.

Review of the literature shows the composition of the outcome meal used to measure energy or nutrient intake in preload studies varies widely between research groups. This lack of consensus has been considered problematic since variation in this *ad lib* meal may potentially influence the primary outcome of the study. Unlike VAS measures of subjective appetite-related sensations which, providing they are administered correctly at regular fixed intervals postprandially, are not influenced by study design, the composition of the outcome meal has the potential to greatly bias the outcome of the trial. Nutrient intake in food choice studies is clearly better addressed by presenting a multi-item buffet meal (Blundell et al., 2010) which allows the participant to make wide choices from foods which differ in energy, energy density and macronutrient content. Multi-item buffet style meals increase the variety of items offered which in turn characteristically promotes greater intake from the meal (Hetherington et al., 2006; Mok, 2010; Raynor & Epstein, 2001; Rolls, VanDuijvenvoorde, & Rolls, 1984; Rolls et al., 1981). Whether such a variable item buffet meal may decrease the sensitivity to detect changes made to a preload when EI is the primary outcome was not previously known, and our current study has shown that, at least in lean men, this does not occur.

In conclusion, in studies using a preload design to assess appetite regulation, increasing the number of items presented within an *ad lib* testmeal may not significantly decrease the sensitivity of the meal to detect changes in hunger and fullness when the primary outcome is EI. Multi-item buffet meals, even those presenting

commonly eaten, moderately palatable items, do however engender some overconsumption compared with restricted, single choice meals. When designing preload studies to measure EI both single-item or multi-item meals may be appropriate to assess changes in eating behaviour, providing that the studies are well designed and conducted.

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