Original Article
CLINICAL TRIALS AND INVESTIGATIONS

Obesity

Effect of Reducing Portion Size at a Compulsory Meal on Later Energy Intake, Gut Hormones, and Appetite in Overweight Adults

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Objective: Larger portion sizes (PS) are associated with greater energy intake (EI), but little evidence exists on the appetitive effects of PS reduction. This study investigated the impact of reducing breakfast PS on subsequent EI, postprandial gastrointestinal hormone responses, and appetite ratings.
Methods: In a randomized crossover design ($n = 33$ adults; mean BMI 29 kg/m^2), a compulsory breakfast was based on 25% of gender-specific estimated daily energy requirements; PS was reduced by 20% and 40%. EI was measured at an *ad libitum* lunch (240 min) and snack (360 min) and by weighed diet diaries until bed. Blood was sampled until lunch in 20 participants. Appetite ratings were measured using visual analogue scales.
Results: EI at lunch (control: 2,930 ± 203; 20% reduction: 2,853 ± 198; 40% reduction: 2,911 ± 179 kJ) and over the whole day except breakfast (control: 7,374 ± 361; 20% reduction: 7,566 ± 468; 40% reduction: 7,413 ± 417 kJ) did not differ. Postprandial PYY, GLP-1, GIP, insulin, and fullness profiles were lower and hunger, desire to eat, and prospective consumption higher following 40% reduction compared to control. Appetite ratings profiles, but not hormone concentrations, were associated with subsequent EI.
Conclusions: Smaller portions at breakfast led to reductions in gastrointestinal hormone secretion but did not affect subsequent energy intake, suggesting small reductions in portion size may be a useful strategy to constrain EI.

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Introduction

Concurrent with the increasing prevalence of obesity has been the increased mass of food consumed per eating occasion (1) and the increased size of commercially available portions (2). Empirical evidence shows larger portion sizes (PS) lead to greater energy intake (EI) at single meals, an effect that continues through 11 days of manipulation (3,4). Reducing PS is a central component in weight management advice, but experimental work to investigate whether PS reduction leads to changes in subsequent EI is limited. Given that there are strong homeostatic mechanisms to guard against low energy intakes (5), energy compensation may occur in an environment where food is widely available. Gastrointestinal hormones and the perception of appetite are important biological and psychological appetitive mechanisms. There has been little simultaneous research of these mechanisms and energy intake responses following modest portion size manipulations of mixed meals.

Moreover, most research in relation to the effect of portion size on intake has been conducted among individuals with a healthy weight where appetite control systems may be at their most robust. In contrast, overweight and obese individuals are poorly studied yet represent the population most in need of interventions to constrain energy intake. This study investigated whether reducing the PS of a compulsory meal is an effective strategy to reduce day-long EI in overweight and obese adults. This study also investigated the impact on gastrointestinal hormones and appetite ratings as measures of biological and psychological appetite control mechanisms.

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Methods

Study design

The study was a randomized crossover design involving three PS conditions, presented to each participant at a standardized breakfast time on separate days: a control PS; PS reduced by 20%; and PS reduced by 40%. The control provided 25% of estimated daily energy requirements for the intended average study participant according to gender (6), the energy content of a representative main meal. The test breakfast was consumed in its entirety. Participants were blinded to the specific aims of the study and foods prepared to draw attention away from the intervention. For each individual, study visits were conducted >1-week apart, on the same weekday and outside the luteal phase of the menstrual cycle for females.

Participants

Healthy, 18-60 year men and women, with a BMI $\geq$25 and <35 kg/m^2 were recruited. Participants were excluded for disordered eating [Eating Attitudes Test (EAT-26) score $\geq$11 (7,8)], depressive symptoms [Zung Depression Scale score $\geq$70 (9)], smoking, excessive habitual alcohol intake (women > 14 units/week, men >21 units/week), weight loss/gain within last 3 months (>4.5 kg) or actively trying to lose/gain weight, medical conditions/medications potentially affecting appetite, inflammatory conditions, diabetes or fasting plasma glucose $\geq$7 mmol/L, pregnancy, breastfeeding or planning pregnancy, extremely high levels of exercise [moderate/vigorous activity >420 min/week assessed with International Physical Activity Questionnaire (IPAQ) (10)], unable to eat test foods, and not regularly consuming breakfast (breakfast $\leq$3/week).

A sample size of 33 was recruited to give 83% power to detect a minimum difference of 500 kJ lunch EI between any pair of experimental conditions assuming an SD of 950 kJ (3,11,12). Biochemical measures were collected in a subgroup of 20 participants.

Recruitment and screening

Participants were recruited from the community, for a study investigating the "relationship between diet and metabolism." Height, weight, waist circumference, body composition (Tanita body composition analyzer BC-418MA), and resting metabolic rate (RMR; IS Gem204 with GEMNutrition 2008.4 software) were measured. Participants completed the EAT-26, Zung depression scale, IPAQ and the Three Factor Eating Questionnaire measuring dietary restraint, disinhibition, and hunger traits (13), and fasting plasma glucose was assessed. Participants were asked to maintain their usual exercise and dietary habits during the study.

Study visits

Participants fasted overnight (11 h prior to each visit) and were asked to refrain from alcohol and avoid strenuous exercise the 24 h before each study day. Provision of the test breakfast marked time zero. Subsequent EI was measured by premeal and postmeal weighing of an *ad libitum* lunch (240 min) and afternoon snack (360 min), plus a weighed diet diary to record the remainder of the day's intake. Visual analogue scale (VAS) questionnaires rating palatability and portion size were given after the first bite of food at breakfast and lunch. Appetite ratings were measured using VAS questionnaires at 30-min intervals until lunch, then immediately after and at 300 and 360 min, then hourly. In a subgroup of 20 participants, blood samples were collected at fasting and 30, 60, 120, 180, and 240 min for the analysis of peptide tyrosine tyrosine 3-36 (PYY_{3-36}), total glucagon-like peptide-1 (GLP-1), total glucose-dependent insulinotropic peptide (GIP), glucose, and insulin (Figure 1).

At the end of the study participants were fully debriefed on the study aims and asked to verbally report if they had noticed the portion size manipulation between visits. They were reimbursed for travel expenses and given an honorarium. Ethical approval for the study was obtained from Cambridgeshire 2 Research Ethics Committee in November 2010 (Ref: 10/H0308/99) and participants gave informed written consent. The study was conducted at Medical Research Council Human Nutrition Research (MRC HNR) between January 2011 and September 2012.

Study foods

The breakfast consisted of a wheat-based breakfast cereal with semi-skimmed milk, scrambled egg, ham, brown toast and butter, and orange juice. For men and women respectively, the control breakfast provided 3,310 and 2,540 kJ; the 20% reduction breakfast provided 2,650 kJ and 2,030 kJ; the 40% reduction breakfast provided 1,990 and 1,520 kJ. The *ad libitum* lunch consisted of a single course amorphous meal of pasta, minced beef, tomato sauce, mixed vegetables, and grated cheese. The lunch provided 8,275 kJ (men) or 6,350 kJ (women). The study breakfast and lunch provided 35% energy from fat, 18% from protein and 47% from carbohydrates (14). The *ad libitum* snack consisted of ten digestive biscuits (plain cookies) on a plate (3,250 kJ).

The completed diet diaries and weighed consumption at lunch and snack for each study day were used to calculate energy intake using MRC HNR's dietary assessment system (15).

Questionnaires

The appetite VAS questionnaires rated hunger, fullness, desire to eat and prospective consumption, and also included five distractor questions on mood. The palatability questionnaire used VAS to rate palatability parameters, as distractor questions, and portion size. The VAS questionnaires asked participants to mark a horizontal line measuring 100 mm with the ends labelled with the extremes of each sensation (e.g. "Not at all" and "Extremely"). The portion size question was "How large is the size of the portion?" anchored with "Extremely small" and "Extremely large." The distance from the left end to the mark was measured to the nearest millimetre.

Analytic methods

Blood samples were separated on collection and plasma stored at −80 °C. Plasma collected on EDTA and treated with dipeptidyl peptidase-IV (DPP-IV) inhibitor immediately on collection (10 μL DPP-IV inhibitor/ml of blood) was analyzed for PYY_{3-36} by radioimmunoassay (Millipore®, MA) (interassay CVs: 15% at 84 pg/mL; 7% at 217 pg/mL), at University College Hospital, London; total GLP-1 using an electrochemical luminescence immunoassay kit on the MesoScale Discovery® multiarray assay platform (MD, USA) (CVs: 16.4%, 11.9% and 11.6% at 5.4, 29, and 83 pg/mL, respectively), at Core Biochemical Assay Laboratory (CBAL), Cambridge; and total GIP using an enzyme-linked immunosorbent assay (Millipore®, MA) (CVs: 6.1, 3.3, 2.3, and 1.8% at 26, 50, 134, and 166

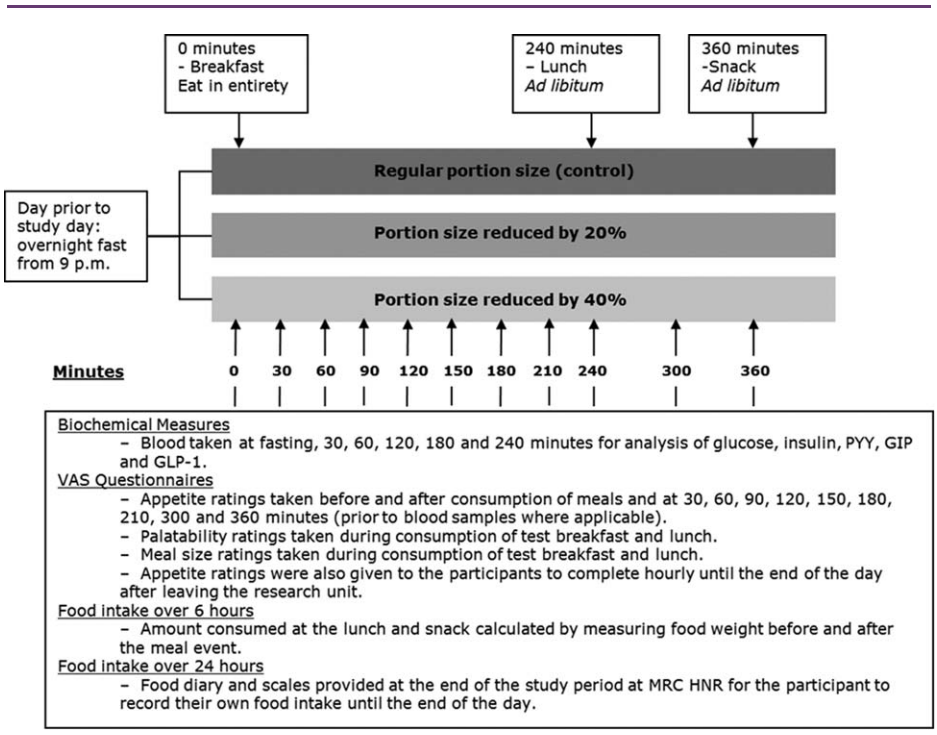

Figure 1 Overview of the time points for meals and measurements taken during a study day. GIP: glucose-dependent insulinotropic peptide; GLP-1: glucagon-like peptide 1; MRC HNR: Medical Research Council Human Nutrition Research; PYY: peptide tyrosine tyrosine; VAS: visual analogue scales.

pg/mL, respectively), at Cambridge Institute for Medical Research. Plasma collected on fluoride oxalate was analyzed for glucose using a Dimension® clinical chemistry system (Siemens, NE) (CVs: 1.69, 2.23, and 2.56% at 6.23, 3.09, and 18.88 mmol/L respectively), at MRC HNR. Plasma collected on lithium heparin was analyzed for insulin on a 1235 AutoDELFIA® automatic immunoassay analyzer using a two-step time resolved fluorometric assay (Perkin Elmer Life Sciences, Wallac Oy, Turku, Finland) (CVs: 3.1, 2.1, 1.9, and 2.0% at 29, 79.4, 277, and 705 pmol/L, respectively) at CBAL, Cambridge.

Statistical analysis

Mixed effects models for continuous responses (16) were used for analysis, which extend standard linear regression to account for correlation between repeated observations within individuals through random effects. EI and PS ratings at breakfast were modeled with PS condition as the explanatory variable, controlling for gender and BMI. Dietary restraint, disinhibition and hunger, were tested for inclusion as covariates, but were omitted for no effects on the associations of interest.

The effect of PS condition on biochemical measures and appetite ratings was assessed by the interaction between condition and time, which estimated differences at each time point. Area under the curve (AUC) was calculated using the trapezoidal rule for the time periods of fasting to the prelunch time point for biochemical measures and appetite ratings, and over the whole day for appetite ratings. Models of whole-day appetite ratings AUC included PS condition as the explanatory variable, controlling for the time over which appetite ratings were made.

Models predicting EI at lunch included explanatory variables of either the prelunch or AUC for each biochemical measure or appetite rating, and controlled for condition, gender and BMI. Similar models assessed the relationship between the whole-day AUC of appetite rating with whole day EI (except breakfast), also controlling for time over which appetite ratings were made.

To examine the relationship between biochemical measures and appetite ratings, appetite ratings were modeled separately with each biochemical measure as the explanatory variable. Time, a quadratic term for time, condition, gender, and BMI were covariates.

Potential carry-over and sequence effects, gender, BMI and age, unless specified above as included *a priori*, were omitted as covariates as there were no effects on the associations of interest. To account for correlation induced by multiple observations/individuals (three visits), a random intercept was incorporated into the models. The models for biochemical and appetite ratings profiles as outcomes had two levels of clustering due to repeated sampling time points and the crossover design. Therefore, a random intercept and slope for time were added to model within-individual variation. Models were fitted using maximum likelihood estimation and likelihood ratio tests were used for model comparison. Plots of residuals were used to check the goodness of fit for each outcome. Insulin and GIP data were transformed (natural logarithm and square root respectively) for analyses, for a symmetrical distribution. All analyses used STATA®12.0 software (StataCorp, TX). Statistical

TABLE 1 Participant characteristics

	All participants ($n = 33$)	Blood sample subgroup ($n = 20$)	Nonblood subgroup ($n = 13$)
Number of men/women	15/18	9/11	7/6
Height (m)	1.69 ± 0.01	1.69 ± 0.01	1.71 ± 0.03
Weight (kg)	83.8 ± 1.5	82.9 ± 2.1	85.3 ± 2.0
BMI (kg/m^2)	29.0 ± 0.4	29.0 ± 0.5	29.2 ± 0.8
Age (years)	42.5 ± 2.0	40.8 ± 2.5	45 ± 3.4
Dietary restraint	7.2 ± 0.7	6.5 ± 0.9	8.2 ± 1.1
Disinhibition	6.7 ± 0.6	6.5 ± 0.7	6.9 ± 1.1
Hunger trait	6.3 ± 0.7	6.2 ± 0.8	6.5 ± 1.1
RMR (kJ/day)	6594 ± 160	6704 ± 224	6425 ± 220
Fasting glucose (mmol/L)	4.8 ± 0.1	4.7 ± 0.1	4.9 ± 0.1
Body fat (%)	32.8 ± 1.5	31.9 ± 1.8	34.2 ± 2.6
Vigorous physical activity (min per week)	65 ± 13	55 ± 14	80 ± 24
Moderate physical activity (min per week)	142 ± 21	173 ± 29	94 ± 26
Walking (min per week)	254 ± 30	270 ± 37	231 ± 53

Mean ± SEM.
BMI: body mass index; RMR: resting metabolic rate.

significance was set at $P < 0.05$. Data are presented as mean ± SEM unless indicated otherwise.

Results

Participant characteristics

The characteristics of the study participants are shown in Table 1.

Energy intake

EI was not different between conditions at lunch (control: 2,930 ± 203; 20% reduction condition: 2,853 ± 198; 40% reduction condition: 2,911 ± 179 kJ) or over the whole day except breakfast (control: 7,374 ± 361; 20% reduction condition: 7,566 ± 468; 40% reduction condition: 7,413 ± 417 kJ) (Figure 2). Daily EI including breakfast was 10,287 ± 395 kJ, 9,897 ± 491 kJ, and 9,161 ± 437 kJ in control, 20% reduction and 40% reduction conditions respectively.

Biochemical measures

Figure 3 shows the postprandial profiles for each of the gastrointestinal hormones. Compared to control, there was a reduction in PYY in 40% reduction condition at 120 and 240 min ($P < 0.05$), but no condition-time interaction for 40% reduction compared to 20% reduction condition ($P > 0.076$), or 20% reduction compared to control ($P > 0.42$). Compared to control, GLP-1 was lower in 40% reduction condition at all postprandial time points ($P < 0.05$). GLP-1 was also lower in 40% reduction condition compared to 20% reduction at 180 min ($P = 0.038$), but there was no condition-time interaction for 20% reduction condition compared to control ($P > 0.056$). GIP was lower in 40% reduction condition compared to control at all postprandial time points ($P < 0.001$), and compared to 20% reduction condition at 30, 120, 180, and 240 min ($P < 0.05$). GIP

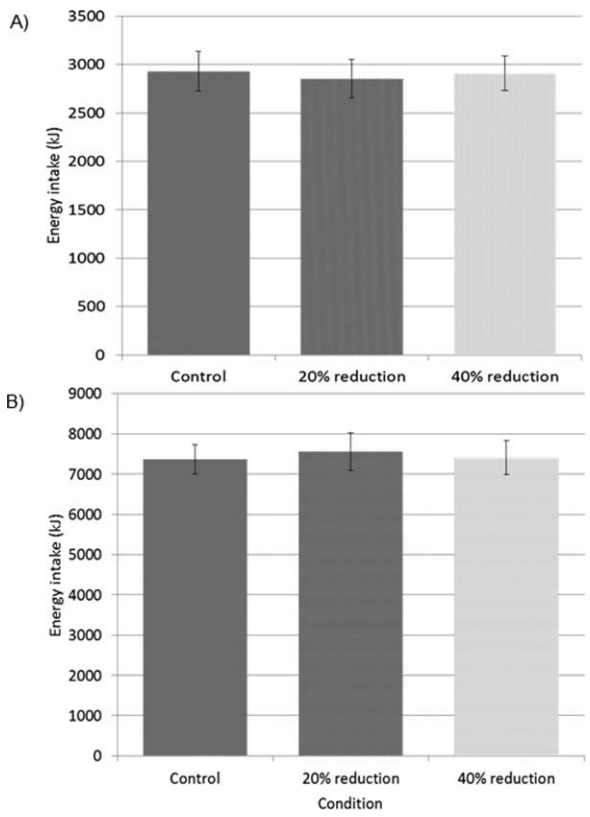

Figure 2 Mean (± SEM) energy intake (**A**) at lunch (control vs. 20% reduction, $\beta = -76.6$, $P = 0.429$; 20% reduction vs. 40% reduction, $\beta = 58.2$, $P = 0.547$; control vs. 40% reduction, $\beta = -18.3$, $P = 0.850$) and (**B**) over the whole day except breakfast (control vs. 20% reduction, $\beta = 192.3$, $P = 0.555$; 20% reduction vs. 40% reduction, $\beta = -152.8$, $P = 0.639$; control vs. 40% reduction, $\beta = 39.5$, $P = 0.904$) did not differ between conditions.

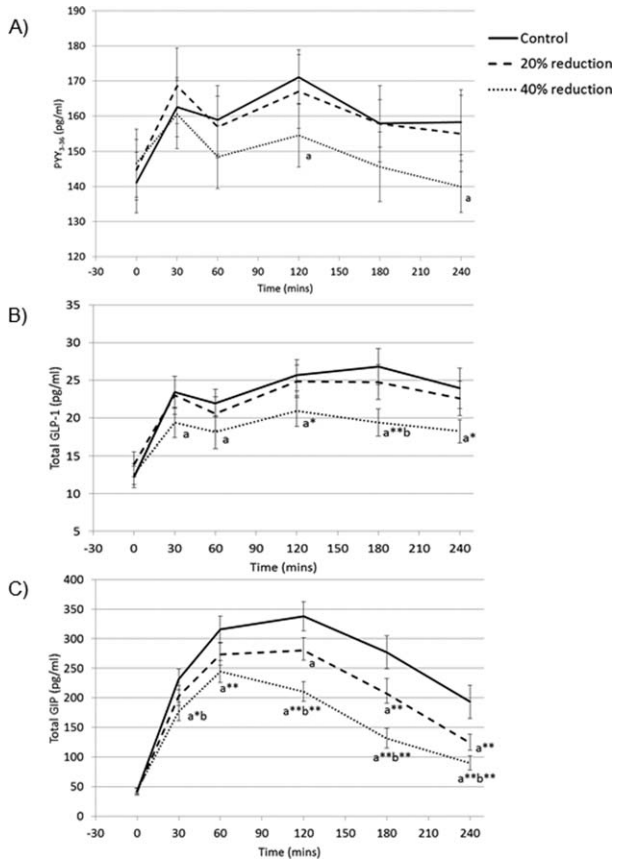

Figure 3 Postprandial response (mean ± SEM) of (**A**) plasma $PYY_{3\text{-}36}$, (**B**) plasma total GLP-1, and (**C**) plasma total GIP, according to condition. "a" indicates the mean of the condition is significantly different from the mean of the control condition at that time point. "b" indicates the mean is significantly different from the mean of the 20% reduction condition at that time point (mixed effects models): $P < 0.05$. Addition of * to the letter indicates $P < 0.01$ and ** indicates $P < 0.001$.

was lower at 120, 180, and 240 min in 20% reduction condition compared to control ($P < 0.05$).

Glucose and insulin profiles are shown in Figure 4. There was no condition-time interaction for glucose ($P > 0.2$ for all comparisons). There was a condition-time interaction such that insulin was less in 40% reduction condition compared to control and 20% reduction condition at 120 and 180 ($P < 0.05$), and compared to control at 240 min ($P < 0.01$). There was no condition-time interaction for 20% reduction condition compared to control ($P > 0.083$).

Appetite ratings

Figure 5 shows the appetite ratings. Hunger was greater after breakfast in 40% reduction condition compared to control ($P < 0.006$) and to 20% reduction condition ($P < 0.021$). There was no condition-time interaction for 20% reduction condition compared to control ($P > 0.291$). There were some differences in postprandial fullness between conditions whereby fullness was lower in 40% reduction condition compared to control ($P < 0.019$). However there were fewer differences when compared to 20% reduction condition, and similarly in 20% reduction condition compared to control. Desire to eat ratings were generally greater in 40% reduction condition compared to control ($P < 0.023$) and compared to 20% reduction condition ($P < 0.037$). There was no condition-time interaction for 20% reduction condition compared to control ($P > 0.223$). Prospective consumption was greater in 40% reduction condition compared to control ($P < 0.011$) but there were fewer differences when compared to 20% reduction condition. There was no condition-time interaction for 20% reduction condition compared to control ($P > 0.068$).

AUCs over the whole day for hunger, desire to eat and prospective consumption were greater in 40% reduction condition compared to control, and smaller for fullness (hunger $\beta = 2423.9$, $P = 0.025$; fullness $\beta = -4857.9$, $P = 0.001$; desire to eat $\beta = 3832.5$, $P = 0.001$; prospective consumption $\beta = 3427.9$, $P = 0.001$). AUC for prospective consumption ratings was greater in 20% reduction condition compared to control ($\beta = 2284.1$, $P = 0.025$), but AUC for hunger ($P = 0.232$), fullness ($P = 0.136$), and desire to eat ($P = 0.118$) did not differ. There were no differences in hunger or fullness when comparing 20 and 40% reduction conditions (data not shown).

Predictors of energy intake

Most of the biochemical measures did not predict EI at lunch ($P > 0.137$) (Table 2). However, AUC ($P = 0.032$) and prelunch ($P = 0.049$) measures of PYY were positively associated with EI at lunch. AUCs and prelunch measures of hunger, desire to eat and prospective consumption were positively associated with lunch EI ($P < 0.02$). Prelunch fullness was negatively associated with lunch

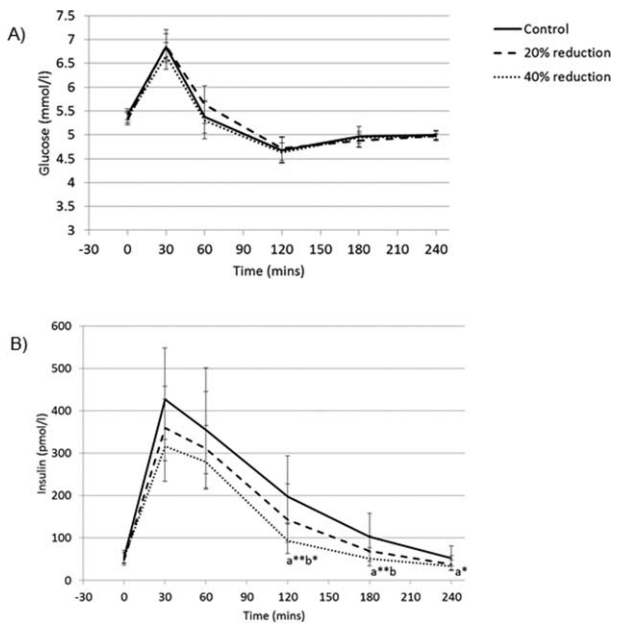

Figure 4 Postprandial response of (**A**) plasma glucose (mean ± SEM) and (**B**) plasma insulin (geometric mean ± 95% confidence intervals), according to condition. "a" indicates the mean of the condition is significantly different from the mean of the control condition at that time point. "b" indicates the mean is significantly different from the mean of the 20% reduction condition at that time point (mixed effects models): $P < 0.05$. Addition of * to the letter indicates $P < 0.01$ and ** indicates $P < 0.001$.

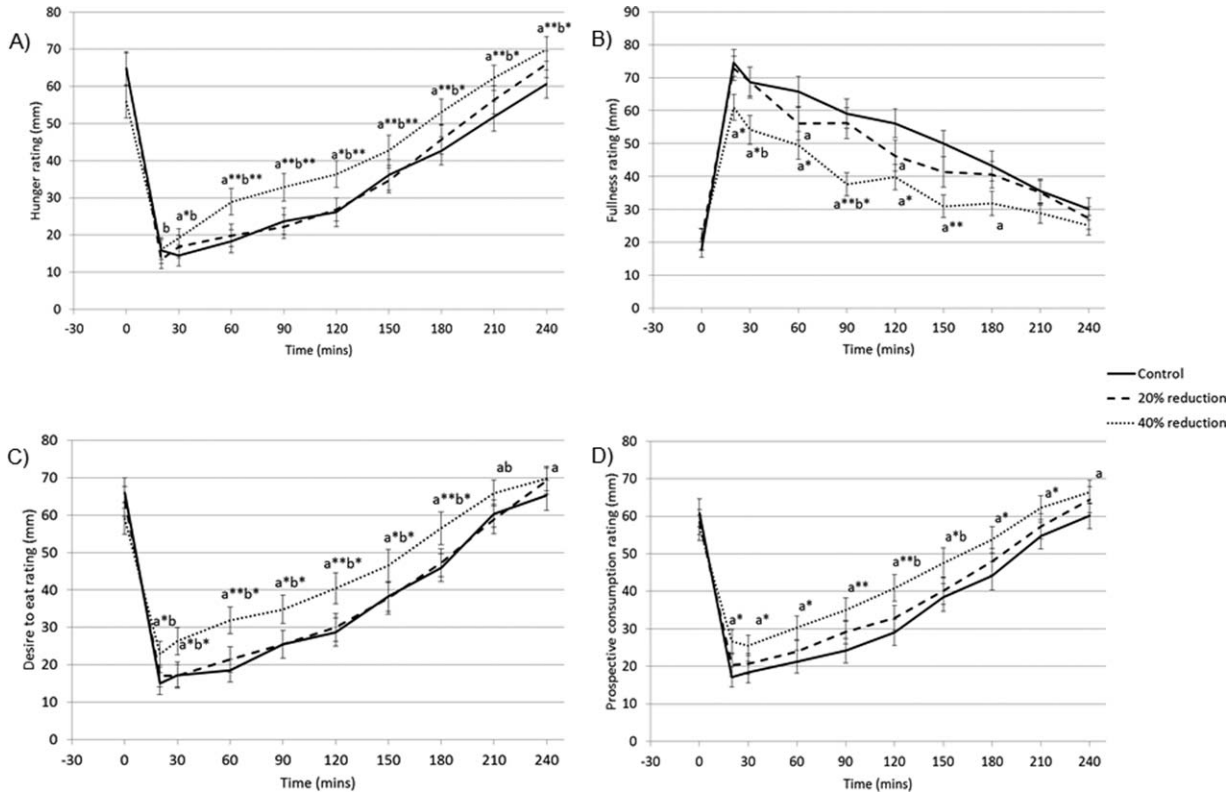

Figure 5 Postprandial ratings (mean ± SEM) for (**A**) hunger, (**B**) fullness, (**C**) desire to eat, and (**D**) prospective consumption, according to condition. "a" indicates the mean of the condition is significantly different from the mean of the control condition at that time point. "b" indicates the mean is significantly different from the mean of the 20% reduction condition at that time point (mixed effects models): $P < 0.05$. Addition of * to the letter indicates $P < 0.01$ and ** indicates $P < 0.001$.

EI ($P < 0.002$), but fullness AUC was not ($P = 0.085$). AUCs for hunger, desire to eat and prospective consumption, but not fullness ($P = 0.469$), were positively associated with EI over the day ($P < 0.026$).

Biochemical measures and appetite ratings associations

GLP-1, GIP, glucose and insulin were negatively associated with hunger, desire to eat, and prospective consumption, and positively associated with fullness ($P < 0.012$). PYY was not associated with any of the appetite ratings ($P > 0.068$) (Table 3).

Perceived portion size

The ratings of portion size at breakfast were different between conditions. Portion size was perceived to be smaller in 40% reduction condition compared to both control ($\beta = -15.6$, $P < 0.001$) and 20% reduction condition ($\beta = -10.8$, $P < 0.001$), and portion size was perceived to be smaller in 20% reduction condition compared to control ($\beta = -4.8$, $P = 0.049$) (portion size rating: control 53 ± 2 mm; 20% reduction 48 ± 3 mm; 40% reduction 37 ± 3 mm). However at debriefing only two participants specifically reported noticing the change in PS at breakfast. None of the participants were concerned about the blinding of the aims of the study and all consented to data inclusion.

Discussion

Reducing PS at a single meal altered biological markers of appetite and appetite ratings, but there was no energy compensation later in the day. EIs at lunch were strikingly consistent in this standardized laboratory setting. These findings indicate reducing PS of a prepared meal could lead to a net reduction in daily EI. However, the effect on gastrointestinal hormones and appetite ratings, particularly after the 40% reduction in PS, questions the sustainability of this strategy to constrain EI.

There were very few differences in the profiles for PYY and GLP-1 between control and 20% reduction conditions. Moreover, there were few differences in the profiles when comparing 20 and 40% reduction conditions suggesting that the responses in these biochemical measures may not be sensitive to the smaller change in PS (660 kJ men and 510 kJ women). Indeed, all previous studies where a reduction in energy load has led to attenuated PYY (17,18), GLP-1 (19,20), or insulin (21,22) profiles, used energy changes between 920 and 2,096 kJ. However, the present study showed distinct differences between all conditions in the postprandial profiles for GIP showing that it is sensitive to energy changes in a clear dose response manner, reflecting its important role as an incretin hormone for the regulation of insulin secretion.

Interestingly, the ratings of perceived PS of the breakfast were different between conditions, although at debriefing most participants

TABLE 2 Estimated regression coefficients to measure associations between biochemical measures and appetite ratings (predictor variables) with energy intake at lunch and over the whole day apart from breakfast (outcome variables), from mixed effects models

	AUC as predictor		Prelunch measure as predictor	
Predictor of lunch EI	Regression coefficient (SE)	*P* value	Regression coefficient (SE)	*P* value
Biochemical measure				
PYY	**0.029 (0.014)**	**0.032**	**4.442 (2.257)**	**0.049**
GLP-1	0.019 (0.071)	0.790	15.95 (11.17)	0.154
GIP	2.666 (4.446)	0.549	39.08 (33.06)	0.237
Glucose	−0.916 (0.710)	0.197	−365.5 (245.8)	0.137
Insulin	−197.0 (259.8)	0.448	−157.0 (168.7)	0.352
Appetite rating				
Hunger	**0.091 (0.022)**	**<0.001**	**11.96 (3.934)**	**0.002**
Fullness	−0.029 (0.017)	0.085	**−10.43 (3.389)**	**0.002**
Desire to eat	**0.087 (0.018)**	**<0.001**	**8.788 (3.783)**	**0.020**
Prospective consumption	**0.100 (0.022)**	**<0.001**	**19.21 (4.384)**	**<0.001**
Predictor of whole day EI	**Regression coefficient (SE)**	***P* value**		
AUC appetite rating				
Hunger	**0.057 (0.025)**	**0.026**		
Fullness	−0.016 (0.021)	0.469		
Desire to eat	**0.057 (0.023)**	**0.013**		
Prospective consumption	**0.068 (0.025)**	**0.007**		

AUC: area under the curve. EI: energy intake. SE: standard error.
Area under the curve was calculated for between the fasting and prelunch time points for predicting energy intake at lunch. Area under the curve for the whole day was calculated for predicting energy intake over the whole day apart from breakfast. Each predictor was analyzed in a separate mixed effects model.
Values are given to four significant figures. Those in bold are significant.

reported not noticing the meal manipulation. The effect size for the difference between perceived PS ratings was considerably smaller when comparing control versus 20% reduction than 20% versus 40% reduction conditions ($\beta = -4.8$; $\beta = -10.8$), although the absolute difference in energy was the same. This difference is likely due to either the relative difference between PS being different (20% for control-20% reduction comparison, and 25% for 20-40% reduction comparison), or due to the Weber-Fechner law, whereby the ability to

TABLE 3 Estimated regression coefficients to measure associations between biochemical measures (predictor variables) and appetite ratings (outcome variables) from baseline to the prelunch time point, from mixed effects models

	Appetite rating							
	Hunger		Fullness		Desire to eat		Prospective consumption	
Biochemical measure	Regression coefficient (SE)	*P* value	Regression coefficient (SE)	*P* value	Regression coefficient (SE)	*P* value	Regression coefficient (SE)	*P* value
PYY	−0.032 (0. 038)	0.409	0.041 (0.041)	0.315	−0.018 (0.040)	0.650	−0.028 (0.031)	0.366
GLP-1	**−0.494 (0.172)**	**0.004**	**0.631 (0.186)**	**0.001**	**−0.442 (0.176)**	**0.012**	**−0.421 (0.138)**	**0.002**
GIP	**−3.271 (0.373)**	**<0.001**	**3.357 (0.416)**	**<0.001**	**−3.143 (0.379)**	**<0.001**	**−2.629 (0.305)**	**<0.001**
Glucose	**−6.650 (1.058)**	**<0.001**	**6.058 (1.186)**	**<0.001**	**−5.493 (1.087)**	**<0.001**	**−4.396 (0.884)**	**<0.001**
Insulin	**−14.07 (1.227)**	**<0.001**	**14.33 (1.391)**	**<0.001**	**−13.63 (1.250)**	**<0.001**	**−11.86 (0.990)**	**<0.001**

SE: standard error.
Each predictor was analyzed in a separate mixed effects model.
Values are given to 4 significant figures. Those in bold are significant.

perceive stimulus change is proportional to the logarithm of the magnitude of the stimulus (23). Thus, as the reference portion size in the first comparison (control versus 20% reduction) was larger than the second (20% versus 40% reduction), the change in PS detectable for the first pairing would have been larger than the second. It is possible that the perception of how much is provided, and thus consumed, could affect appetite ratings. The smaller effect size of perceived PS between control and 20% reduction conditions could in part account for fewer differences in appetite ratings between these conditions.

Postprandial biochemical responses were poor predictors of subsequent EI, consistent with much of the existing evidence (24-26). However, appetite ratings tended to predict EI at lunch and the rest of the day. This is in agreement with some (12,27-29), but not all (30,31), previous studies. The mixed evidence likely reflects the subjective nature of the perception of appetite which leads to measurement variability, but differences are more easily detected in crossover than parallel design studies (32). Although associations between appetite ratings and EI in the present study were highly significant, the effect sizes were small. This, coupled with relatively small differences in postprandial appetite ratings responses to the manipulated meal, could in part explain the lack of compensation for the changes in energy. In contrast with the known function of PYY, where exogenous administration reduces EI (17,33,34), there was a small but significant positive effect of AUC and prelunch PYY on subsequent EI. However, the effect decreased after adjustment for additional participant characteristics, indicating it may be confounded by other factors. Thus there is uncertainty about these present findings relating to PYY. In contrast to the clear exogenous effect, endogenous postprandial responses in PYY were not associated with subsequent EI (12,25,35), possibly as exogenous PYY tends to be used at supra-physiological levels (12).

GLP-1, glucose and insulin were positively related to fullness and negatively related to hunger, desire to eat and prospective consumption consistent with previous research (22,24,36-38), indicating that these biochemical measures are likely to play roles in the perception of appetite sensations. However, some studies have found no relationship, or mixed results, between glucose or insulin and appetite ratings (24,29), possibly because they have reported correlations between the mean AUC or peak values rather than examining within-person relationships. Previous findings with respect to the relationship between postprandial PYY and appetite are mixed, including positive associations between PYY and perceived fullness (36,39), while others, consistent with the present findings, have found no associations (12,38), or associations in lean but not obese participants (35). Thus, the robustness of the association of endogenous PYY with appetite ratings is questionable. It is unclear whether GIP plays a role in influencing appetite and EI (40), however the present findings showed GIP was associated with appetite ratings. The distinct similarity between GIP and appetite ratings profiles may have led to these associations, but causality cannot be assumed. The lack of association between GIP and subsequent lunch EI is in agreement with the perspective that GIP does not influence EI.

The present findings support the concept that reducing the PS of prepared meals or unit foods could constrain EI and contribute to prevention of weight gain. However as weight control advice is inherently overt, it is important to establish whether similar effects are seen when participants are aware of the reduction in PS.

There are several limitations to this study. It was conducted in a laboratory setting and, although the specific hypothesis was concealed, participants were aware of their eating behavior being observed. The frequency and type of food provided at lunch was fixed, thus only the amount could vary potentially limiting compensation by removing some of the environmental cues that are profuse in a free-living environment and can influence EI. This setting also prevented any self-initiated eating episodes between breakfast and lunch. Some of the appetite and hormone profiles suggest effects of PS reduction may have diminished over time and compensation might be seen in a free-living environment during this period. The use of a different control PS (in terms of percentage of estimated daily energy requirements) may alter the relative effects of a percentage reduction. The overweight and obese sample may limit comparison with previous work, however it was most appropriate to study portion size reduction in this group as those with an elevated BMI are most in need of interventions to constrain energy intake, and are to date an understudied population in this field. The study was conducted over a single day and it is possible that a longer period of consuming portions set to provide energy below requirements could lead to adaptation and energy compensation. Future studies should attempt to examine PS reduction in a more realistic setting and with prolonged exposure to smaller portions.

Conclusion

Compulsory reductions in PS at breakfast did not lead to differences in subsequent energy intake over the day, despite changes in biological and psychological measures that tend to favor energy compensation. Although the effect size was small, if sustained this will be of public health benefit in constraining energy intake. O

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