Title

The effect of covertly reducing food portion size at a single meal on daily energy intake and appetite control in overweight and obese adults.

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What is already known about this subject:

- Larger portion sizes are linked with increased energy intake.
- There is an innate asymmetry to the appetite control system.
- There is a notable paucity of evidence on specifically reducing portion size in an overweight and obese population.

What this study adds:

- This study for the first time examines the effects of covert portion size reduction on later daily energy intake and appetite control in overweight and obese adults.
- This study argues that covert portion size reduction could be a useful approach in attempts to constrain energy intake, particularly for weight gain prevention.

Abstract

Background: Larger portion sizes (PS) are associated with greater energy intake (EI), but little evidence exists on appetitive effects of PS reduction.

Objective: To investigate covertly reducing breakfast PS on subsequent EI, postprandial gastrointestinal hormone and perceived appetite responses.

Design: A randomized crossover study in 33 adults (mean BMI 29kg/m^2). Condition A provided breakfast (25% of gender-specific estimated daily energy requirements); PS was then reduced by 20% (condition B) and 40% (condition C). EI was measured at an *ad libitum* lunch (240mins) and snack (360mins), and by weighed diet diaries (rest of the day). Blood was sampled after breakfast from 20 participants. Perceived appetite was measured using visual analogue scales.

Results: Postprandial profiles of PYY, GLP-1, GIP, insulin and fullness were lower and hunger, desire to eat and prospective consumption higher in condition C compared to A. Despite this, EI at lunch (A:2930±203; B:2853±198; C:2911±179kJ) and later that day (A:3865±332; B:4011±369; C:3798±357kJ) did not differ. Hormones were not consistently associated with subsequent EI, but perceived appetite profiles were.

Conclusions: Covert PS reduction does not lead to subsequent energy compensation that day, suggesting it could constrain daily EI. Further research is required given altered perceived appetite and gastrointestinal hormones responses.

Introduction

Concurrent with increasing prevalence of obesity has been increased mass of food consumed per eating occasion (1-3) and the size of commercially available portions (4-6). Empirical evidence shows larger portion sizes (PS) lead to greater energy intake (EI) at a single meal; an effect that continues with 11 days of manipulation (7-15). Reducing PS is a central component in weight management advice, but experimental work to investigate whether PS reduction leads to reduced EI is limited (7-15). Given the asymmetry of appetite and homeostatic mechanisms to achieve energy balance (16), energy compensation may occur in an environment where food is widely available. Understanding the response of short-term appetite control mechanisms to a PS reduction is important to understand the likely impact on EI.

This study investigated whether covertly reducing the PS of a meal is an effective strategy to reduce day-long EI in overweight and obese adults and the impact on gastrointestinal hormones and perceived appetite as measures of biological and psychological appetite control mechanisms.

Methods

Study Design

This was a randomised crossover design involving three PS conditions, presented to each participant at a standardised breakfast time on separate days: a control PS (condition A); PS reduced by 20% (condition B); and PS reduced by 40% (condition C). The control provided 25% of estimated daily energy requirements for the intended average study participant according to gender (24), (3310kJ for men and 2540kJ for women). Participants were blinded to the specific aims of the study and foods prepared to make the intervention as covert as

possible. For each individual, study visits were conducted >1 week apart, on the same day of the week and outside of the luteal phase of the menstrual cycle for females.

Participants

Healthy, 18-60y men and women, with a BMI ≥25 and <35kg/m^2 were recruited. Participants were excluded for disordered eating assessed with Eating Attitudes Test (EAT-26) score ≥11 (17-19), depressive symptoms using the Zung Depression Scale score ≥70 (20), smoking, excessive habitual alcohol intake (>14 units/week for women, >21 units/week for men), weight loss/gain within the last three months (>4.5kg) or actively trying to lose/gain weight, medical conditions or medications potentially affecting appetite, inflammatory conditions, diabetes or fasting plasma glucose ≥7mmol/l, pregnancy, breastfeeding or planning a pregnancy, extremely high levels of exercise (moderate or vigorous level for more than 420min/week assessed with International Physical Activity Questionnaire (IPAQ) (21)), unable to eat test foods, and not regularly consuming breakfast (breakfast ≤3/week).

A sample size of 33 was recruited to give 83% power to detect a minimum difference of 500kJ EI at lunch between any pair of experimental conditions assuming an SD of 950kJ (8,10,22). Biochemical measures were conducted in a sub-group 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 analyser BC-418MA), and resting metabolic rate (RMR; IS Gem 204 with GEMNutrition 2008.4 software) were measured. Participants completed the EAT-26, Zung depression scale, IPAQ and the Three Factor Eating Questionnaire (TFEQ) measuring the traits dietary restraint, disinhibition, and hunger (23) and fasting plasma

glucose assessed. Participants were asked to maintain their usual exercise and dietary habits during the study.

Study visits

Participants fasted overnight (11h prior to each visit) and were asked to refrain from alcohol and avoid strenuous exercise for the 24h before each study day. Provision of the test breakfast marked time zero. Subsequent EI was measured by pre- and post-meal weighing of an *ad libitum* lunch (240min) and afternoon snack (360min), plus a weighed diet diary to record the remainder of the day's intake. Visual analogue scale (VAS) questionnaires rating palatability and meal size were given during breakfast and lunch. Perceived appetite ratings were measured using VAS questionnaires at 30min intervals until lunch, then immediately after and at 300 and 360min, then hourly. In a subgroup of 20 participants, blood samples were collected at fasting and 30, 60, 120, 180 and 240min 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, 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 study breakfast and lunch provided the average reported macronutrient composition of the UK diet (35% energy from fat, 18% from protein and 47% from carbohydrates (25)). The breakfast consisted of a wheat-based breakfast cereal with semi-skimmed milk, scrambled

egg, ham, brown toast and butter, and orange juice. The *ad libitum* lunch consisted of a single course amorphous meal of pasta, mince, tomato sauce, mixed vegetables and grated cheese. The lunch provided 1978kJ (men) or 1518kJ (women). The *ad libitum* snack consisted of ten digestive biscuits on a plate.

The completed diet diaries and recorded consumption at lunch and snack for each study day were coded by the Dietary Assessment Team at HNR using the in-house dietary assessment system. Dietary data was then extracted from the system for analysis.

Questionnaires

The mood and appetite VAS questionnaires rated hunger, fullness, desire to eat and prospective consumption, and also included five distractor questions. The palatability questionnaire used VAS to rate the pleasantness of the food appearance, aroma, taste and texture, desire to eat the food, and the size of the portion. The VAS questionnaires asked participants to mark a horizontal line measuring 100mm with the ends labelled with the extremes of each sensation (e.g. “Not at all” and “Extremely”). The distance from the left end to where the participant mark was drawn was measured to the nearest millimetre.

Analytic methods

Blood samples were separated on collection and plasma stored at -80^{o}C until analysis. Plasma samples collected on EDTA and treated with dipeptidyl peptidase-IV (DPP-IV) inhibitor immediately on collection (10µl DPP-IV inhibitor/ml of blood) were analysed for PYY_{3-36} by radioimmunoassay (Millipore®, Massachusetts, USA) (interassay CVs: 15% at 84pg/ml and 7% at 217pg/ml), at University College Hospital, London; total GLP-1 using an electrochemical luminescence immunoassay kit on the MesoScale Discovery® multi-array assay platform (Maryland, USA) (CVs: 16.4% at 5.4pg/ml, 11.9% at 29pg/ml and 11.6% at

83pg/ml), at Core Biochemical Assay Laboratory (CBAL), Cambridge; and total GIP using an enzyme-linked immunosorbent assay (Millipore®, Massachusetts, USA) (CVs: 6.1% at 26pg/ml, 3.3% at 50pg/ml, 2.3% at 134pg/ml and 1.8% at 166pg/ml), at Cambridge Institute for Medical Research. Plasma samples collected on fluoride oxalate were analysed for glucose using a Dimension® clinical chemistry system (Siemens, Newark, USA) (CVs: 1.69% at 6.23mmol/L, 2.23% at 3.09mmol/L and 2.56% at 18.88mmol/L), at MRC HNR. Plasma collected on lithium heparin were analysed 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% at 29pmol/L, 2.1% at 79.4pmol/L, 1.9% at 277pmol/L and 2.0% at 705pmol/L) at CBAL, Cambridge.

Statistical analysis

Mixed effects models for continuous responses (26) were used for analysis, which extend standard linear regression to account for within-person variation through random effects. EI and perceived PS at breakfast were modelled 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 perceived 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 pre-lunch time-point for biochemical measures and perceived appetite ratings, and over the whole day for perceived appetite ratings. Models of whole-day perceived appetite AUC included PS condition as the explanatory variable, controlling for time over which appetite ratings were made.

Models predicting EI at lunch included explanatory variables of either the pre-lunch or AUC for each biochemical measure or perceived appetite rating, and controlled for condition, gender and BMI. Similar models assessed the relationship between the whole-day AUC of perceived 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 perceived appetite, perceived appetite ratings were modelled separately with each biochemical measure as the explanatory variable. Time, a quadratic term for time, condition, gender and BMI were included as 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/individual (three visits), a random intercept was incorporated into the models. The models for biochemical and perceived appetite profiles as outcomes had two levels of clustering due to repeated sampling time-points and the crossover design. Therefore, a random intercept and a random 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, Texas, USA). Statistical 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)

EI was not different between conditions at lunch (**Figure 2A**; A vs. B, β=-76.6, p=0.429; B vs. C, β=58.2, p=0.547; A vs. C, β=-18.3, p=0.850), or the remainder of the day (**Figure 2B**; A vs. B, β=192.3, p=0.555; B vs. C, β=-152.8, p=0.639; A vs. C, β=39.5, p=0.904). Daily EI was 10287 ± 395kJ, 9897 ± 491kJ and 9161 ± 437kJ in conditions A, B and C respectively.

Biochemical measures

Figure 3 shows the postprandial profiles for each of the gastrointestinal hormones. Compared to condition A, there was a reduction in PYY in C at 120min (β=-22.05, p=0.022), and 240min (β=-23.9, p=0.013). There was no condition-time interaction for conditions C compared to B (p>0.076), or B compared to A (p>0.42). Compared to condition A, GLP-1 was lower in C at 30 (β=-4.4, p=0.024), 60 (β=-4.2, p=0.032), 120 (β=-5.1, p=0.009), 180 (β=-7.8, p<0.001), and 240min (β=-6.1, p=0.002). GLP-1 was also lower in condition C compared to B at 180min (β=-4.1, p=0.038). There was no condition-time interaction for condition B compared to A (p>0.056). GIP was lower in condition B compared to A at 120 (β=-1.6, p=0.014), 180 (β=-2.3, p<0.001) and 240min (β=-2.5, p<0.001). GIP was lower in condition C compared to A at 30 (β=-2.2, p=0.001), 60 (β=-2.4, p<0.001), 120 (β=-4.2, p<0.001), 180 (β=-5.5, p<0.001) and 240min (β=-4.6, p<0.001), and compared to B at 30 (β=-1.3, p=0.046), 120 (β=-2.6, p<0.001), 180 (β=-3.2, p<0.001) and 240min (β=-2.0, p<0.001).

Glucose and insulin profiles are shown in **Figure 4**. There was no condition-time interaction for glucose for condition B compared to A (p>0.224), condition C compared to A (p>0.655) or condition C compared to B (p>0.210). There was a condition-time interaction such that insulin was less in condition C compared to A at 120 (β=-0.7, p<0.001), 180 (β=-0.7,

p<0.001), and 240min (β=-0.4, p=0.008), and insulin was also less in condition C compared to B at 120 (β=-0.5, p=0.001), and 180min (β=-0.4, p=0.014). There was no condition-time interaction for condition B compared to A (p>0.083).

Perceived appetite ratings

Figure 5 shows the perceived appetite ratings. Compared to condition A, hunger was greater in C at all time-points from 30-240min (p<0.006). Hunger was also greater in condition C at all time-points postprandially (p<0.021) when compared to B. There was no condition-time interaction for condition B compared to A (p>0.291). Compared to condition A, fullness was lower in C at all time-points from 20-180min (p<0.019). Fullness was lower in condition C at 30 (p=0.017) and 90min (p=0.003) when compared to B. Also fullness was lower in condition B compared to A at 60 (p=0.041) and 120min (p=0.040). Desire to eat ratings were greater in condition C at all time-points postprandially (p<0.023) compared to A, and at all time-points from 20-210min (p<0.037) compared to B. There was no condition-time interaction for condition B compared to A (p>0.223). Prospective consumption was greater in condition C compared to A at all time-points postprandially (p<0.011) and compared to B, at 120 (p=0.018) and 150min (p=0.027). There was no condition-time interaction for condition B compared to A (p>0.068).

AUCs over the whole day for hunger, desire to eat and prospective consumption were greater in condition C compared to A, and smaller for fullness (hunger β=2423.9, p=0.025; fullness β=-4857.9, p=0.001; desire to eat β=3832.5, p=0.001; prospective consumption β=3427.9, p=0.001). AUC for prospective consumption ratings was greater in condition B compared to A (β=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 conditions B and C (data not shown).

Predictors of energy intake (EI) at lunch and over the whole day

Most of the biochemical measures did not predict EI at lunch ($p>0.137$) (**Table 2**). However, AUC ($p=0.032$) and pre-lunch ($p=0.049$) measures of PYY were positively associated with EI at lunch. AUCs and pre-lunch measures of hunger, desire to eat and prospective consumption were positively associated with lunch EI ($p<0.02$). Pre-lunch fullness was negatively associated with lunch 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 ($p<0.026$).

Associations between biochemical measures and perceived appetite ratings

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 perceived appetite ratings ($p>0.068$) (**Table 3**).

Perceived portion size (PS)

At debriefing, none of the participants were concerned about the study's covert nature and consented to data inclusion. Only two participants noticed the change in PS at breakfast. However the ratings of perceived meal size at breakfast were different between conditions. Perceived breakfast size was smaller in condition C compared to both A ($\beta=-15.6$, $p<0.001$) and B ($\beta=-10.8$, $p<0.001$), and perceived meal size smaller in B compared to A (data not shown).

Discussion

Reducing PS at a single meal alters psychological and biological markers of appetite, but there is no energy compensation later in the day. EIs at lunch were strikingly consistent in

this standardized laboratory setting. These findings indicate covertly reducing PS of a prepared meal could lead to a net reduction in daily EI. However, the effect on perceived appetite and gastrointestinal hormones, 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 the standard PS and the 20% reduction. Moreover, there were few differences in the profiles when comparing the 20% and 40% reduction conditions suggesting that the responses in these biochemical measures may not be sensitive to the smaller change in PS (660kJ men and 510kJ women). Indeed, all previous studies where a reduction in energy load has led to attenuated PYY (27,28), GLP-1 (29,30), or insulin (31,32) profiles, used energy changes between 920-2096kJ. 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 reported not noticing the meal manipulation. The effect size for the difference between perceived PS ratings was considerably smaller when comparing conditions A *versus* B than B *versus* C (β=-4.8; β=-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% A-B, and 25% B-C), or due to the Weber-Fechner law, whereby the ability to perceive stimulus change is proportional to the logarithm of the magnitude of the stimulus (33). Thus, as the reference portion size in the first comparison (A *versus* B) was larger than the second (B *versus* C), 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 energy is provided, and thus consumed, could affect appetite ratings. The smaller effect size of perceived PS between conditions A and B could in part account for fewer differences in perceived appetite ratings between these conditions.

Postprandial biochemical responses were poor predictors of subsequent EI, consistent with much of the existing evidence (34-36). However, perceived appetite ratings tended to predict EI at lunch and the rest of the day. This is in agreement with some (22, 37-39), but not all (40,41), 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 (42). Although associations between perceived appetite and EI in the present study were highly significant, the effect sizes were small. This, coupled with relatively small differences in postprandial perceived appetite response 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 (27, 43, 44), there was a small but significant positive effect of AUC and pre-lunch 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 PYY. In contrast to the clear exogenous effect, endogenous postprandial responses in PYY were not associated with subsequent EI (22,35,45), possibly as exogenous PYY tends to be supra-physiological (22).

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 (32,34,46-49), 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 perceived appetite ratings (34,39), 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 perceived appetite are mixed, including positive associations between PYY and perceived fullness (46,50), while others, consistent with the present findings, have found no associations (22,49,51), or associations in lean but not obese participants (45). Thus, the robustness of the association of endogenous PYY with perceived appetite is questionable. It is unclear whether GIP plays a role in influencing appetite and EI (52), however the present findings showed GIP was associated with perceived appetite ratings. The distinct similarity between GIP and perceived appetite 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 covertly reducing the PS of commercially available unit foods or pre-prepared meals 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 behaviour 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 study was conducted over a single day and it is possible that a longer period of

consuming PSs 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.

Conclusions

Covert reductions in PS lead to lower EI, despite changes in biological and behavioural measures that tend to favour energy compensation. Although the effect size is small, if sustained this will be of public health benefit, in the prevention of weight gain.

Conflict of interest

SAJ is the independent Chair of the Department of Health Responsibility Deal Food Network in England, which includes voluntary agreements with industry to reduce the portion size of some food and drinks. No other authors declare a conflict of interest.

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HBL and SAJ were responsible for project conception. HBL, ALA and SAJ developed the protocol. IS-T advised on statistical analysis. HBL conducted research, analysed data, interpreted results, and drafted the manuscript. ALA, IS-T, CGW, FR, FMG and SAJ contributed to the data interpretation and critical revision of the manuscript. HBL had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1: Participant characteristics.

Mean ± SEM.

BMI: Body Mass Index. RMR: Resting metabolic rate.

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

AUC: area under the curve. EI: energy intake. SE: standard error.

Area under the curve was calculated for between the fasting and pre-lunch 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 analysed in a separate mixed effects model.

Values are given to 4 significant figures. Those in bold are significant.

Table 3: Estimated regression coefficients to measure associations between biochemical measures (predictor variables) and perceived appetite ratings (outcome variables) from baseline to the pre-lunch time-point, from mixed effects models.

SE: standard error.

Each predictor was analysed in a separate model.

Values are given to 4 significant figures. Those in bold are significant.

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).

Figure 2: Mean (± SEM) energy intake at A) lunch and B) over the whole day, not including breakfast, according to condition.

Figure 3: Postprandial response (mean ± SEM) of A) plasma PYY_{3-36}, B) plasma total GLP-1, and C) plasma total GIP, according to condition. Letter indicates the condition where the mean is significantly different at that time point (mixed effects models): $p<0.05$.

Figure 4: Postprandial response of A) plasma glucose (mean ± SEM), and B) plasma insulin (geometric mean ± 95% confidence intervals), according to condition. Letter indicates the condition where the mean is significantly different at that time point (mixed effects models): $p<0.05$.

Figure 5: Postprandial ratings (mean ± SEM) for A) perceived hunger, B) perceived fullness, C) perceived desire to eat, and D) perceived prospective consumption, according to condition. Letter indicates the condition where the mean is significantly different at that time point (mixed effects models): $p<0.05$.

Table 1

Participant characteristic	All participants (n=33)	Blood sample subgroup (n=20)	Non-blood 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 (mins per week)	65 ± 13	55 ± 14	80 ± 24
Moderate physical activity (mins per week)	142 ± 21	173 ± 29	94 ± 26
Walking (mins per week)	254 ± 30	270 ± 37	231 ± 53

Table 2

Predictor of lunch EI	AUC as predictor		Pre-lunch measure as predictor	
	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
Perceived 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 perceived 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**		

Table 3

	Perceived 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**

Figure 1

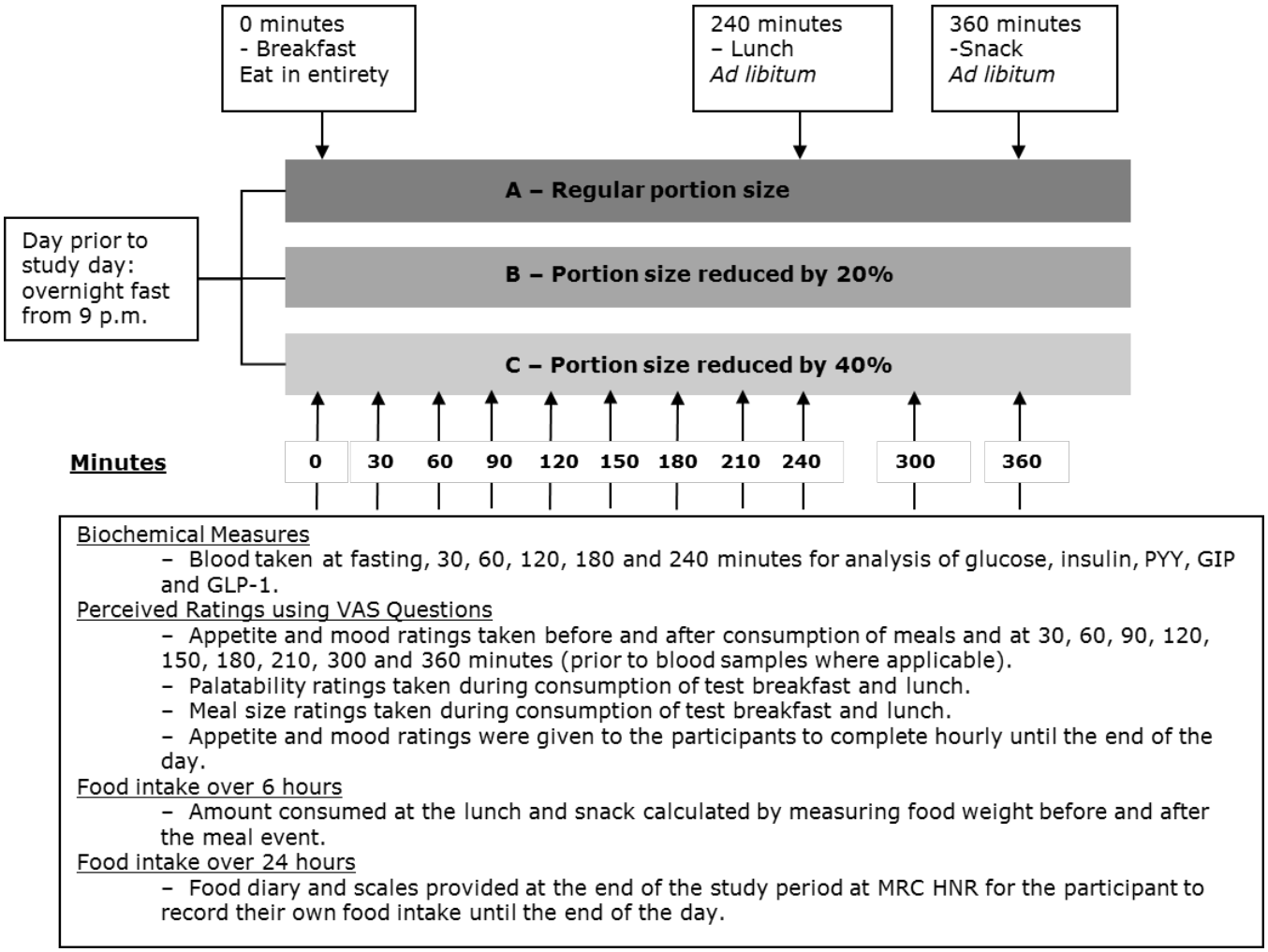
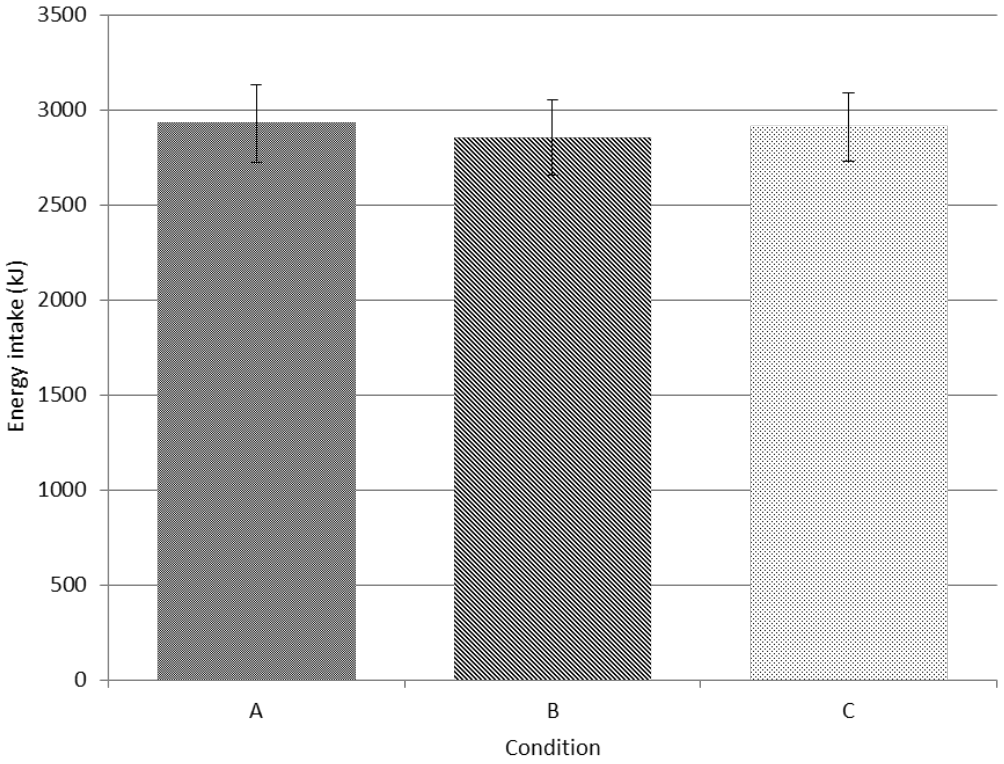

0 minutes
- Breakfast
Eat in entirety
240 minutes
– Lunch
Ad libitum
360 minutes
-Snack
Ad libitum
A – Regular portion size
B – Portion size reduced by 20%
C – Portion size reduced by 40%
Day prior to study day: overnight fast from 9 p.m.
Minutes
0
30
60
90
120
150
180
210
240
300
360
Biochemical Measures
– Blood taken at fasting, 30, 60, 120, 180 and 240 minutes for analysis of glucose, insulin, PYY, GIP and GLP-1.
Perceived Ratings using VAS Questions
– Appetite and mood ratings taken before and after consumption of meals and at 30, 60, 90, 120, 150, 180, 210, 300 and 360 minutes (prior to blood samples where applicable).
– Palatability ratings taken during consumption of test breakfast and lunch.
– Meal size ratings taken during consumption of test breakfast and lunch.
– Appetite and mood ratings were given to the participants to complete hourly until the end of the day.
Food intake over 6 hours
– Amount consumed at the lunch and snack calculated by measuring food weight before and after the meal event.
Food intake over 24 hours
– Food diary and scales provided at the end of the study period at MRC HNR for the participant to record their own food intake until the end of the day.

Figure 2

A)


3500
3000
2500
2000
1500
1000
500
0
Energy intake (kJ)
A
B
C
Condition

B)

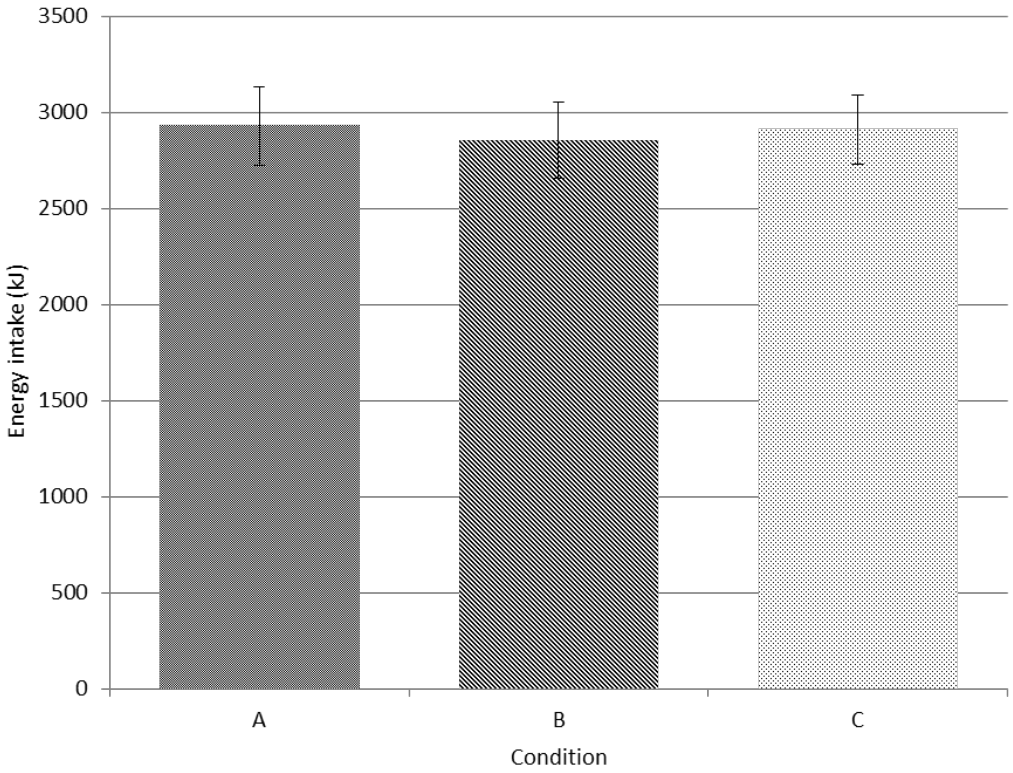
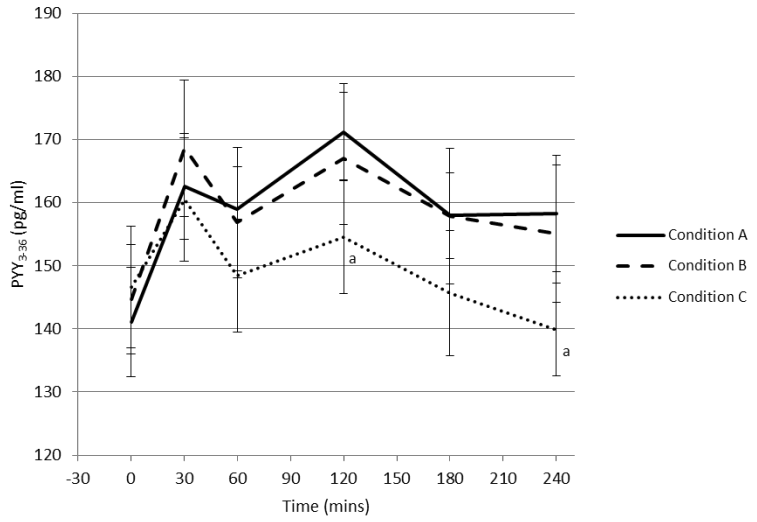
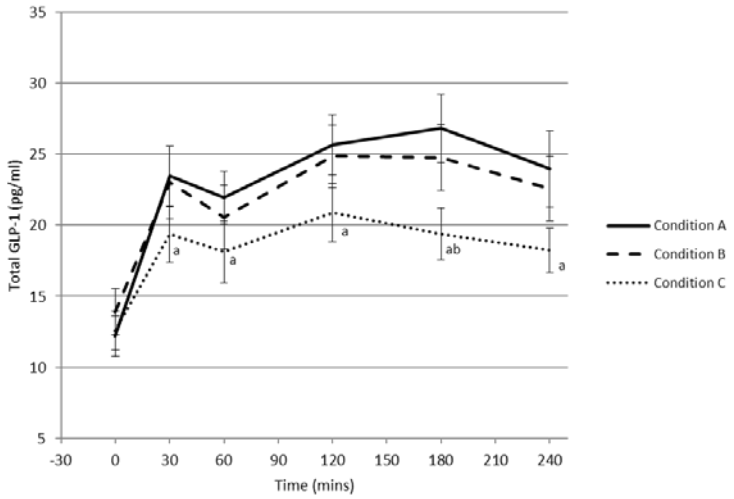

3500
3000
2500
2000
1500
1000
500
0
Energy intake (kJ)
A
B
C
Condition

Figure 3

A)


190
180
170
160
150
140
130
120
PYY3-36 (pg/ml)
-30
0
30
60
90
120
150
180
210
240
Time (mins)
a
a
Condition A
Condition B
Condition C

B)


35
30
25
20
15
10
5
Total GLP-1 (pg/ml)
-30
0
30
60
90
120
150
180
210
240
Time (mins)
a
a
a
ab
a
Condition A
Condition B
Condition C

C)

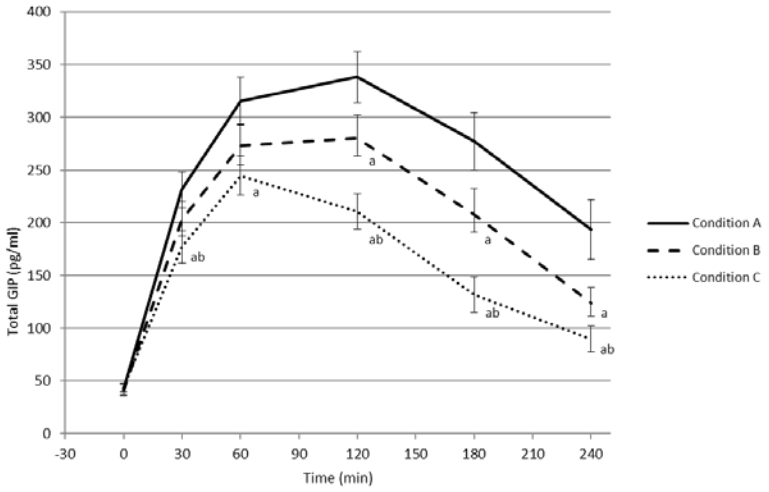
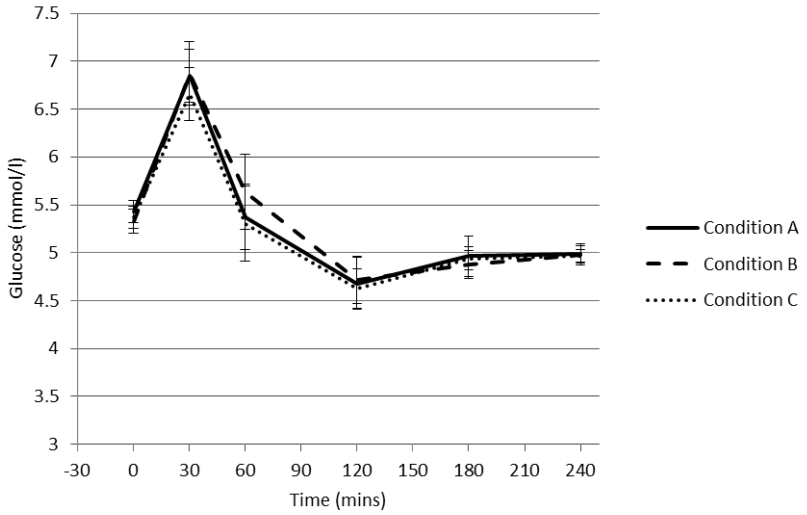

400
350
300
250
200
150
100
50
0
Total GIP (pg/ml)
-30
0
30
60
90
120
150
180
210
240
Time (min)
ab
a
a
ab
a
ab
a
ab
Condition A
Condition B
Condition C

Figure 4

A)


7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
Glucose (mmol/l)
-30
0
30
60
90
120
150
180
210
240
Time (mins)
Condition A
Condition B
Condition C

B)

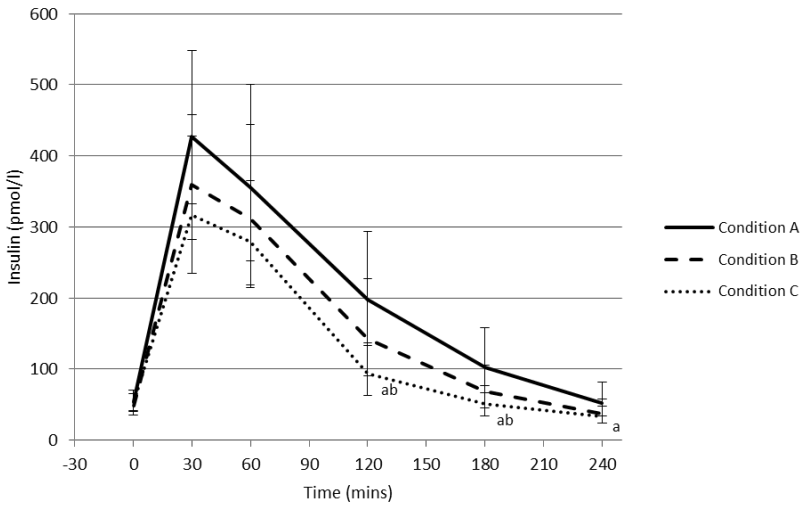
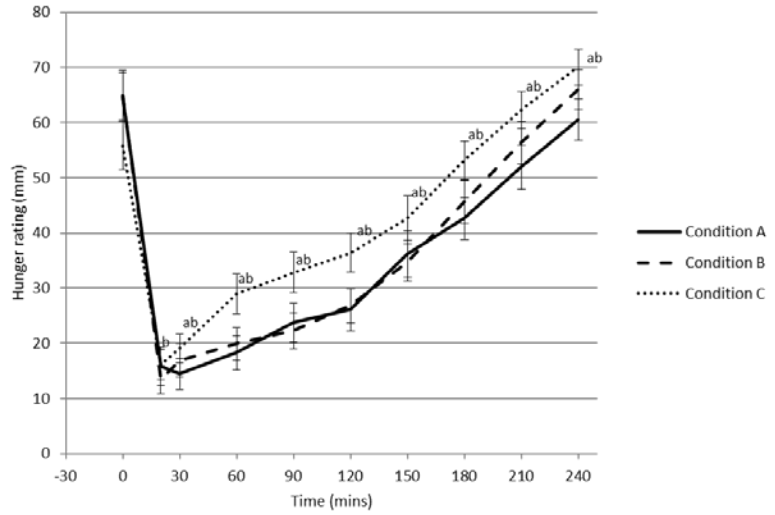
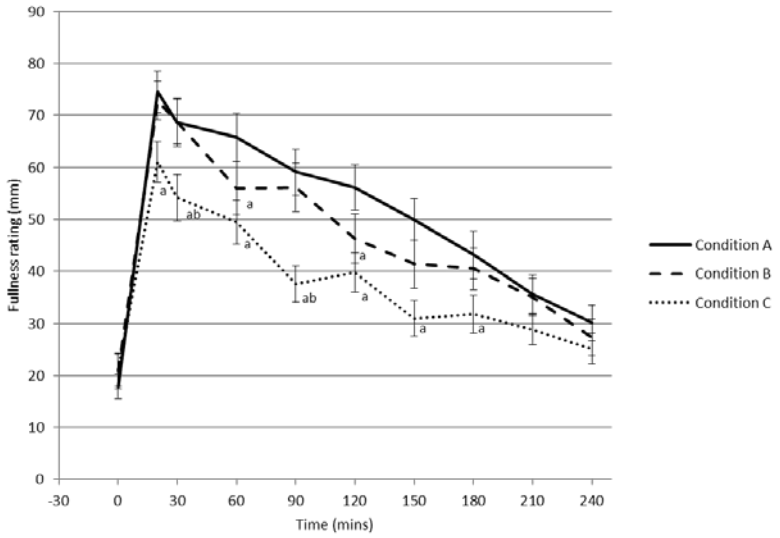

600
500
400
300
200
100
0
Insulin (pmol/l)
-30
0
30
60
90
120
150
180
210
240
Time (mins)
ab
ab
a
Condition A
Condition B
Condition C

Figure 5

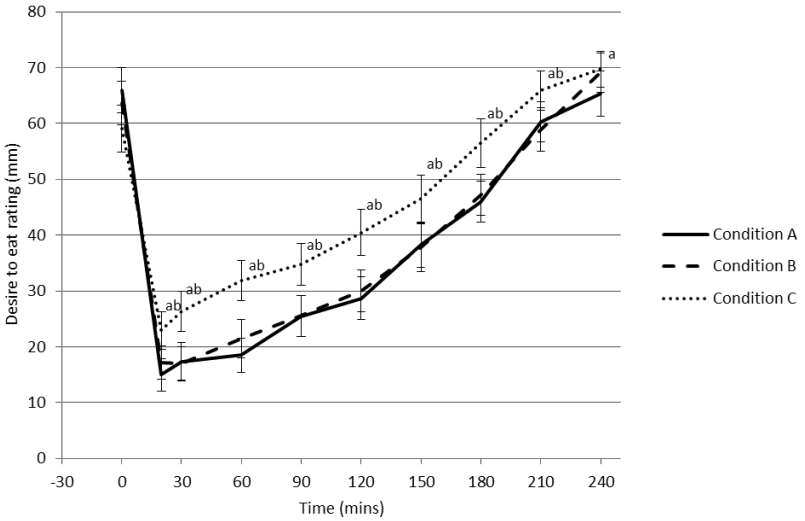
A)


Hunger rating (mm)
Time (mins)
Condition A
Condition B
Condition C

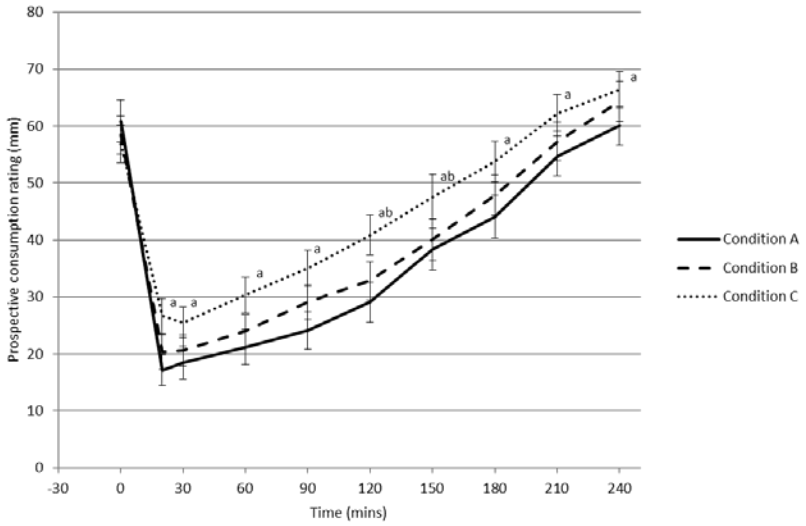
B)


Fullness rating (mm)
Time (mins)
Condition A
Condition B
Condition C

C)


Desire to eat rating (mm)
Time (mins)
Condition A
Condition B
Condition C

D)


80
70
60
50
40
30
20
10
0
Prospective consumption rating (mm)
-30
0
30
60
90
120
150
180
210
240
Time (mins)
a
a
a
a
ab
a
ab
a
a
Condition A
Condition B
Condition C