

Chapter 2. METHODS.

2.1. Study design.

This study employed a within-subjects, repeated measures, cross-over design, with each participant returning for four separate test sessions, generally spaced a week, but never less than five days apart (Rolls et al. 2002). Each subject estimated the portion size of eight foods and drinks with low, medium or high energy densities once under each of four conditions: (F) fullness; (H) hunger; (FPS) fullness with perceived satiation (PS) cue and (HPS) hunger with PS cue. The order in which subjects were exposed to each condition was randomised using a Williams Latin Square (Williams, 1949) (Appendix 1).

The independent variables were fullness, hunger, PS and energy density. Fullness, hunger and PS were measured using Visual Analogue Scales (VAS) based on Hill and Blundell (1982) (Appendices 2 and 3). Energy density was measured according to the classification system from Rolls and Barnett (2000) (see List of Abbreviations). The dependant variable was estimated portion size, measured using a questionnaire designed based on previous literature (Blake et al. 1989; Beasley et al. 2004) (Appendix 3).

Quantitative data was collected for 19 weeks, between June and December 2008.

2.2. Population, sample and sample size estimation.

Sample size was estimated using a power calculation, in consultation with a statistician (Colin Sinclair, University of Chester) and based on data from Blake et al. (1989). Results indicated that inclusion of 32 subjects was necessary in order to detect significant differences between food/drink portion size estimates under different conditions (alpha 0.05; 80% power). Due to problems with recruitment beyond the investigator's control, only 27 male participants took part in the study, which, as a result, lost a small degree of power.

Participants were recruited from the University of Chester campus and surrounding area using advertisements (Appendix 4) and flyers. Based on methods by Tsuchiya, Almiron-Roig, Lluch, Guyonnet and Drewnowski (2006) and Flood, Roe and Rolls (2006), eligibility criteria were set as: being aged between 18 and 45 years; being of normal weight (BMI between 18 and 27.9¹); being non-dieting; non-smoking and consuming breakfast regularly. Exclusion criteria were set as: having a history of weight cycling over the last ten years; having any condition affecting diet, appetite or food intake; having a food allergy or intolerance or following any food restriction; taking prescription medications/supplements which may have affected appetite and/or weight; being an athlete in training; being involved in intense physical activity for >10 h per week; disliking or being unfamiliar with any of the eight test foods or drinks; having prior knowledge of the study or relevant qualifications in nutrition.

¹ The 'normal weight' cut off point was raised from 24.9 to 27.9 to prevent exclusion of subjects with considerable muscle mass.

Individuals willing to participate were initially checked for eligibility via a telephone pre-screen interview (Appendix 5). Those who appeared to meet the study criteria were subsequently invited to the laboratory to confirm their weight, height and BMI; and determine liking and familiarity ratings for test foods and drinks, using 100 mm VAS scales based on Raudenbush and Frank (1999); Yeomans, Blundell and Leshem (2004) and Prescott, Johnstone and Francis (2004) (Appendix 6). Prior to rating liking and familiarity for each test food and drink, participants were told that liking and familiarity referred to how much a person liked or was familiar with the taste and feel of a food/drink in their mouth and stomach. Raudenbush and Frank (1999) considered foods with liking or familiarity scores of ≤ 49 as disliked or unfamiliar. However, in order to optimize recruitment levels, cut-offs in this study were lowered to ≤ 9 and ≤ 29 for a food/drink to be considered disliked or unfamiliar, respectively.

Subjects' eating habits (cognitive restraint, disinhibition and hunger) were also assessed during laboratory screening, using the Three Factor Eating Questionnaire (TFEQ) from Stunkard and Messick (1985) (Appendix 7). Subjects with TFEQ scores of ≥ 9 on the disinhibition scale, or those with a score of ≥ 10 on the cognitive restraint scale plus ≥ 7 on the hunger scale were excluded.

Each participant received a Participant Information Sheet (PIS) and provided written informed consent prior to any laboratory procedures taking place (Appendices 8 and 9).

The information from laboratory screening was collated using a laboratory screening sheet (Appendix 10). Once eligibility had been confirmed, volunteers

received a letter of invitation stating the date, time and location for each of the four study sessions (Appendix 11). To standardise baseline appetite levels, participants were asked to report to the laboratory at 8.30am on the same day each week, having eaten or drunk nothing for the previous 12 h, except for non-carbonated water. To decrease variability, participants were also asked to refrain from drinking alcohol and to keep evening meals and activity levels similar on the day before each test.

Ethical approval for the study was obtained from the Research Ethics Committee, Faculty of Applied and Health Sciences, University of Chester (Appendix 12). All 27 participants completed the study and received £20 reimbursement for their participation.

2.3. Materials and Procedures.

2.3.1. Test meal.

Participants under conditions F and FPS were provided with a breakfast pre-load prior to the test. The pre-load consisted of sliced white bread (80.6 g; Warburtons); spreadable butter (10 g; Anchor); sliced cheddar cheese (50 g or 60 g; Tesco or Cathedral City, respectively); digestive biscuits (31.6 g; Tesco); fresh orange juice (150 ml; Del Rivo) and still water (150 ml; Tesco) and was presented on an individual tray (Appendix 13). The pre-load provided 737 kcal, contributing approximately one-third of the estimated average requirement for energy (2550 kcal/d) for inactive males aged 19-49 years (Department of Health, 1991). Previous

research (Tsuchiya et al. 2006) suggested that this amount of energy was enough to elicit sufficient satiety up to 90 minutes post consumption. Levels of protein, carbohydrate and fat contained in the pre-load were 26.5 g, 69.4 g and 37.8 g, respectively.

2.3.2. Visual analogue scale (VAS) ratings.

Participants rated hunger, fullness and thirst using 100 mm visual analogue scales (VAS) (Appendix 2). The use of VAS scales in assessment of appetite sensations has been validated previously (Raben, Tagliabue and Astrup, 1995; Flint, 2000) and is common practice in appetite research (Hill and Blundell, 1982; Rolls, Morris and Roe, 2002; Provencher et al. 2007). The scales in this study were provided in booklet form, one scale per page and were anchored at each end with the labels “not at all” and “extremely”. For example, for the question, “How full do you feel?” the scale ranged from “not full at all” to “extremely full”. In order to prevent experimental bias, three distracting VAS questions on alertness, tiredness and sleepiness were also included in the VAS booklet (Livingstone, Robson, Welch, Burns, Burrows and McCormack, 2000). Data from these three VAS were not used in the study.

2.3.3. Test foods and drinks.

Details of test foods and drinks used in the study, including brands, energy densities, volumes and amounts displayed are included in Appendix 14. The cola drink, crisps and chocolate bar were presented as sold (Appendix 15). Hot chocolate was presented in the form of an empty takeaway container with heat proof lid, as supplied by a popular coffee company. The ice-cream was presented as an empty tub. The banana and muffin were presented as sold, on individual plates of 15.5cm in diameter. The cornflakes were presented dry, in a cereal bowl, 14cm in diameter, with a depth of 7.5cm.

All brand names, weights and/or nutritional information visible on test foods/drinks were covered, using colour coordinated materials in keeping with the original packaging. A fresh banana was purchased each week and individual weights were recorded, with the mean weight \pm standard deviation (SD) being 140.4 ± 2.6 g. The shape of the banana was maintained between tests using a template based on the shape of the banana purchased in week 1. The muffin was frozen in between tests and replaced with any change in appearance. The weight of the muffin was recorded each week, with the mean weight \pm SD being 139.1 ± 4.6 g.

Test foods included in the study were selected to incorporate solid, liquid and amorphous types, with varying energy densities, i.e. VLED = very low energy density (<0.6 kcal/g); LED = low energy density (0.6-1.4 kcal/g); MED = medium energy density 1.5 to 3.9 kcal/g) and HED = high energy density (≥ 4.0 kcal/g or ml)

(Rolls and Barnett, 2000) (Appendix 14). The amounts presented were based on commercial pack or container sizes for the products chosen.

Based on the methods of Brunstrom and Shakeshaft (2009) and to encourage participants to estimate a portion size according to their immediate need, rather than the time of day, test foods and drinks included in the study were those which may be consumed at any given time, rather than at specific mealtimes. The only breakfast food included in the study was the cornflakes.

Actual portion sizes of the foods and drinks included in this study were calculated based on four portion guidance size schemes created by the following organisations: Dietitians in Obesity Management (DOM) UK (2005); the Food Standards Agency (FSA) in Crawley (2002); the American Dietetic Association (ADA) (2007) and the US Food and Drug Administration (FDA) (2001) (Appendices 14 and 16). These schemes were selected to represent portion size guidance schemes from two different countries, based on different criteria and created for different purposes (see Introduction, section 1.4).

Ten different sequences for the order of presentation of test food/drinks were created by random selection amongst 8 numbers (Appendix 17). These sequences were used in the first 10 weeks of the study and then again in the following 9 weeks. Changing the order of presentation of test foods/drinks was essential to avoid memory effects amongst participants (Higgs, 2002), who were never exposed to a single sequence on more than one occasion.

In order to prevent the smell of test foods/drinks from affecting appetite status and portion size estimation (Sorensen et al. 2003; Ferriday and Brunstrom,

2008), foods/drinks were selected to include those which are normally served cold or could be realistically presented in the empty form of their original container. Each food/drink was presented in an individual booth, in a room separate from the waiting room, so that the appearance of one food/drink could not interact with and influence portion size estimation of another.

Any objects or pictures which may have acted as food cues were removed from the test room prior to study commencement.

2.3.4. Estimation of food/drink portion sizes.

Instructions on how to estimate a portion were provided on pages 2-3 of the Portion Size Question Booklet (Appendix 3). Participants were provided with a definition of the word **portion** (the quantity of food or drink that they would consume on one eating or drinking occasion) and told to consider this definition in order to estimate the portion size of each food and drink on display. For example, when presented with the ice-cream, subjects were asked, “How many portions of ice-cream are in this tub?” In order to answer this question, participants were told to ask themselves, “Would I be able to consume all of that food or drink at this moment in time?” If the answer was yes, then participants were told to consider that as one portion. If the answer was no, then participants were asked to estimate portion size in relation to the amount of food/drink presented before them. For example, if they thought the tub of ice-cream on display was twice the amount they could consume at that moment in time, then this was equivalent to two portion sizes. Participants were

also permitted to express portion size estimates as fractions of whole numbers, e.g. $\frac{1}{2}$, $1\frac{1}{2}$, etc.

Under conditions FPS and HPS, prior to estimating the portion size of each food/drink, participants were asked to respond to the following PS cue, “How full do you think you would be after consuming this amount of food or drink?” For example, when estimating the portion size of the ice-cream, participants were asked the following question: “How full do you think you would be after consuming this tub of ice-cream?” Study participants rated PS on a 100 mm VAS scale and were asked to consider their response before estimating and recording the portion size of the food/drink displayed before them. The exact wording of the PS question was pre-piloted for optimal understanding amongst a group of 20 males prior to study commencement (see section 2.3.6).

The portion size of each food/drink was recorded in the Portion Size Question Booklets. The order of the questions in the booklets corresponded to the order of presentation of the foods/drinks on display. Participants received one of two types of booklet each week (F/H or FPS/HPS), according to whether their condition involved the PS cue. Examples of the booklets used in Week 1 are provided in Appendix 3. Participants were asked to read the instructions in their booklets at each of the 4 study sessions, prior to entering the test room. After reading the instructions, the main points of the test were discussed. In order to ensure portion sizes were estimated in the presence of standardised levels of hunger and fullness, the importance of estimating a portion size and considering the PS question “at that moment in time” was emphasised (Brunstrom and Shakeshaft, 2009).

On arrival at the test room, participants were asked to locate themselves in front of one of the eight booths. Booth numbers were pre-allocated according to participant ID numbers (Appendix 18). Participants received a set of reminder instructions and were asked not to talk or touch any of the foods/drinks on display. Based on methods by Burger et al. (2007) participants were given 60 seconds to estimate and record the portion size of the first food/drink presented before them, after which time, under instruction from the lead researcher, they moved one booth to the right and repeated the process of evaluation. This rotation continued until the portion size of all 8 foods/drinks on display had been estimated.

At the end of the test, participants received reminder cards stating the date and time of their next test session. Participants under fasting conditions also received a chocolate bar (55 g; Nestle) and carton of orange juice (250 ml; Tesco) to compensate for the lack of breakfast.

2.3.5. Procedures.

Subjects arrived at 08:30, having fasted for the previous 12 h and were seated in a waiting room in two separate groups (F + FPS and H + HPS) according to whether or not their condition involved breakfast consumption. The waiting room was free from objects or pictures which may have acted as food cues. To enhance protocol compliance each participant was asked to complete a food and activity diary (Tsuchiya et al. 2006) (Appendix 19) and subsequently informed of their ID number and test condition for that day.

At 08:35, participants received their Portion Size Question Booklets and instructions for the test. The first set of VAS questions (Time 0 or baseline) was completed at 08:45, after which participants under conditions H and HPS were asked to consume 100 ml water and taken to the test room (08:55) to complete the test.

Meanwhile, participants under conditions F and FPS received the breakfast meal (08:55), which they were asked to consume within 10 minutes. At 09:05 participants completed the second set of VAS scales (VAS 1), whilst waiting 45 minutes to allow stomach distension and gastric secretion to evoke feelings of satiation (Brand et al. 1982). During this time, participants were allowed to read, listen to music with earphones or use a computer. Internet access and publications containing visual cues which may have affected appetite were forbidden (Livingstone et al. 2000).

At 09:50, participants under conditions F and FPS verified their satiety levels by completing the final set of VAS (VAS 2) and were taken to test (09:55).

The activities involved in each test session according to condition and the order in which they occurred are provided in Appendix 20. At the end of the 4 study sessions, each participant was reimbursed for taking part in the study and asked to complete an optional discharge questionnaire. This was used to determine whether participants were aware of the purpose of the study (Appendix 21). Throughout the study, participant data were collected and stored in a locked filing cabinet and/or password protected computer, only accessible to the lead researcher, academic supervisor and technician. Data were coded with an ID study number to protect the identity of participants.

2.3.6. PS cue pilot test.

Two pilot questionnaires were tested on campus at the University of Chester, in order to select the PS cue (Appendix 22). Based on methods by Yeomans, Lartamo, Procter, Lee and Gray (2001), twenty male volunteers, aged 18-45 years, were approached on campus during work or leisure time and asked to respond to two cues in relation to a picture of (a) a cup of café latte and (b) a piece of flapjack. The cues were as follows:

- 1) How full do you think you would be after consuming this amount of food/drink?
- 2) How much of another product do you think you would be able to consume after eating/drinking this amount of food/drink?

Volunteers were asked to rate anticipated fullness in response to each cue for both the café latte and the flapjack on 100 mm VAS and asked: “Of the two questions above, which one do you find (a) easier to understand, (b) simpler/clearer and (c) easier to answer. Volunteers were provided with a choice of answers for (a) – (c) for both the café latte and the flapjack, including: none; 1; 2; both. Where volunteers provided mixed responses for (a) – (c) it was assumed they had no preference for either cue 1 or 2 and were therefore categorised as liking both PS cues equally.

2.3.7. Practice test.

In order to identify any potential problems with the study, a test rehearsal involving 7 volunteers was carried out at the University of Chester. Participants arrived at 08:30, having fasted for the previous 12 h and followed test procedures as detailed in section 2.3.5.

At the end of the test session, participants were asked to provide verbal feedback. This revealed that the volume of liquid served with the breakfast meal was insufficient, which resulted in the addition of a 150 ml glass of water to the meal and an increase in the volume of orange juice from 100 ml to 150 ml.

2.4. Data management and data analysis.

Statistical tests were carried out using SPSS for Windows, Version 16.0 (SPSS Inc., Chicago). Liking, familiarity and appetite sensation scores, measures of PS and portion size estimates were assessed for normality using the Kolmogorov-Smirnov (KS) test. One-way within subjects repeated measures ANOVAs were used to compare mean appetite sensation scores (hunger, fullness and thirst) at each time point and portion size estimates under each condition. Mauchly's sphericity test was used to validate the use of all repeated measures ANOVAs. Minke (1997) suggests violations of sphericity are trivial where there are only two levels of comparison, plus ANOVA is reasonably robust to violations of sphericity where group size by condition is similar (Pallant, 2007) as in our case. However, to be sure of the

appropriateness of ANOVA, the Geisser-Greenhouse correction was used to determine levels of significance where data were not homogenous (Mauchly's test, $p < 0.05$).

Nested ANOVAs, with Bonferonni correction where appropriate, were used to assess the independent and interactive effects of hunger and PS on mean portion size estimates for each food and drink.

Paired sample t-tests were used to compare mean PS ratings for each food and drink under full and hungry conditions. The Pearson's product-moment correlation test was used to investigate the relationship between PS VAS ratings and energy density; PS VAS ratings and weight and PS VAS ratings and energy load of the test foods. Tests were carried out on foods only, under full and hungry conditions¹.

Percentage error of mean estimates for each food and drink under full and hungry conditions were calculated using standards from all four guidance schemes. Percentage error was calculated as follows: $[(\text{mean estimate} - \text{standard}) / \text{standard}] \times 100$ (Blake et al. 1989). One-sample t-tests were used to compare mean portion size estimates under full and hungry conditions with actual portion sizes based on standards from Dietitians in Obesity Management (DOM) UK (2005), the Food Standards Agency (FSA) (2002) (Crawley, 2002), the American Dietetic Association (ADA) (2007) and the US Food and Drug Administration (FDA) (2001). The relationship between percentage error and energy density; percentage error and weight and percentage error and energy load was explored using Pearson's product-

¹ Pearson's r values were interpreted using cut offs from Arch (2008). (See Appendix 23).

moment correlation test. Each test was carried out in the presence and absence of drinks, under full and hungry conditions.

Mean ratings of liking and familiarity for each food and drink were compared using one-way within subjects repeated measures ANOVA, with the Bonferonni correction where appropriate. The Pearson's product-moment correlation test was used to investigate the relationship between liking ratings and portion size estimates; and liking ratings and measures of PS. Tests were carried out under full and hungry conditions, for all foods and drinks. The Pearson's test was also conducted to explore the relationship between familiarity ratings and portion size estimates; and familiarity ratings and measures of PS. Tests were carried out under full and hungry conditions, for all foods and drinks.

The level of significance for all statistical tests was set to <0.05 .