METABOLIC PHENOTYPING GUIDELINES

Studying eating behaviour in humans

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Abstract

The study of human appetite and eating behaviour has become increasingly important in recent years due to the rise in body weight dysregulation through both obesity and eating disorders. Adequate control over appetite is paramount for the control of body weight and in order to understand appetite, it is necessary to measure eating behaviour accurately. So far, research in this field has revealed that no single experimental design can answer all research questions. Each research question posed will require a specific study design that will limit the findings of that study to those particular conditions. For example, choices will be made among the use of laboratory or free-living studies, time period for examination, specific measurement techniques and investigative methodologies employed. It is important that these represent informed decisions about what design and which methodology will provide the most meaningful outcomes. This review will examine some of the 'gold standard' study designs and methodologies currently employed in the study of human appetite and eating behaviour.

Key Words
- eating behaviour
- satiety
- satiation
- eating behaviour methodology
- appetite

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Homeostatic vs hedonic eating behaviour

Traditionally, homeostatic regulation has been associated with the regulation of the internal milieu as developed by Claude Bernard during the 19th century. In the field of appetite, homeostasis is used to explain the quantitative changes in eating and food intake such as those that occur in order to correct any energy deficit. Richter (1943) subsequently used the term 'behavioural regulation of internal states' to indicate how eating behaviour operated to maintain physiological functioning. Hedonic aspects of appetite are those that are concerned with the influence of reward, pleasure and palatability on eating. The 'homeostatic system' comprises a network of gastrointestinal peptides and brain neurotransmitters, and also peripheral neural signalling and adipokines such as leptin. This system has been described, for example, by Schwartz *et al.* (2000) and updated by Halford & Blundell (2000) and Blundell *et al.* (2012), and it illustrates how physiological signals of energy requirements are integrated with the motivation to eat via sensations of hunger and fullness. This review was commissioned to strictly address the behavioural aspects of appetite control, which provide the foundation for obtaining valid and reliable measured changes in the actual behaviour of eating (and food selection) and in the associated motivational states that often determine the initiation, direction and duration of any feeding event. However, it is recognised that these behaviours emanate from a complex and sophisticated set of physiological pathways in the periphery and the brain. The actual behavioural expression of human appetite should be interpreted against this background physiological state. Description of the physiology is not within the scope of this chapter, but the reader is directed to recent reviews in order to put the behavioural aspects of appetite within a physiological context (Konturek *et al.* 2004, Yi & Tschöp 2012).

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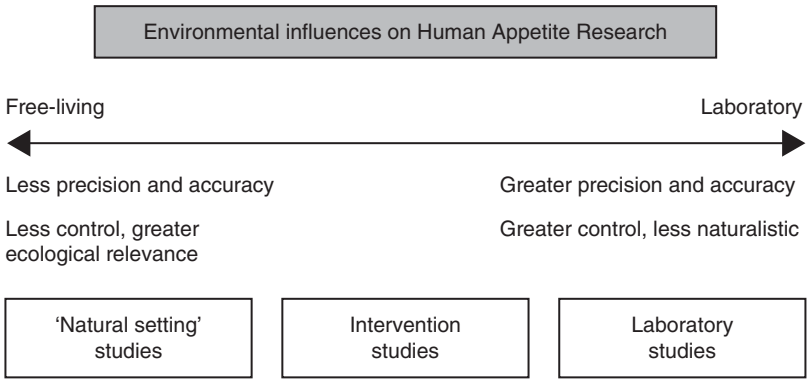

Figure 1
Conceptual description of the relationship between laboratory and free-living research (taken from Blundell *et al*. (2009)).

The 'hedonic system', coordinated by the brain's reward circuitry (that is the network subserving pleasurable aspects of eating), responds primarily to sensory properties and thoughts about food that reflect the explicit and implicit cognitions of liking and wanting. Similar to the homeostatic system, there is a network of brain pathways and neurotransmitters that encode hedonic aspects of eating. Importantly, these brain pathways are influenced by hormones such as leptin and ghrelin that arise in the periphery. Consequently, hedonic responses are not simply due to a brain signal but arise due to interactions between food sensory input and underlying hormonal status. It is known that these pathways are also involved in the addictive response to drugs such as cocaine and amphetamine. Although the homoeostatic and hedonic systems are based on distinctive neural substrates, both systems evolved to maintain appropriate levels of energy and nutrients; therefore, it could be expected that there will be considerable functional overlap between these domains in the control of food intake (Finlayson *et al*. 2007). This overlap means that we should review the classical distinction between homeostasis and hedonics. As there are physical connections and integration of actions between the two systems, they do not appear to be functionally separate and it may be disadvantageous to continue to regard them as separate domains. This is one area of appetite research in which methodological refinement can lead the way.

Laboratory vs free-living studies

Human eating occurs in discrete episodes throughout the day but in a variety of different environments. Therefore, while considering the study of eating behaviour, a decision must be made between laboratory and free-living studies. This decision is a balance between the strong control when using laboratory studies and the more realistic setting in free-living studies as shown in Fig. 1.

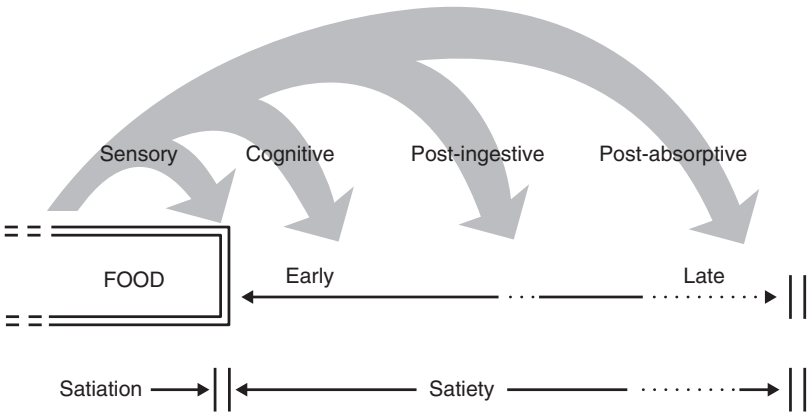

Figure 2
Illustration of the difference between satiety and satiation (Blundell *et al*. 1987).

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The study hypotheses and theoretical background of the study will determine the decision. Laboratory studies are not intended to replicate the free-living environment but rather to examine a number of aspects associated with appetite and eating behaviour, which are free from external influences typically present in the free-living environment. Laboratory studies allow specific factors to be isolated in order to study their effects on the expression of appetite (Blundell *et al*. 2009). On the other hand, free-living studies are extremely valuable for answering large-scale research questions in the natural environment. Different methods are required for collecting data in the laboratory vs the free-living environment. At present, the methods implemented to measure actual eating behaviour in the free-living environment are not comparable to laboratory techniques with regard to their validity and reliability; hence, the preference would be to measure appetite and eating behaviour under the strict, controlled conditions achievable only in the laboratory. The choice of approach represents a trade-off between internal and external validity or between precision and naturalness (Hill *et al*. 1995, Blundell *et al*. 2009). Early experiments investigating eating behaviour began with single foods to establish the basic relationships between variables involved in food consumption. Progression has been made from examining liquid diets, to single, bite-sized solid foods, to lunches and dinners that more closely represent what many people eat today (Stellar 1992). Since 1992, further developments have included examining food choice and food preferences, different eating situations, multiple meals and snacking behaviour. In addition, in order to address the gap in the literature between strictly controlled laboratory studies and free-living situations, there has been a move towards studying some of the social and environmental issues that may have an influence on eating behaviour under free-living conditions, but doing this under laboratory conditions, for example, eating while watching television and in contact with other people (Stroebele & de Castro 2004, Salvy *et al*. 2007, Temple *et al*. 2007, Robinson *et al*. 2011).

While progress has been made towards increasing the accuracy of free-living studies (see later), the uncertainty and heterogeneity in real-life measurement of eating behaviour require objective and quantitative research to be done within the laboratory. The following text will summarise terminology, study design and methodologies employed in laboratory studies measuring appetite and eating behaviour in humans, but will include a brief discussion of methodologies employed in the free-living environment.

Appetite terminology: satiety and satiation

Food consumption is episodic; it occurs in discrete bouts a certain number of times each day giving rise to a variety of patterns. In laboratory research, a single eating episode is often the focus of the study and has become a fundamental measure of eating behaviour. Of interest are the processes occurring around such an episode of eating and the processes involved in the initiation and termination of the eating episode. It is therefore paramount that studies incorporate measurements during appropriate time intervals, for example, preprandial, prandial or postprandial. The satiety cascade developed over 20 years ago identifies the overlapping processes occurring after food intake until the next period of eating (Blundell *et al*. 1987). The satiety cascade embodies two distinct processes: satiation and satiety. In turn, the satiety cascade highlights two phases of satiety – 'early' and 'late' that occur in the between-meal period. Satiety can be measured through subjective appetite ratings, biomarkers, such as appetite-related peptides, and measures of energy intake (Blundell *et al*. 2010). Satiation is a term used to describe within-meal inhibition, and the size of meal that the subjects are allowed to eat *ad libitum* can be sensitively measured under controlled laboratory conditions as a measure of eating behaviour. Satiety and satiation can be thought of as integrated processes but which can be separated theoretically and which permit different designs for separate measurement.

Study design and good laboratory practice

Procedures and methodologies to measure appetite and eating behaviour in studies within the laboratory setting, when conducted with the appropriate degree of scientific control, should result in high-quality data. Of importance is the application of an appropriate study design, which identifies the control and isolation of specific variables to test the research question under investigation. Alongside this, quality control over the actual methods employed in the laboratory environment is paramount. The study of human behaviour is never simple; therefore, experimental work should be governed by prescribed carefully monitored procedures applied in a standard and consistent manner on every occasion with every participant. Good laboratory practice (GLP) in the field of appetite control (as in branches of physiological sciences) should be ensured through the application of Standard Operating Procedures (SOPs).

Standard operating procedures

SOPs are of the utmost importance and they comprise clear and unambiguous statements describing the

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instructions for each task the researcher will conduct. Examples include the conduct between a researcher and a participant, directions on food preparation/weighing/cooking times, state of the environment and instructions to participants, all of which should be written in formal SOP documents. SOPs should be periodically updated and new versions authorised by an expert researcher. There should also be an SOP carrying instructions regarding the preparation of all other SOPs. All SOPs should be readily available for laboratory inspections. When more than one researcher is working on the same project, each should observe the others to ensure that all are carrying out the same tasks in exactly the same way. In addition, any calculations carried out on the data and the transfer of written data to electronic files should be systematically controlled through specific SOPs. Human error is inevitable; therefore, processes should be in place to minimise this and for additional experimenters to check and double check that recorded electronic data files are correct. These processes are essential to ensure that best quality data are collected and to avoid contamination through informal approaches or casual practice during experimental work. SOPs should be reviewed and maintained regularly in the light of methodological improvements.

Appetite and food intake laboratory specifics

Food intake laboratories are often purposely built facilities specifically designed to carry out measurements on appetite and eating behaviour by identifying a critical number of variables. At the centre of these laboratories, there is usually a metabolic kitchen for the accurate and hygienic preparation of test foods. These laboratories are also required to provide small rooms, cubicles or shielded spaces where participants can be provided with test foods in a secluded and controlled environment away from other distractions. It is important to insulate laboratory studies from the turbulence that exists in the natural free-living environment. Most studies will be designed in such a way that environmental and social factors do not interfere with eating behaviours. However, some studies will be designed to test the effects of social aspects or TV watching for example; therefore, group dining and other provisions may be required. Other measurements involved in the study should be carried out in rooms separate from the feeding cubicles. These can include anthropometric and physiological variables. Therefore, food intake laboratories differ from those that measure only eating to laboratories with specific rooms and allotted spaces for the measurement of energy expenditure, body composition, blood factors and metabolic activity. Whatever the structure be, all eating behaviour laboratories must share the same objective of strict control over experimental procedures that affect human behaviour.

Measuring satiety

Study designs focussed on satiety are required to measure the effects following consumption of food. The satiety cascade shown in Fig. 2 identifies a number of processes that occur after the consumption of food; therefore, it is important in satiety studies to minimise the number of variables changing simultaneously and, ideally, to ensure that only one variable differs between the active and control conditions. In experiments in which a specific food is the object of study, the manipulation of the active test condition should be sufficiently large to elicit differences when compared with the control condition (however, this may be beyond the experimenter's control in some instances). These types of food studies (sometimes called preload studies) usually provide the participants with a pre-test snack/meal/drink of fixed weight, volume, energy content, macronutrient composition, consistency, sensory qualities, etc. Only the single variable under investigation should be allowed to vary and all others should remain uniform between active and control conditions. In practice, this is extremely difficult to do, but it is important that participants are not able to detect differences between the two experimental conditions being compared. The general rule is to provide participants with experimental food(s) and give them a specific amount of time to consume the whole portion before a schedule of measurements takes place over the following hours, often with intake of a subsequent meal measured a few hours later (see Fig. 3; Rolls *et al.* 1991, 1994). The interval between the preload and the subsequent test meal needs to be realistic and anticipated with regard to the supposed action of the active variable. During this inter-meal period, participants should be supervised and monitored, whenever it is possible, to exclude the possibility of additional eating and drinking that would contaminate the study. The closer the resemblance between this schedule and normal eating patterns is, the more meaningful the results will be. For example, inter-meal periods of 3–4 h would be similar to the eating patterns that most study participants would follow on a daily basis. Obviously, shorter studies are easier for monitoring the participants within the laboratory; longer studies may require that participants have food intake

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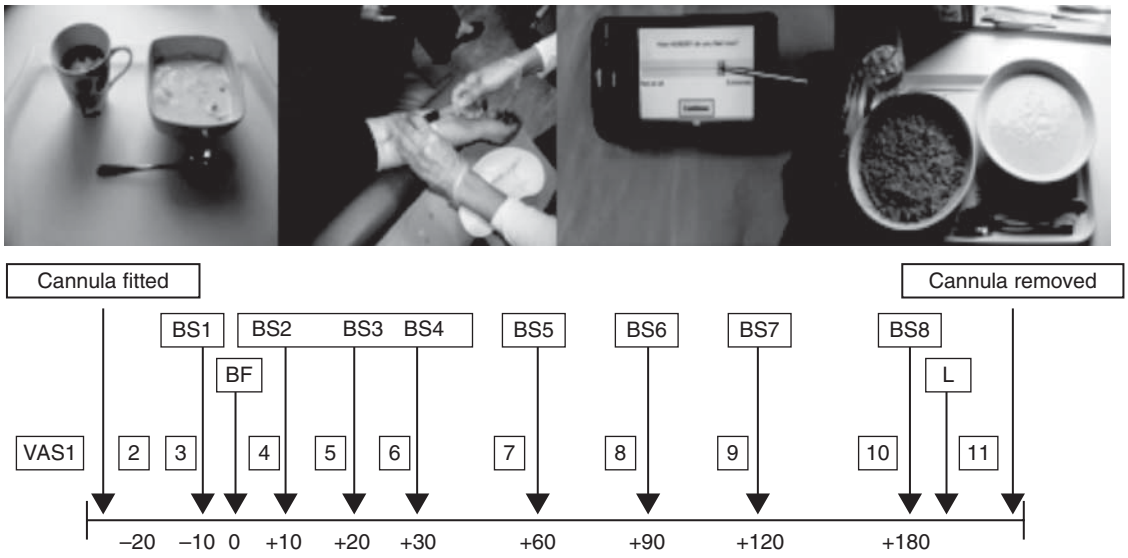

Figure 3
Schematic diagram of a systematic study measuring satiety and satiation. (BF, breakfast; BS, blood sample; VAS, visual analogue scale; L, lunch) as used in Gibbons *et al.* (2013).

measured within the laboratory, but are allowed to leave the laboratory between meals (with strict instructions of what is and is not allowed during the study period). In addition to the preload, the nature and structure of the subsequent test meal itself are crucial to the outcomes of the study. For example, varying the palatability of the test meals will affect the compensatory response to preloads that vary in size (Robinson *et al.* 2005) and having one large meal compared with a buffet style meal will also affect the results (Raynor & Epstein 2000). For more detailed information regarding the methodological aspects of eating frequency, the reader may refer to the review by Gatenby (1997). Owing to the large inter-individual variability in eating behaviour and perceptions of subjective appetite, these studies are optimally carried out with repeated-measures designs whereby all participants act under their own control.

Appetite ratings scales

Satiety can be measured through subjective appetite ratings and/or appetite-related peptides. Subjective appetite ratings (as a measure of the motivation to eat) are measured through visual analogue scales (VASs) and have been used in clinical and research settings to continuously monitor a range of subjective sensations such as depression, pain and appetite (Stubbs *et al.* 2000). These measures provide valuable information on sensations that are difficult to monitor using alternative methods (Gwaltney *et al.* 2008). VASs typically take the form of 100 mm horizontal lines anchored at both ends by extreme subjective feelings (Hill & Blundell 1982). This horizontal line represents a continuum and allows the participant to place a mark on the scale reflecting the intensity of a subjective sensation at a particular time (i.e. state), allowing the sensation to be measured and quantified. The interpretation of VASs is usually unambiguous as the descriptive terms are already present (Stubbs *et al.* 2000). VASs can be used to ask a variety of questions regarding appetite and often include four basic terms: hunger, fullness, prospective food consumption and desire to eat (originally devised and validated by Rogers & Blundell (1979)). Traditionally, VASs were administered using pen and paper (P&P), which was quick and relatively easy to use. However, data collection by the P&P method is often time consuming as each line needs to be measured manually and entered into a spreadsheet individually, a procedure that introduces the possibility of human error. To eliminate the problems of using P&P, portable handheld computers were developed in order to administer electronic appetite scales, which became the electronic appetite rating system (EARS) (Delargy *et al.* 1996). The transition to the use of handheld computers was driven by their relatively inexpensive cost and their associated practical benefits (Whybrow *et al.* 2006). Additional benefits of electronic VAS systems include the use of an audio alarm as a reminder of when ratings needed to be completed, leading to improved compliance rates (Hufford & Shields 2002). All entries can

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be date- and timestamped. The first EARS to be developed used a VAS software program that was designed to administer a VAS using a Psion handheld personal digital assistant (PDA) (University of Leeds, UK). This software employed a 100 mm horizontal line with a vertical marker present at the mid-point. The arrow keys were used to move the cursor left or right to a particular position. A comparison of the EARS with the standard P&P method used two different energy preloads to manipulate subjective appetite sensations (Delargy *et al.* 1996). Both techniques detected a significant difference between the high- and low-energy lunches. A number of electronic devices have now been validated for administering a VAS with the main development requiring the participants to use a 'stylus' to mark their responses on the screen of the device – which is ergonomically similar to placing a mark on a paper VAS using a pencil (Stubbs *et al.* 2001, Stratton *et al.* 1998, Whybrow *et al.* 2006, Gibbons *et al.* 2011; Fig. 4). All electronic devices can be accepted as a valid method for measuring appetite, but should not be used interchangeably with P&P. A number of studies have implemented the use of VASs to measure appetite and have shown a high degree of reproducibility (Delargy *et al.* 1996, Stubbs *et al.* 2001, Stratton *et al.* 1998, Whybrow *et al.* 2006), with a number of reviews commenting on their validity and reliability (De Graaf 1993, Flint *et al.* 2000, Stubbs *et al.* 2000). It is important to note that key experimental studies have confirmed the validity and reliability of the laboratory test meal procedure (Gregerson *et al.* 2008) and the use of VASs as a measure of the strength of the motivation to eat (Flint *et al.* 2000).

The satiety quotient

From hunger and fullness ratings, a calculation of a satiety quotient relative to the energy/weight content of the food provided can be carried out (Green *et al.* 1997, Chapman *et al.* 2005, Yeomans *et al.* 2005).

Subjective appetite and biomarkers

The strength of satiety can also be inferred through circulating levels of appetite-related peptides, such as ghrelin, cholecystokinin (CCK), glucagon-like peptide 1 (GLP1) and peptide YY (PYY) among others, which are expected to play a role in the short-term control of appetite. The level of ghrelin, regarded as being orexigenic, is high during periods of fasting before falling in response to food intake. The levels of CCK, GLP1 and PYY are low during fasting and increase in response to food

Figure 4
Example of an electronic appetite rating system currently used by the Human Appetite Research Unit at the University of Leeds (Caudwell *et al.* 2011).

consumption. Owing to the similar episodic patterns of these peptides and subjective appetite, they are often measured simultaneously as indicators or biomarkers of satiety. However, it has been noted recently that the evidence for a role of these peptides in short-term appetite is far from clear. It is known that when supra-physiological levels of these peptides are infused, this usually provides good evidence for their role in appetite and eating behaviour; however, their influence under normal circulating physiological levels, for example, in response to a normally consumed breakfast meal, is not so profound (Gibbons *et al.* 2013).

Considering the characterisation of the gold standard techniques to measure appetite-related peptides, these postprandial studies are extremely difficult and expensive to carry out. Many of the peptides degrade extremely quickly. It is therefore critical to 'treat' blood samples with protease inhibitors immediately following the collection of blood. Combinations of inhibitors are often necessary according to the range of peptides to be measured. SOPs should be in place to ensure whether all samples are exposed to exactly the same procedures, i.e. centrifuged and pipetted (separating the plasma component) before freeze-storing the samples, preferably at −80 °C until later analysis. There are a number of analysis techniques that can be employed to measure most of these peptides – for example, RIA and ELISA. More recent techniques allow 'multi-plexing', i.e. the measurement of a large number of peptides in one assay. These are becoming more cost-effective and are therefore useful in large-scale studies. Of primary importance is that the analysis is as systematic as possible, for example, all assay kits from the same manufacturing 'lot', all samples from the same participant completed on the same plate and so on. Quality control measures should be employed to ensure that samples are

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analysed similarly on all plates and that the coefficient of variation is as low as possible. Experienced laboratory technicians with previous knowledge of conducting the analysis using similar kits should handle these wherever possible. Data outputs from this kind of analysis should be checked thoroughly before data are stored in their final form. One interesting theoretical and methodological question is whether or not these peptide biomarkers provide more convincing evidence for satiety than do changes in rating scales.

Measuring satiation

Inspecting the satiety cascade indicates that satiation is the process that brings a meal to an end. This means that satiation reflects processes controlling the meal size (energy and/or weight). The frequency of eating has not been shown to differ between normal-weight and obese individuals (Bellisle *et al*. 1997), yet obese individuals consume more calories, therefore indicating the importance of meal size as a contributor to over-consumption and the development of obesity. When participants have been interrogated about their reasons for stopping eating, statements often refer to fullness and changes in perceived taste sensations (Hetherington 1996, Tuomisto *et al*. 1998). Clearly, the number and nature of foods provided are important, with single foods being more likely to elicit sensory responses whereas provision of several foods may divert the focus to other sensations. Researchers should be aware of how such decisions about study design can influence the responses of subjects and therefore the interpretation of underlying processes. It is also important that any foods provided are equally palatable to the participants; this should be verified during the screening process of the study. Consequently, the nature of the food materials provided exerts a significant influence on the measured strength of satiation.

Appropriate methodologies for measuring satiation consider a variety of parameters, for example, the properties of food and the environmental/contextual factors that may be involved in meal termination (Blundell *et al*. 2009). Even if foods provided are not particularly liked or disliked, people tend to consume most (if not all) of the food on their plate. From the satiety cascade, it is clear that the sensory properties of food and the palatability of foods will affect the meal size. It is therefore important that researchers choose experimental foods that are liked to a similar extent. Cognitive factors involved in meal termination imply that, from over thousands of eating episodes, we 'learn' about the satiating effects of food and can therefore estimate the amount needed of each food/meal to elicit satiation. Energy density is a particularly important parameter when providing meals to measure satiation. On visual inspection, portion sizes of low-energy-dense (salad, fruit, etc.) foods tend to be larger than portion sizes of high-dense foods (chocolate, peanuts, etc.). This feature of energy density is now well documented (Blundell *et al*. 1995), and it is important that satiation studies take this into account. Texture of food is also important, as liquid foods are consumed at a faster rate than solid foods (Zijlstra *et al*. 2007). Consequently, more calories are likely to be consumed when liquid meals are provided and subjects are allowed to consume them *ad libitum*; texture of test foods should therefore be a controlled variable when other aspects of food are under investigation. Cognitive knowledge concerning when the next eating episode will occur has been shown to have an effect on the termination of a meal, highlighting that people consume relative to the next availability of food (De Graaf *et al*. 1999). Researchers should incorporate into protocols whether participants are told about the timings of measurements or not; however, standard procedures are necessary and must always be governed by SOPs. When measuring eating behaviour, it is important that the participants are maintained in a similar state of satiety, as hunger is obviously a determinant of food intake. Participants have to fast (limit food and drink intake) for a long time before they are allowed to have a meal that they are permitted to consume *ad libitum* to ensure a similar level of hunger between participants.

Clearly, a number of factors (texture, energy density, palatability, appetitive state and cognitive factors) can influence satiation, and for a true test of meal termination, only one of these factors should be allowed to vary. However, some experimental designs can incorporate serial measures of satiety and satiation within a single experimental protocol. This could take the form of a fixed portion of food provided (active and control foods, for example, high fat vs high carbohydrate, but of fixed energy content, texture, palatability, etc.) before monitoring subjective appetite (and appetite-related peptides) over a fixed period of time (to measure satiety). Participants would then be provided with the same test meal on both the active and control condition days that they would be permitted to consume *ad libitum* (to measure satiation). One requirement of a meal provided for consumption *ad libitum* is the provision of ample food to prevent the participant from cognitively estimating how much has been consumed. One tactic is to provide large portions of food(s) to the participant with instructions that more is available if required.

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Measuring hedonic eating behaviour

The translation of 'food hedonics' into measurable, behavioural operations is not without its challenges. Successful procedures must encompass the ability to not only reflect the existence of the different components of reward, but also prevent confounding one component with another in order to allow for dissociations to be detected. Explicit measures of food 'liking' and 'wanting' most commonly use psychometric techniques such as numerical scales and VASs. Questions such as 'How pleasant would it be to taste some of this food now?' and 'How pleasant is the taste of this food?' are often used to measure explicit liking for food, whereas questions such as 'How strong is your desire to eat this food?' and 'How much do you want this food?' are often used for the assessment of explicit wanting. These techniques are limited by the accuracy of self-reporting and methodological issues such as 'end avoidance' and social desirability. However, if used carefully they can be quite sensitive to even subtle experimental manipulations and they frequently predict ingestive behaviours. While people tend to be very good at estimating and reporting their explicit 'liking' for food, they are often unable to accurately gauge their implicit 'wanting' for food. Implicit wanting concerns the core motivational aspects of reward-seeking behaviours. Therefore, the measures that reflect motivational responses to food and related cues can be said to contain at least an element of implicit 'wanting'. The more spontaneous the response is, the more that behaviour is likely to reflect the core process of 'wanting' without contamination from subjective processes. Importantly, implicit 'wanting' may not be adequately captured by the non-specific desire for food in general. Wanting implies a target with a direction, not just a force. In recent years, a range of techniques have been adapted or developed to assess more implicit forms of wanting. These methods tend to involve tasks that require an instrumental response such as a button press or mouse click in relation to the simulated or actual presence of food or food cues. Techniques tend to fall into one of the two categories. The first type depends on the subjects' willingness to expend effort in order to obtain a target food – usually something highly palatable and suitable to the subject's personal preference. These measures operationalise wanting as the reinforcing value of the food or how hard an individual is willing to work to gain access to food compared with an alternative reward (Epstein *et al*. 2007). The second type of technique depends on the compatibility of a food or food cue with a time-critical approach-related response. These techniques such as the Stroop task, the visual probe task and stimulus–response compatibility task measure reaction times following exposure to a food compared with the control or alternative food category (for example, Nathan *et al*. 2012). The resulting 'approach bias', affected by the attention grabbing/maintaining properties of the food and reflected in the speed of the response, is interpreted as a measure of motivational value or 'wanting' (Finlayson *et al*. 2008, Brignell *et al*. 2009).

Measuring appetite and eating behaviour in the free-living environment

Studies within the laboratory using appropriate stringent methodologies to measure human appetite and eating behaviour can reveal important findings. However, laboratory studies are not applicable to all study questions and cannot be used to explain all aspects of eating behaviour. It is argued that free-living studies do provide data from real people in real situations (Meiselman 1992), for example, in restaurants, cafeterias, food courts, school lunchrooms, sports bars, supermarkets and movie theatres. Army cadets are a group who have previously been used a number of times to investigate eating behaviour in a free-living but controlled environment (Widdowson *et al*. 1954, Edholm *et al*. 1955). For free-living studies on children, separate methodologies are needed to balance appropriate engagement with children's capabilities and levels of performance. Alternative techniques have been developed and refined by researchers such as Jane Wardle (Wardle *et al*. 2001).

However, free-living studies involve collection of complex data, which needs to be observed, coded, measured, analysed and reported (Schachter 1971). Studies of this type are difficult to gain ethical approval for, harder to staff and harder to analyse (Wansink 2009). It is also clear that studies measuring free-living eating behaviour are rife with unexplained findings and inconsistent results; therefore, the contribution of field studies is often regarded as lesser when compared with that of laboratory studies. Perhaps, the reason for this is the lack of agreement in establishing a set of procedures, methods and analyses in these types of studies (Wansink 2009), which would be similar to those used to measure satiety and satiation in the laboratory environment. Of primary importance in free-living studies becoming more reliable is the employment of a framework that uses consistent models, methods, measures and analyses across studies (Wansink 2009). The objective of this framework would be

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to heighten the sensitivity of measuring eating behaviour by increasing the effect sizes through the reduction of systematic variation. Identification of sources of noise is important in order to control the factors that might affect the results relevant to the hypothesis being tested. Even if these factors are not controlled, identifying them in the process allows the possibility that they can serve as covariates or enables appropriate *post hoc* tests to be carried out.

Selecting appropriate methodology can be complex in free-living studies. Methods used range from food acquisition (i.e. purchase of food products) to the assessment of food consumption (measured, inferred or recalled intake). Wansink (2009) identified different levels at which intake can be measured in the free-living environment, for example, pre-intake, post-intake and scenario-based measures of eating behaviour. Each has its merits and limitations, for example, measuring acquisition of food through scanner data can result in reliable data for foods consumed over short periods of time, whereas other foods may stay in storage and never be consumed. Moreover, the purchase of a product does not provide information regarding who actually consumed the product, which may range from one individual to an entire family.

Post-intake measures include recall of a person's eating behaviour by asking them what was consumed and when or by residual intake based on what food was not consumed from a given portion. Of course, these measures are dependent on a number of variables such as the participants having convenient access to a range of foods and, perhaps more importantly, rely on the participant's ability to remember what they have consumed, and their willingness and motivation to truthfully report all food and beverage items consumed. Furthermore, evidence indicates that recording food intake may result in the individual consuming less than they normally would due to an increase in self-monitoring (Baker & Kirschenbaum 1993, Goris *et al*. 2000). Dietary recall procedures, such as the United States Department of Agriculture's Automated Multiple Pass Method (AMPM; Moshfegh *et al*. 2008)), can reduce the influences of such issues. The AMPM is a frequently used semi-structured interview that takes between 30 and 45 min during which a trained researcher gathers information from participants on all food and beverage items consumed over the previous 24 h through five stages (see Table 1). Particularly useful in this technique is the use of visual aids to decipher portion sizes. For reliable data, this technique is recommended to be completed on separate occasions over a short period of time and to include at least 1 weekend day.

Scenario-based intake measures involve presenting consumers with a scenario from which they are asked to predict their intake under various manipulated situations. These studies are low in validity compared with actual measured food intake or even compared with reported food intake but may provide interesting information as a pre-study. However, further research is required in order to determine how closely results from these studies relate to actual food intake. Large-scale research on eating behaviour traits can also be carried out using psychometric questionnaires. These questionnaires can be employed as to obtain an estimate of eating behaviour and are a technique that can be used to gather a lot of information in a timely and cost-effective manner. Eating behaviour traits such as binge eating (Gormally *et al*. 1982), restraint (Stunkard & Messick 1985) and emotional eating (Van Strien *et al*. 1986) can all be measured through questionnaires. Further benefits of measuring traits such as these are that they are relatively stable and reflect a 'trait' rather than an appetite 'state' such as those measured using VASs.

Technological advancements

In the modern era, technological advancements are sought for a range of applications. New methodological

Table 1 The five stages of the Automated Multiple-Pass Method for 24-h dietary recall

Step	Procedure
Quick list	The participant freely recalls all of the food and beverage items they have consumed over the preceding 24-h period without interruption from the researcher
Forgotten foods	The researcher cues recall of nine frequently forgotten food categories that include non-alcoholic and alcoholic beverages, fruit, cheeses and bread items
Time and occasion	The reported food and beverage items are reviewed and each item is assigned to an eating occasion (e.g. breakfast and snack)
Detail and review	Detailed information is gathered about brand names, recipes, portion size, added items (including condiments and fats), source (homemade or pre-packaged) and location of consumption
Final probe	The participant reviews the information and is given the chance to recall any foods they may have missed, or report any small items of food they may not have felt as worth reporting. Finally, they assess whether their reported food intake was more, less or typical of their habitual intake

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developments are helping to bridge the gap between relatively controlled laboratory studies for measuring appetite and eating behaviour and the more difficult to control free-living environment studies. For example, the measurement of subjective appetite using VASs on P&P has limited flaws when the participants are closely monitored in the laboratory, but in free-living situations the P&P method has considerable limitations; for example, when unsupervised, compliance is low (Stone *et al*. 2002) and questions may be omitted, wrongly marked or not filled in at the correct time resulting in invalid data (Stratton *et al*. 1998). However, the newly developed electronic devices are now being used for the measurement of subjective appetite and, as these devices have the capacity for researchers to set alarms for participants to fill them in, there is no reason as to why these cannot be employed in free-living studies.

Further to this, there are a number of mobile phone applications that allow dietary records to be stored more easily than traditional P&P techniques, as they allow photographs of consumed foods to be taken and for portion sizes to be weighed and recorded (Tsai *et al*. 2007, Martin *et al*. 2009). Such advances are facilitating the more accurate assessment of appetite and eating behaviour in the free-living environment.

Overview

To summarise, there are advantages and disadvantages of both laboratory and free-living studies. While there have been some developments in laboratory studies becoming more realistic and free-living studies becoming more controlled, there is a strong argument that there is room for both types of study, as they each answer different questions using different methodologies (Kissileff 1992). Perhaps, combining the measurement of actual eating behaviour under laboratory conditions and free-living eating behaviour through dietary recall or questionnaires measuring traits of eating behaviour is where future research should be focussed, for example see Dalton *et al*. (2013).

This manuscript has identified the 'gold standard' methodologies for the study of human appetite and eating behaviour, but it is also important to understand how the use of these designs and techniques has resulted in vital findings regarding appetite and human eating behaviour. Through complex, multi-level study designs, we have been able to show a relationship among fat-free mass, resting metabolic rate and meal size. Additionally, the relationship between appetite-related peptides under normal feeding conditions has revealed relationships between ghrelin and GLP1 with subjective appetite and eating behaviour. These relationships have only been fully established using the most appropriate study design and gold-standard methodologies.

In order for the measurement of human appetite and eating behaviour to be reliable and valid, the following steps are advised. First, a true understanding of an appropriate study design to answer the research question must be in place. Secondly, the selection of standardised methodologies and procedures to collect and store the data must be agreed. Finally, the conduct of the researchers both in their use of study techniques and participant interaction must represent GLP. Only when these steps are fulfilled will there be confidence in the generated data.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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