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Symposium on 'Measuring nutrient intake'*

Methodological issues relating to the measurement of food, energy and nutrient intake in human laboratory-based studies

R. J. Stubbs[1†], A. M. Johnstone[1], L. M. O'Reilly[1] and S. D. Poppitt[2]

[1]*Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, UK*
[2]*Department of Medicine, University of Auckland, Auckland, New Zealand*

The relevance of measuring intake to the nutritional and behavioural sciences

Understanding the mechanisms controlling human appetite and energy intake (EI) is fundamental to nutritional science since it is through a balance between EI and energy expenditure (EE) that body weight and composition are maintained and functional integrity is sustained. The recent resurgence of interest in the study of feeding behaviour and the physiological control of food intake (FI) is mainly due to the rapidly increasing proportion of overweight and obese individuals in Western society (White *et al.* 1991; Department of Health, 1995). It is widely accepted that the uncoupling of EI from EE is largely responsible for these secular trends in body weight. The ability to measure food, energy and nutrient (FEN) intake is critical to our understanding of the processes producing these secular trends. The mechanisms controlling feeding are multifactorial and complex in nature and are generally characterized by an on-going interaction between physiology and behaviour. In recent years an increasing number of studies have incorporated both behavioural and nutritional measures of intake in attempting to understand the quantitative importance of a number of factors (e.g. diet composition, exercise, disease) thought to exert important influences on appetite and energy balance (EB).

The measurement of intake in the laboratory is also critical in establishing mechanistic links between other aspects of diet and disease. The relationship between dietary antioxidant intake and oxidative damage to somatic DNA, the effects of dietary lipid profile on immune function and the influence of fibre on mineral bioavailability are three of many possible examples. Such carefully controlled laboratory protocols are crucial in establishing the existence and importance of diet in the promotion of health and the development of disease.

There are, thus, two basic forms of intake measurement in the laboratory. The first allows subjects to control their own FEN intake within the constraints of the experimental design. Under these conditions measures of FEN intake result from the subject's behavioural response to the experimental manipulation and any other factors which may influence their behaviour at the time. The second type of FEN intake measurement in the laboratory is a measure of a fixed mandatory intake (termed 'fixed-intake' studies here). In such studies the subject's feeding behaviour has been clamped and the measurement is not as susceptible to 'behavioural noise' provided the subject is compliant with the protocol and the experiment is rigorously designed. Fixed-intake studies are therefore less fraught with methodological difficulties relating to the effects of the experimental environment on behaviour. The majority of errors under these conditions will be of a technical nature, while studies which allow subjects to alter their own intakes are subject to both technical and behavioural errors. Because behaviourally-oriented studies are subject to both types of error, these designs will be given most attention in the present discussion.

Why measure intake in the laboratory? The investigator's dilemma

It is useful to consider why researchers should attempt to measure FEN intake in the laboratory at all, since intake can be measured in a number of settings which are more pertinent to the normal feeding behaviour of human subjects (Meiselman, 1992). Epidemiological and dietary survey studies have the advantage over laboratory studies of using large numbers of subjects who are going about their everyday activities in their natural setting (Colditz *et al.* 1990; Black *et al.* 1991; Bingham *et al.* 1994; Briefel *et al.* 1995). The ecological

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validity of such studies is theoretically high. However, there are a number of methodological problems associated with epidemiological and dietary survey studies which inevitably limit the conclusions derived from them. (1) The errors in data collection are high and are not necessarily random (e.g. under-reporting of EI in the obese; Schoeller, 1990). (2) In many studies subjects are not randomly selected and the population is not always entirely representative of the general population. (3) Many epidemiological studies are cross-sectional and assume that the processes influencing the phenomena under investigation are uniform over time and hence over the age groups who constitute the cross-sectional population (Lissner & Heitman, 1995). Clearly this is not always the case. (4) It has recently become apparent that ecological studies which rely on self-reported dietary intakes are subject to mis-reporting, which itself is biased towards under-reporting (Schoeller, 1990; Black *et al.* 1991). (5) Other (unmeasured) variables may exert important confounding effects on relationships under investigation. (6) The 'environment' in which data are collected for ecological studies, while often claimed to be 'naturalistic' may not actually represent the environment in which subjects spend most of their lives or consume most of their FEN intakes.

Intervention studies often represent a good compromise between the artificiality of the laboratory and the lack of control over both manipulation and measurement that occurs in epidemiological studies (Gatenby *et al.* 1995; Aaron *et al.* 1998; Zimmermans & van Het Hof, undated). Typically, subjects adhere to a given manipulation (e.g. consuming a number of low-fat foods made available *ad libitum* by the investigator) but go about their normal lives so that the impact of the manipulation, (e.g. on fat and feeding behaviour) can be assessed. Because they share the techniques of ecological and laboratory studies, intervention studies also share the errors, artifacts and limitations of studies conducted in both environments.

It is often claimed that a key advantage of laboratory measures of intake is that they afford a far greater degree of control over the intervention, the experimental environment and the outcome measures being made. The laboratory enables rigorous control of nutrient intake in studies where it is essential that all components of the diet are known and where dietary intake is controlled. Furthermore, laboratory studies facilitate detailed, sometimes invasive measurements that cannot be achieved in either epidemiological or intervention studies. Laboratory measures of nutrient intake are essential to establish the quantitative relationship between nutrient intake and physiological and/or behavioural outcomes which can only be hypothesized from the results of less tightly controlled epidemiological studies. In the laboratory, outcomes (e.g. hunger, energy and nutrient intakes) can be measured with considerable precision and accuracy and in good experimental designs there is little contamination from extraneous influences. However, in the laboratory, control of inputs and environment may also constrain subject responses, and it is not always clear whether control of the experimental situation is as comprehensive as is supposed by the investigator.

Laboratory studies of dietary intake are also bedevilled by a number of serious constraints which limit their external (or ecological) validity (Spitzer & Rodin, 1981; Hill *et al.* 1995; Blundell & Stubbs, 1997). Experiments typically use small numbers of non-randomly selected subjects in the artificial environment of the laboratory using protocols and techniques which are often unfamiliar to the subject. It is therefore important to understand the limitations of the laboratory approach when attempting to extrapolate the results of experiments conducted in the laboratory (where the signal : noise ratio may be artificially elevated) to everyday life. The clinical setting can also be employed to make very precise and accurate manipulations of energy or nutrient intake, for instance by infusing pure nutrients intravenously (e.g. Gil *et al.* 1991). This experimental environment can, therefore, be even more artificial than the laboratory setting and can be subject to additional errors, artifacts and constraints. Because the experimental setting can influence the variable under investigation it is advantageous to assess the effect of various influences on FEN intake (e.g. the effect of dietary fat on EI) in different experimental environments. If broadly similar phenomena are apparent in each of these experimental conditions it is reasonable to accept that the phenomenon under scrutiny is robust and not an artifact of the experimental conditions themselves (Blundell & Stubbs, 1997). These considerations suggest that there is good reason for measuring FEN intake in the laboratory since this experimental setting has certain advantages over more ecological (real-life) settings. The laboratory also has a number of disadvantages when compared with more ecological studies and cannot, therefore, replace those studies, but can provide crucial experimental data to complement them. In general the internal validity of laboratory studies is high but their external validity can be limited, compared with more ecological (naturalistic) studies.

What is being measured: energy and nutrient intake or feeding behaviour?

Whether a person forages in the bush, laboratory or the supermarket, he or she generally seeks, obtains, selects and ingests food rather than nutrients *per se*. If asked to describe their foraging or feeding behaviour, few if any subjects would outline a profile of energy and nutrients which they were selecting or ingesting, nor would they be able, except in the broadest terms, to make informed estimates of their current state of energy and nutrient balance. However, it is implicit in numerous scientific reports concerned with the measurement of *ad libitum* dietary intakes that subjects are selecting energy and nutrients with a hypothesized goal of regulating the balance of one or more of these variables during the time-course of the experimental protocol. Subjects may select and ingest certain amounts of specific foods for a variety of reasons that do not relate causatively to the energy and nutrient intake results calculated by the investigator. These computations are often treated as the primary outcome of the experiment. They are not. They are the secondary outcome of the subject's feeding behaviour. As far as the subject's motivations and intentions are concerned, energy and nutrient intake values may be a coincidental outcome of their feeding behaviour, itself possibly determined

by other influences (e.g. preference, food avoidance or prior learned experience). As discussed later, these concerns become greater as the time window of measurement contracts.

The logic and assumptions behind the behavioural, nutritional and physiological measures made in the laboratory

What is being measured and why?

The main assumption in studies of human feeding behaviour and EB is that behavioural, nutritional and physiological variables are causatively linked (Blundell, 1995). The major objective of measuring these variables in the laboratory is to establish the existence and magnitude of causative relationships between them. The same is true for studies where intakes are fixed; only the behaviour (pattern of FEN intake) is assumed. A number of models for the role of nutrients in EB regulation assume that behavioural changes in feeding are a direct consequence of unconditioned physiological signalling (Mayer, 1955; Mellinkoff et al. 1956; Fernstrom & Wurtman, 1972; Flatt, 1987). This is, however, not necessarily correct. Eating behaviour comprises a large learned and anticipatory component, such as timing of ingestive events (meals or snacks), and whilst physiological events, such as changes in plasma glucose (Van Itallie et al. 1952) or altered gastric emptying rate (Hunt, 1980) may correlate with altered energy and nutrient intakes, it does not necessarily follow that the relationship is directly causative. Additionally, the demonstration of a relationship between a measured physiological change and an alteration of feeding behaviour may not resolve the issue of which caused which.

Measurements of feeding behaviour

Dietary intake is essentially a behavioural phenomenon in that changes in energy and nutrient intakes are the result of a change in feeding behaviour in terms of meal size, frequency or the composition of foods selected. In fixed-intake studies a certain behavioural profile is assumed. In laboratory studies of feeding behaviour, specific aspects of the appetite control system are manipulated at the cognitive (Booth et al. 1982), sensory (Rolls et al. 1981; Johnstone et al. 1998a), gastrointestinal (Welch et al. 1985; Stratton et al. 1998) or even the metabolic level (Thompson & Campbell, 1977; Gil et al. 1991) by, for example, deceiving subjects about the energy content of foods, altering the sensory variety of the diet, administering nasogastric infusions or parenteral infusions respectively. Researchers are careful to control possible confounding influences out of the experiment. It is often assumed that any response is attributable to that part of the system that has been manipulated (e.g. sensory stimulation through altering the sensory variety of a nutritionally controlled diet). In reality any response is due to those parts of the system which have been manipulated under those experimental conditions and operating in the absence of those factors which have been controlled-out of the experiment. This point should be remembered when extrapolating laboratory data to processes operating in everyday life.

Nutritional measures in laboratory studies of intake

Both energy and individual nutrient intakes can be measured in the laboratory. In reality the majority of studies measure changes in FI and estimate changes in energy and nutrient intake from standard food tables. Estimation of nutrient intakes from standard food tables involves a number of assumptions including the use of average metabolizable energy coefficients, the errors from which are not always negligible (Bingham, 1987).

It is sometimes claimed that epidemiological and diet-survey studies are bedevilled by problems of misreporting while laboratory studies are uncontaminated by these behavioural artifacts. However, any study which relies on self-reported intakes of human subjects will be susceptible to two potential errors: misreporting and altered feeding behaviour in response to the demand characteristics of the experiment. A recent study (Poppitt et al. 1996) has demonstrated how subjects under-reported alcohol and fat intakes when asked to recall the amounts of foods which they ate in the laboratory (the intakes of which were covertly measured) on the preceding day. Subjects may also alter their feeding behaviour in the laboratory in relation to their perception of the experimenter's expectations, concern about their own feeding behaviour or other influences of the experimental environment. The use of within-subject designs helps to minimize these errors since the experimental design may bias EI on a certain direction, but if the experimental environment is constant, this effect should be of a similar magnitude on different treatments or conditions. While the plausibility of unusual EI can be roughly assessed by reference to the expected energy requirements of the subject in diet surveys, this approach requires greater caution in the laboratory, where a number of factors (e.g. unfamiliar diets) may lead to unusual FEN intakes.

It may be useful to distinguish between the cognitive effect of under-reporting and the technical discrepancy of misrecording. In a recent study subjects were given ad libitum access to thirty-nine common supermarket foods, and asked to weigh all foods before and leftovers after ingestion using a PETRA weighing system (Johnstone et al. 1998a). These foods were also independently weighed before and after ingestion by the investigator. Intakes of weight, energy, fat, water and fibre were up to 10 % lower when estimated by subjects themselves than when estimated by the investigators' independent record of their own weights. Thus, an ostensibly similar procedure produced technical discrepancies of 10 % for some (but not other) dietary variables.

Physiological measures made in intake studies

Protocols relating dietary intakes to physiological or behavioural outcomes require intensive physiological investigations which may range from the analysis of blood samples for levels of substrates and hormones (e.g. Yamada, 1985), to long-term measurement of changes in body composition (Keys et al. 1950). Physiological changes thought to be important in controlling feeding behaviour are often measured in relation to an experimentally-induced change in that behaviour (Van Itallie et al. 1952; Smith & Gibbs, 1985; Stubbs et al. 1995). The demonstration of a correlation

between a behavioural outcome and a physiological signalling system does not necessarily demonstrate that the physiological change induced the behavioural change. For example, we have found that there is a significant inverse correlation between carbohydrate oxidation and hunger in the inter-meal period, when subjects are given, *ad libitum*, low-, medium- or high-fat diets (Stubbs, unpublished results). Glucose oxidation is thought to be an important process underlying metabolic satiety signals (Van Itallie *et al.* 1952; Mayer, 1955; Thompson & Campbell, 1977; Raben, 1995). However, carbohydrate is usually the main substrate being oxidized in the inter-meal interval. Other signals such as gastric emptying and nutrient absorption from the gut may also be involved and a conclusion that carbohydrate oxidation was the main signal underlying satiety in the inter-meal period would be premature. In reality carbohydrate oxidation was the main putative signal that we measured and detected in the experiment, which may play a role in the maintenance of inter-meal satiety. Studies that have manipulated the putative signalling system and produced directional changes in feeding consistent with the hypothesized role of that signalling system strengthen the case for its involvement in appetite control. For example, it has been shown that pharmacological inhibition of glucose metabolism has been shown to increase hunger and food intake in human subjects (Thompson & Campbell, 1977). It follows from this that no single laboratory study of intake is likely to produce conclusive findings that unequivocally demonstrate the role of a physiological system in appetite control.

A number of studies do manipulate putative signalling systems and examine their effects on subsequent feeding behaviour. It does not automatically follow that the outcomes of such experiments are directly relevant to normal feeding. For example, it has been demonstrated that lipid infusions into the small intestine of human subjects lead to potent suppression of feeding, perhaps mediated by intestinal lipid receptors (Welch *et al.* 1985). However, the stomach normally regulates delivery of nutrients into the small intestine on an approximately energetic basis (Hunt & Stubbs, 1975; Hunt, 1980). Could the potent suppression of fat intake by intestinal lipid infusions be due to the supra-physiological saturation of lipid receptors in the experiments concerned? Manipulations of putative signalling systems are often conducted at or beyond the extremes of the physiological range. The results of such studies should be considered in relation to the likely physiological role of such systems in the normal feeding behaviour or functioning of the unencumbered subject. The manipulation of one aspect of physiology in an intact subject is also likely to influence other physiological processes. For instance it has been shown that parenteral nutrient infusions can slow the rate of gastric emptying (de Myttenaere *et al.* 1994). It is also important that experiments using extensive physiological measurements do not clutter the protocol to the extent that they constrain the feeding behaviour under investigation.

There is, of course, considerable interaction between physiological signalling and eating behaviour, although as previously discussed, feeding behaviour contains a large learned component and as such is not controlled entirely by current minute-to-minute physiological signals (Blundell &

Stubbs, 1998). The failure of a physiological manipulation to influence feeding behaviour may not be due to the non-involvement of that system in feeding behaviour, it may be due to the fact that the subject is responding during the experiment on the basis of previously learned experience. Thus, the measures made of outcomes in laboratory studies of FEN intake are not as straightforward to interpret as they might ostensibly appear. Careful consideration needs to be given to study designs and the measurements made. Interpretation of results should emphasize errors and limitations in the methodology used and avoid over-generalizing results to everyday life. There are numerous methods that have been used to measure intake which are discussed in the following section.

Methods of measuring food energy and nutrient intake and feeding behaviour in the laboratory

The measurements that are made in laboratory studies of food and nutrient intake

The major methods of measuring FI in the laboratory and brief descriptions of their advantages and disadvantages are shown in Table 1. We have divided these methods, for the sake of categorization, into (1) direct quantitative measures of FI, (2) semi-quantitative measures of motivation to eat and measures related to feeding behaviour and (3) techniques that are used to validate and/or verify laboratory measures of FI and feeding behaviour.

Quantitative measures of food intake. Methods of directly measuring FEN intake in the laboratory include continuous weighing of foods (Hill *et al.* 1995), food dispensing machines (Silverstone & Fincham, 1978; Silverstone *et al.* 1980; Wurtman & Wurtman, 1981; Rising *et al.* 1992) and laboratory weighing of food before and after consumption by volunteers (Cotton *et al.* 1994; Stubbs *et al.* 1995; Poppitt *et al.* 1996). Continuous weighing of foods requires specific equipment (e.g. the Universal eating monitor) where the investigators can monitor the changes in weight of food as it is eaten using a concealed electric balance on which the eating vessel is positioned. This allows an accurate continuous measurement of FI over time. If used over a number of meals in sequence the apparatus is a valuable means of quantifying meal size, frequency and intra-meal rate of ingestion. It is restricted by its use only within the laboratory and limits the experiment to the use of one food at a time (Hill *et al.* 1995).

The use of solid food units which are small bite-size portions of food presented in excess out of view of the subjects, creates a situation where individuals are not subject to cognitive and visual cues that normally help self-monitoring of FI (Spiegel *et al.* 1989; Spiegel & Stellar, 1990). This approach is again limited in the nature of the food used (often sandwich items). The use of liquid diets where subjects also cannot view the foods presented and thus are prevented from assessing quantity consumed and quantity remaining also deprive subjects of cognitive and visual control (Campbell *et al.* 1971). These studies may also provide subjects with a source of energy and nutrients which are not integrated into the food

Table 1. Major methods for measuring food intake in the laboratory, with brief descriptions of their advantages and disadvantages

Experimental environment	Experimental manipulations	Advantages	Disadvantages	Example reference
Direct quantitative measures of food intake				
Continuous weighing (Universal eating monitor)	Using specific equipment, investigators can monitor the changes in the weight of food as it is being eaten. This is achieved by the continuous weighing of the subject's plate with a concealed electric balance on which the eating vessel rests.	Accurate readout of changes in the weight of food consumed. Is a useful device to assess the impact of novel foods on appetite control, for solid and liquid foods alike. Continuous monitoring of intake yields a cumulative intake curve over time.	Can only be used in the laboratory environment – limited numbers of volunteers. Short-term studies (within day) only. Only provides information about rate of food disappearance from the eating vessel. Food choice cannot be studied. Expensive equipment required.	Kissileff *et al.* (1980) Hill *et al.* (1989)
Laboratory weighing	Volunteers are given a selection of foods to choose from, which have been pre-weighed and recorded by the investigator.	Allows quantitative data (amount of food eaten) and qualitative (nutrients) when the food composition is known. Allows more accurate measurements to be recorded, particularly over longer periods of time (weeks).	Very labour intensive and there is a large amount of waste if fresh produce is used. Expensive. When a selection of food items is offered this may restrict choice. Laboratory environment can create methodological artifacts.	Blundell *et al.* (1993) Stubbs *et al.* (1995) Poppit *et al.* (1996)
PETRA (portable electronic tape recording automated) scale	A weighing scale combination with a tape recorder enabling subjects to record a verbal description of each food item.	It is a user-friendly device. Weights of food eaten are automatically digitally recorded, thus avoiding errors in reading and recording weights from dietary scales.	The raw data require more effort to analyse compared with more conventional weighing methods. Subjects still need to keep a food diary if additional written record of intake and/or time of eating is required.	Bingham (1987) Barker *et al.* (1988) Johnstone *et al.* (1998*a*)
Weighed food intake and food diary	Aims to assess daily variation in intake. Subjects given a set of electronic scales to record weight of food consumed and leftovers. In addition, a food diary should be used to record time and name of food intake.	Experiment can be easily carried out in a naturalistic setting. Particular use in epidemiological studies where a 7 d record is used to determine nutrient intake.	Labour-intensive for subject. Scales with a taring facility can cause confusion and lead to inaccurate records. Validity of records may decline as the length of recording increases. Between 3 d and 14 d records required for different manipulations. Can lead to alteration in feeding patterns for convenience or due to demand characteristics of the experiment.	Bingham (1987)
Chemical analysis (i) Duplicate diets (ii) Aliquot sampling (iii) Equivalent composite	(i) Duplicate portions of all food and drinks are collected for chemical analysis. (ii) The volunteer keeps a weighed record of intake and keeps aliquot samples of food for later analysis, or the laboratory weighed intake is replicated at a later date. (iii) The volunteer keeps a weighed record of intake. The investigator makes up a composite sample.	Useful for calculating intake of nutrients which are not published in food tables or variable due to seasonal or cooking effects.	Very expensive technique. Can influence actual feeding behaviour. Most appropriate under some conditions (e.g. detailed studies, where *n* is small), less appropriate with large populations. Particularly useful for post-hoc validation of actual nutrient composition of foods ingested, by chemical analysis of duplicate diets.	Ralph (1993)
Liquid diet assessments	Subjects have *ad libitum* access to a nutritionally controlled liquid diet, the composition of which is often covertly manipulated. The amount of liquid consumed is often concealed from the subject.	Creates a situation where subjects are deprived of usual cognitive and visual cues which help self-monitoring of food intake. Allows very tight control of nutritional interventions. Easy to quantify intake by weighing. Particularly relevant in the clinical setting.	Feeding situation is highly artificial. Diet can be monotonous. There is some debate as to whether the regulation of fluid balance via thirst mechanisms contaminates the assessment of feeding behaviour when using liquid diets. Limited number of manipulations and situations in which it can be used.	Jordan *et al.* (1966) Jordan (1969) Campbell *et al.* (1971) Stratton *et al.* (1998)
Fixed portions (solid food unit)	Small bite-sized pieces of food, typically spiral shaped sandwiches, are presented in excess to subjects. They are typically presented in a covered box with a circular hole for hand access.	Creates a situation where subjects are deprived of usual cognitive and visual cues which help self-monitoring of food intake.	An adaptation session is necessary to familiarize subjects with the technique before the manipulation. Feeding situation artificial but useful for within-subject design, preloading tests.	Spiegel *et al.* (1989) Spiegel & Stellar (1990)

Table 1. *Continued.*

Experimental environment	Experimental manipulations	Advantages	Disadvantages	Example reference
Food dispensing machine	Adaptation of commercially available vending machines to provide food items 'ad-libitum' combined with laboratory weighing	'Real' food items can be offered with the frequency, quantity, choice and time of eating recorded automatically if it is linked to a computer system.	Can only be used in the residential laboratory environment. Necessity for detailed instructions, for example to eat foods immediately. Use of foods is limited and therefore not suitable for total *ad libitum* studies.	Silverstone & Fincham (1978) Silverstone *et al.* (1980) Wurtman *et al.* (1981)

Semi-quantitative measures of motivation to eat and measures related to feeding behaviour

Experimental environment	Experimental manipulations	Advantages	Disadvantages	Example reference
Observed eating style	Investigator observes and records aspects of eating style, such as total number of bites, bite size, number of chews per mouthful, number of chews per meal.	This information has been used to try to define lean–obese differences in obese eating style, but there is no clear distinction between the groups.	Findings difficult to interpret. Qualitative information only, little quantitative data. Useful for ecological evaluations. Interventions are not possible.	Hill (1974) Rogers & Blundell (1979) Spitzer & Rodin (1981) Hill *et al.* (1995)
Hunger and appetite ratings	Subjects record feelings such as hunger, appetite or fullness on a visual analogue rating scale. These are horizontal lines (100 mm) anchored at one end with an extreme state such as 'not at all hungry' to 'as hungry as I have ever felt'.	Relatively easy for volunteers to complete and can be used for long-term studies to monitor subjective feelings. The use of a hand-held computer increases ease and reliability of data-capture. Provide important supplemental information to measures of feeding behaviour.	Appetite ratings are not a good proxy for food intake. In the laboratory they tend to be more sensitive in response to an intervention than actual changes in behaviour. For detailed analysis appropriate statistics are required. Do not necessarily predict food intake in free-living subjects. Show good correlation over time with changes in some physiological variables.	Blundell (1979) Hill & Blundell (1982) Mattes (1990) Stubbs *et al.* (1997)
Salivation	Dry cotton swabs are weighed and placed in the mouth of the subject for a fixed time interval. The wet swabs are removed and weighed. Change in weight is an index of saliva production.	Has been claimed to be an objective assessment of hunger or motivation to eat. Relatively short-term, inexpensive measurement.	Can be intrusive to volunteers, especially in *ad libitum* feeding studies. Difficult to establish any quantitative relationship between salivation and food or energy intake or between salivation and subjective hunger. Salivation can be influenced by some treatments under investigation e.g. anorectic drugs.	Wooley & Wooley (1973, 1981)
Subjectively expressed food choice	Subjects are asked to complete a forced choice checklist stating which of a number of paired foods they would choose between. Foods in the list can be structured in relation to diet composition or sensory characteristics. Alternatively subjects are asked to list the foods they would most like to eat at that moment in time.	Allows subjects to express motivation to eat in relation to a variety of familiar foods that may not be available in the laboratory. Inexpensive. Good for estimating possible responses in motivation to select certain types of food.	The relationship between expressed food selection and actual food selection is not understood. Subjects may select foods in relation to factors not related to the experimental manipulation e.g. general preference.	Hill *et al.* (1986)
Palatability of food	Subjects complete a visual-analogue rating 15 min after consuming a meal. Questions ask about pleasantness and satisfaction of the food consumed.	Easily implemented technique to evaluate subjective perception of food. Sensory variables can be quantitatively expressed by the subject. Pleasantness assumed to be symptomatically related to palatability.	Palatability can also be influenced by the previous experiences of the individual via learning. Also, external influences (e.g. experimental environment) can influence ratings at the time of eating.	Hill & Blundell (1982)
Restraint, emotionality and externality of eating	Commonly used questionnaires are the Dutch eating behaviour questionnaire and three-factor inventory	Psychometric questionnaires which can pseudo-quantitatively assess influences of variables in relation to food, energy and nutrient intake. Shown to be sensitive in different groups of subjects e.g. lean–obese and male–female.	Does not give a quantitative estimation of the variables or how they will quantitatively affect measures of food intake.	Stunkard & Messick (1985) Van Strien *et al.* (1986)

Table 1. *Continued.*

Techniques used to validate laboratory measurement of intake and feeding behaviour

Experimental environment	Experimental manipulations	Advantages	Disadvantages	Example reference
Body weight as an index of energy balance	Subject weighed on calibrated scales and weight gain/loss (corrected to nude) used as a proxy for energy balance (EB).	Can be used as a check when energy intake (EI) and/or expenditure (EE) is also known.	Minimum of 7–10 d record for this method to provide indications of changes in EB. Time of weighing, clothing and emptying the bladder need to be accounted for. Errors can be very large.	Prentice *et al.* (1985)
Labelled bicarbonate	Subject wears a small pump which infuses labelled bicarbonate (^{14}C). Will provide daily measurement of EE which can be applied to check EB.	Allows objective assessments of 24 h EE, which can be invaluable in laboratory studies. Of particular value in studies concerned with control of EB and the day-to-day relationship between EI and EE.	Uses radiolabelled bicarbonate. Is invasive and requires a continuous subcutaneous microinfusion.	Elia (1991)
Doubly-labelled water (DLW)	The use of DLW to independently measure EE, together with estimates of physical activity as multiples of BMR can be combined to assess plausibility of intakes with reference to misreporting over periods of 10 d–3 weeks.	This is an objective means of demonstrating non-compliance in long-term studies where EI and body weight are both continually monitored. Of particular value in studies concerned with control of EB.	Does not quantify mis-reporting; it assesses the plausibility of EI data assuming subjects to be in EB. Tends to ignore over-reporting. Is only approximate in most studies. Only useful in longer term studies.	Prentice (1990)
Biomarkers: PABA	A known amount of PABA can be incorporated into foods when intakes are mandatory. Total recovery of PABA indicates compliance. For *ad libitum* studies a fixed concentration of PABA in a diet would be required.	Can be of particular use in demonstrating compliance in metabolic balance studies where total urine collections are made routinely. Potentially useful for validating intakes on diets of a constant measurable composition.	Incomplete urine collections could erroneously suggest non-compliance. Cannot be used in common food items.	Bingham (1987)
Biomarkers: N	Urine samples (24 h urinary N for protein intake).	As with all excretion methods, additional markers (e.g. PABA for urine) should be used for checking completeness of collection. Good to validate intake but poor proxy for intake – as only suggests EI based on assumed constancy of protein : energy ratio of the diet.	Not always an acceptable method for the general public. Problems with loss of collection. N balance is a good proxy for EB if an independent assessment of food intake (e.g. weighed records) is used. Rough guide only. Assumes subjects are in energy and N balance.	Bingham (1987)

PABA, *p*-amino benzoic acid.

matrix as is the case with solid foods. Interestingly Campbell *et al.* (1971) showed lean subjects to compensate, while overweight subjects without exception lost weight while feeding *ad libitum* on such diets.

Automated food dispensing machines can be adapted from commercially available equipment to provide food items *ad libitum* (Silverstone & Fincham, 1978; Silverstone *et al.* 1980; Wurtman & Wurtman, 1981, Rising *et al.* 1992). This allows a record of the frequency, quantity, choice and time of eating to be recorded automatically if linked via a computer system. This is also useful only within the laboratory and requires subjects to consume the foods immediately they are removed from the vendor. Use of subject-access codes and timing devices enables the time at which subjects select certain foods to be recorded. A clear advantage of the food dispensing machine is that real foods can be used with minimal labour costs on the part of the investigator. A clear disadvantage is that the machine is only capable of dispensing certain foods of a certain portion size.

In laboratory-based, weighed-intake studies all foods are of a known composition and are weighed before and after each meal or snacking occasion (Obarzanek & Levitsky, 1985; Cotton *et al.* 1994; Stubbs *et al.* 1995). These approaches provide robust quantitative data, but are extremely labour intensive and, since food must be provided *ad libitum*, are often very expensive in terms of food wastage. In order to avoid the labour-intense nature of researcher-weighed foods, or to enable subjects to select their normal diet, subjects may be asked to self-weigh or self-record their own FI. Provided a subject weighs all foods eaten and food-remains accurately, quantitative estimates of intake are theoretically more precise than semi-quantitative observational measures (e.g. food diary, 24 h recall) (Bingham, 1987). However, the more intrusive and quantitative a technique is, the more the normal feeding behaviour of the subject is likely to be disrupted. Substantial errors can still also accrue, for example, due to mixing of food remains and misclassification of foods. Subjects often fail to record all foods eaten or alter their feeding patterns to simplify the food-weighing process. Bingham (1987), in a meticulous review of dietary assessment methodology, notes that there are at least nine sources of error in methods used to assess dietary intake. These are errors derived from food tables, coding errors, wrong weights of food, reporting errors, variation with time, wrong frequency of consumption, change in diet, response bias and sampling bias. Most observational and quantitative methods of EI assessment used in the laboratory are subject to several of these errors. If subjects have access to a diet of a constant measurable composition then it is possible to verify that composition by chemical analysis. This, in turn, depends on the precision with which energy or nutrient intake measures are required.

Semi-quantitative measures of motivation to eat and measures related to feeding behaviour. A specific advantage of studying the effects of various agents on appetite, feeding behaviour and hence EB in man, is that it is possible to ask subjects structured questions about their motivation to eat or not eat, which type of food and what amount they would consume. A number of behavioural or motivational measures have been made in laboratory studies of feeding.

These include assessments of hunger, fullness, or even salivation as measures of disposition to eat (for reviews, see Spitzer & Rodin, 1981 and Hill *et al.* 1995). The measurement of hunger using visual analogue scales (VAS) has yielded valuable information regarding the effects of dietary, pharmaceutical and physiological manipulations on motivation to eat (Hill & Blundell, 1982; Silverstone & Goodall, 1986; Leathwood & Pollet, 1988; de Graaf, 1993). Inasmuch as it is possible to quantify, VAS exhibit a good degree of reliability and validity in that they predict, with reasonable certainty, meal initiation and are sensitive to experimental manipulations. It appears that VAS are best used in within-subject repeated measures designs where the effects of different treatments can be compared under similar circumstances. VAS are psychometric tools and the results obtained from them are neither objective nor entirely quantitative. They yield the most valuable information when combined with other aspects of feeding behaviour and EB. It is important to recognize that subjectively-rated motivation to eat is not an inevitable outcome of underlying physiological processes. Rather it is the subject's own interpretation of their own sensations and motivations, which are influenced *inter alia* by underlying physiological processes (Blundell, 1979). Furthermore, human subjects often eat for a variety of reasons in addition to their state of hunger and can express hunger or appetite for reasons in addition to the physical sensation that they associate with meal initiation (Blundell, 1979).

Psychometric techniques may also be used to assess appetite for specific food groups, such as 'appetite for something sweet' or 'appetite for something savoury' (de Graaf, 1993). Use of these techniques may allow assessments of feeding motivation which overcome some of the constraints of limited food selection in some laboratory studies.

There are a number of other behavioural measures that are particularly relevant to understanding patterns of FEN intake in laboratory studies. These include assessments of the palatability of foods (Hill & Blundell, 1982), differences in the sensory and physical characteristics of the foods (Watts *et al.* 1989) and the subject's psychological profile with respect to restraint, emotionality and externality (van Strien *et al.* 1986).

From a methodological viewpoint the palatability of a food is often thought of as the momentary orosensory pleasantness of a food, which can affect the intake of that food. Thus, differences in the perceived palatability of experimental foods may affect the intake of those foods during an experiment. However, there is currently considerable controversy as to the exact definition of palatability, let alone how to measure it. The reader is referred to a recent debate regarding these issues (Booth, 1990; Kissileff, 1990; Ramirez, 1990; Rogers, 1990). Since there are no standardized approaches to these measures, the methodology of Hill & Blundell (1982) is currently recommended. The use of VAS to assess the subject's perception of the pleasantness of a food 15 min after its consumption has been shown to relate to the amount eaten (Hill *et al.* 1984), is sensitive to sensorially different meals, and shows significant effects between meals and menu days during studies (e.g. Stubbs *et al.* 1995; Stubbs & Harbron, 1996).

It is not possible to make statements about the quantitative influence of palatability on feeding behaviour because it is a psychometric, pseudo-quantitative variable. This does not mean that the measure of palatability is not important in laboratory studies of intake. Any claim that a manipulation of diet composition affected feeding behaviour needs to be supported by evidence that the change in dietary intake was not a function of differences in the palatability of foods between treatments which attended changes in diet composition. The lack of a statistically significant difference in the palatability of foods ingested during the study is often cited as evidence that changes in palatability of foods did not contaminate the experimental outcome (e.g. Stubbs *et al.* 1995; Stubbs & Harbron, 1996). This is acceptable as long as the experimenter can demonstrate that the technique used to assess palatability is sensitive under the conditions in which it was used. Palatability assessments can also be used in conjunction with sensory assessments (e.g. discrimination) of foods before the use of those foods in a study protocol.

'Dietary restraint' is a term used to describe aspects of the feeding behaviour of people who are attempting to limit or reduce their body weight by means of cognitive energy restriction (dieting) (Herman & Polivy, 1991). In doing so it is proposed that they are placing their motivation in relation to feeding at odds with physiological feeding stimuli. Placing cognition at odds with physiological drives can influence feeding behaviour. The consequences of this effect are a subject of some debate (Agras, 1990; Booth *et al.* 1990; Cooper & Charnock, 1990; Herman & Polivy, 1990; McClusky, 1990; Treasure, 1990; Tuschl, 1990; Westenhoefer *et al.* 1990). Measures of restraint can predictably assess a subject's likelihood of cognitively modifying their intake whilst being studied in the experimental environment. Restraint can be assessed by use of validated questionnaires, the two most popular being the Dutch eating behaviour questionnaire (van Strien *et al.* 1986) and the three-factor eating inventory (Stunkard & Messick, 1985). Because the concept of restraint has predictable behavioural outcomes its utility in understanding the feeding behaviour of subjects in the laboratory is growing. Understanding restraint may therefore become important in understanding factors that influence human feeding behaviour in terms of notably low EI, especially relative to EB (e.g. Johnstone *et al.* 1998*a*) and specific patterns of feeding under laboratory conditions (Herman *et al.* 1987), which is characterized by seemingly spontaneously excessive intakes under the conditions of the study concerned, and may be of relevance for higher levels of intake seen when restrained eaters are given modified low-fat foods (Miller *et al.* 1995).

Techniques that are used to validate and/or verify laboratory measures of food intake and feeding behaviour. Theoretically, the use of biomarkers offers an objective, independent assessment of dietary intake that is precise, accurate, unobtrusive and does not disrupt the behaviour of the subject concerned. The use of doubly-labelled water to measure EE can be an independent means to assess the plausibility of dietary intakes (Schoeller, 1990; Goldberg *et al.* 1991), provided both EI and EE are measured over 10 d or more. It is perhaps more difficult to define 'plausible' intakes in the laboratory. Unfortunately, most laboratory studies which measure intake are of a shorter duration than this. In this context the recent development of the labelled bicarbonate technique offers a method of examining FEN intakes relative to EE over periods of 24 h (Elia, 1991). Similarly, urinary N output (derived from 24 h total urine collections) offers a means of validating protein intake, based on the fact that healthy subjects in EB are usually in N balance (Bingham & Cummings, 1983, 1985; Bingham, 1994). These methods are essentially a means of verifying dietary intakes relative to an assumed habitual EB, rather than a direct means of measuring energy or nutrient intakes. The development of precise and accurate biomarkers for the assessment of energy and nutrient intakes is still in its infancy, with much work to be done before they replace more traditional measures of dietary intake in human subjects.

It will be apparent from Table 1 that different techniques for the measurement of EI are most useful under different experimental conditions, which range from micromeasures such as latency to eat subsequent to the ingestion of different preloads, to macromeasures such as changes in EI and body weight over 2 weeks as result of changes in the nutrient composition of a covertly manipulated diet (for a review, see Hill *et al.* 1995).

Experimental designs

Laboratory experiments which attempt to measure FEN intakes involve a number of interventions or manipulations of the appetite control system by cognitive, behavioural, environmental, nutritional, physiological or pharmacological means. These manipulations do not fall within the scope of the present discussion. Here the consideration of experimental designs is constrained to those aspects of the design used to measure the experimental outcomes in terms of FEN intakes. While the limitations and constraints of experimental designs are highlighted later, it is pertinent to point out that each has its own advantages which yield important data.

Preloading studies

With the exception of observational approaches the most basic form of experimental design used to measure food and EI in the laboratory is the 'preload–test meal' paradigm, which has been used to assess the short-term effect of a wide range of manipulations on subjective motivation to eat and often intake at a subsequent test meal (Spitzer & Rodin, 1981; Kissileff, 1984; Rolls *et al.* 1991, 1994; Hulshof & de Graaf, 1993). These experiments are most profitably conducted using a within-subject repeated measures design. Hill *et al.* (1995) note that 'For many years a good deal of research on appetite control has been concerned solely with the effects of a variety of variables on the short-term consumption of foods presented to young adults, typically university students, often in a somewhat contrived context. Many of these studies have revealed little of lasting scientific value in proportion to the investment of experimenter labour'. Despite these caveats most researchers (including ourselves) have employed (and some rely on) this design to

detect, experimentally, changes in intake in the laboratory. There are a number of factors relating to the preload–test meal paradigm which should be borne in mind, before employing this approach. First, the preload–test meal paradigm cannot make statements regarding the likely effects of a given manipulation on EB, or indeed at the meal subsequent to the last test meal. Within the test period any differential effect of the preload manipulation will decay as the time between the preload and test meal increases (Rolls *et al.* 1991). Experimenters use intervals between the preload and the test meal ranging from 20 min to several hours. Second, this design is particularly vulnerable to type 2 errors (Blundell, 1995) and evidence of the sensitivity of the experimental system in use should be provided. Third, the nature of the test meal will also affect the outcome in terms of FEN intake. Research reports should state why a given test meal was chosen. Fourth, the preload paradigm is also particularly subject to a number of influences that impinge on the cause–effect relationship under scrutiny, due to the short time-window of measurement.

Recently Lawton *et al.* (1993) have extended the preload–test meal design, by altering the composition of possible test meals available. This means that the test meal is an outcome variable in relation to the preload and the preload plus test meal together become the input manipulation, whose effects on subsequent intake can be assessed. While subject to some of the constraints of the preload design this adaptation enhances the ecological validity of the experiment by producing a feeding sequence similar to that encountered in everyday life, and measures feeding over the course of a day, which appears to be the shortest time-window that can be used to make any statements about the possible effects of a given manipulation on EB. A further adaptation to the preload design has been used by Foltin *et al.* (1988, 1990, 1992) who have provided subjects in the laboratory setting with a variety of familiar food items and covertly manipulated one mandatory meal, usually lunch. This again has the advantage of enhancing the ecological validity of the experimental design, and enables the experiment to be conducted over more than 1 d. We have recently used a multiple-preload design to assess the effect of snack composition on the FI and EI of an otherwise *ad libitum* diet of fixed composition, over 7 d per treatment (Shannon *et al.* 1998). Thus, while the original preload–test meal paradigm has certain limitations, this basic experimental design has been developed and usefully exploited in laboratory studies of feeding behaviour and its likely effects on EB.

The use of manipulated diets

As interest has focused on the effect of dietary variables (e.g. nutrient composition, energy density, sensory characteristics) on EI and EB the use of manipulated diets in laboratory studies of feeding has become common (e.g. Lissner *et al.* 1987; Kendall *et al.* 1991; Tremblay *et al.* 1991; Lawton *et al.* 1993; Stubbs *et al.* 1995; Stubbs & Harbron, 1996; O'Reilly *et al.* 1997). Under these conditions the diet can represent aspects of both the manipulated input and the measured outcome variable in an experiment. The degree of manipulation can vary from highly precise systematic

manipulation of the nutrient ratios and/or energy density of all foods on the diet, to partial manipulations of the whole diet (Stubbs *et al.* 1995; Stubbs & Harbron, 1996; O'Reilly *et al.* 1997), for example, manipulations that use foods with a food quotient above or below 0·85, as high- or low-fat foods respectively (Tremblay *et al.* 1991). It is important in such studies for the experimenter to describe meticulously the nature of the manipulation, for instance, whether energy density was also altered with the nutrient ratio of the diet, or what was the range of variation in the composition of foods with a food quotient above 0·85. Whenever a study uses a manipulated diet the design of that diet places certain constraints on the behaviour of the subject. In real life, subjects are able to vary the energy density, composition, amount and solid food : fluid ratio of the foods they select and ingest. Very few laboratory designs enable this degree of behavioural flexibility and often view variations in more than one dietary variable as a contamination of the cause–effect relationship under scrutiny. Herein lies a further dilemma to the investigator, namely that facilitating an increased flexibility of the subject's behavioural response decreases the signal : noise ratio in the manipulation and so may weaken the detection of the cause–effect relationship under investigation. On the other hand, the more controlled the dietary manipulation, the more constrained the subject is in their behavioural response. It is important to emphasize, rather than understate or rationalize, the limitations of the experimental design in research reports. This allows the influence of the experimental context on experimental outcomes to be assessed. For example, comparing studies that use overtly and covertly manipulated diets suggests that cognition and/or learning play an important role in mechanisms of energy compensation (Stubbs, 1995).

As regards the issue of learned behaviour in relation to the type of manipulated diet used, an interesting phenomenon has been observed in the growing number of studies that use covertly manipulated diets over periods ranging from a few days to a few weeks. These studies are characterized by a general tendency for subjects not to alter their FI in response to the dietary manipulation, unless the manipulation produces a particularly large (physiological or orosensory) effect. Even then compensatory responses are somewhat blunted, and FI changes little. This means that there are a growing number of studies which appear to suggest that maintaining a constant weight or volume of food intake appears to be a major goal of subjects feeding in the laboratory. Why should weight and volume appear as important features that affect FI in some studies? (A litre of water would have weight and volume but would provide no energy or nutrients.) Blundell (1995) has given the most coherent explanation of this phenomenon. He argues that the ultimate function of satiety signals is to monitor the biological value of foods and to play a role in the processing of ingested nutrients. During the acquisition of learned feeding patterns, weight and volume of food will have become associated with (conditioned to) the important biological components of food, namely energy value and nutrient composition. Blundell further argues that weight and volume become learned cues with high functional validity (proximal cues which correlate well with more distal cues such as hormone release,

contact with gastrointestinal receptors etc.). In other words subjects learn to associate the weight and volume of specific, familiar foods that they eat habitually with the physiological consequences of ingesting those foods. However, in an experiment using manipulated foods, subjects are presented with foods that are not of a similar composition to those normally ingested, even if they look similar. Indeed, studies using covertly manipulated diets tend to strip learning cues out of the experiment by covertly altering food composition, dissociating the sensory and nutritional attributes of the foods, randomizing the order of experimental conditions to avoid learned order effects and often, using relatively unfamiliar foods. In the absence of familiar feeding cues, weight and volume of food may attain greater significance, under these experimental conditions. Furthermore, in studies where subjects feed *ad libitum* on covertly manipulated diets of a constant composition, they cannot alter the type, energy density or composition of foods they eat, to the extent that they can in real life (e.g. Lissner *et al.* 1987; Kendall *et al.* 1991; Stubbs *et al.* 1995; Stubbs & Harbron, 1996; O'Reilly *et al.* 1997). Hence any compensatory feeding responses are likely to be more blunted under these experimental conditions relative to those encountered in real life. Because of these important constraints of the experimental design on feeding behaviour, equal attention should be given to comparisons of overt and covert manipulations, and studies using familiar foods, in order to distinguish whether feeding responses are due to the overt or covert nature of the experiment or the nutritional nature of the dietary manipulation.

The use of familiar foods

Ostensibly it might appear that the use of familiar foods creates a microcosm of the real-life feeding situation and overcomes the constraints of using manipulated diets. However, the choice of foods provided in the laboratory is inevitably limited and few reports ever give scientific explanations of why a certain range or selection of foods was made available to a group of subjects. Given that a number of dietary factors can influence FEN intakes in laboratory studies considerable attention should be paid to this aspect of dietary design. The use of familiar foods in the laboratory also decreases the precision of measurement since it is more difficult to quantify energy and nutrient intakes especially if the foods are mixed as in real life. The use of familiar foods in discrete units de-naturalizes the experimental context with as yet unquantified consequences. Other studies, particularly those of DeCastro (DeCastro, 1987; DeCastro & Elmore, 1988) employ subjects to record their own intakes in the natural setting. While these studies are subject to all of the errors associated with dietary surveys, they provide invaluable information regarding patterns of behaviour in free-living subjects in their natural setting. It is, therefore, valuable to attempt to compare more artificial manipulations in the laboratory (e.g. Lissner *et al.* 1987; Kendall *et al.* 1991; Tremblay *et al.* 1991; Lawton *et al.* 1993; Stubbs *et al.* 1995; Stubbs & Harbron, 1996; O'Reilly *et al.* 1997) with less precise but more naturalistic studies (DeCastro, 1987; DeCastro & Elmore, 1988).

Diets designed to detect changes in food or nutrient selection

A couple of decades ago the serotonin theory proposed that diet-induced alterations in the neurotransmitter serotonin led to oscillations in feeding behaviour between protein and carbohydrate (Fernstrom & Wurtman, 1972). This led to a number of dietary designs aimed at enabling experimental detection of changes in protein and carbohydrate selection, some (e.g. Wurtman & Wurtman, 1982/83) ignored the presence of fat in the foods being selected. At the present time interest is focused more on factors which may influence the selection of high- or low-fat foods. Again, subjects are often presented with a (usually small) selection of high- and low-fat versions of a food in order to detect selective differences between them. Not surprisingly, few studies have shown directional changes in the selection of foods enriched in a particular macronutrient over foods enriched in another macronutrient. The reason for choosing certain foods, the sensitivity of the dietary design to experimental manipulations and the limitations of the design should all be reported by the investigator. If the object of an experiment is to detect changes in the selection of foods enriched in a certain macronutrient over foods enriched in others, then unless there is good scientific justification for not doing so, the three main macronutrients that subjects normally encounter during eating (protein, carbohydrate and fat) should, perhaps, be represented in the design. There are clear scientific reasons for including or excluding alcohol, depending on the nature of the investigation. When considering the issue of diet selection in the laboratory it is impossible to simulate the degree of food choice that is often available in real life. Many human feeding studies are not designed to detect changes in nutrient selection. Indeed some studies have constrained macronutrient selection to the extent that it is impossible to derive conclusions relating to qualitative patterns of feeding (i.e. composition of foods selected). We have recently designed and tested a model to detect changes in the selection of macronutrient-rich foods in the laboratory, after consideration of animal models of selection and the statistical factors which should define a model which discriminates between three independent variables (macronutrients) (Stubbs *et al.* 1997). The model provides subjects with access to a counterbalanced range of macronutrient sources (Leibowitz, 1992). This takes the form of ten foods rich in an individual macronutrient (thirty in total) with the remaining energy in each food evenly split (as far as possible) between the other two macronutrients. Changes in diet selection in animals generally require a period of conditioning during which the animal learns to associate the sensory characteristics of a food with its postingestive consequences (Forbes, 1995). For this reason the design uses common, familiar foods. Different subjects prefer different foods. It would not be possible to tailor all food items to the preference profiles of each individual subject, since this would require a near infinite variety available in the laboratory. The model therefore aims to provide sufficient variety for subjects to be able to select foods from each food category (high protein, high carbohydrate or high fat) without their choice being heavily constrained by the avoidance of most foods within any one category, simply because they did not like those foods. Despite these

considerations the model has several major limitations. It does not simulate the situation encountered in everyday life and is only of use in repeated-measures, within-subject designs. Furthermore the initial test of the model's sensitivity has led to further refinements of the model to enable it to be expanded to a 3 d rotating menu using forty-five foods available per day. The considerable expense and labour involved in this dietary design are likely to preclude its extensive use. The ecological validity of the model has yet to be evaluated. This model does not allow human macronutrient selection *per se* to be detected in the laboratory. It allows changes in the selection of common, familiar foods enriched in certain macronutrients to be detected.

It is important to realize the limitations of the experimental environment, the methods used and the techniques employed to measure FEN intakes in order to obtain results that add to scientific knowledge about eating behaviour and its consequences for health. With these concerns in mind there are a number of further issues (some touched on earlier) that are pertinent to those about to embark on the measurement of FEN intakes in the laboratory. The major issues are dealt with in the following section.

General methodological issues that are of relevance to the design and interpretation of laboratory measures of feeding behaviour and food, energy and nutrient intake

(1) Precision *v*. naturalness. The experimental environment itself can (and often does) affect the outcome of experiments (see previous discussion). It is important to demonstrate the existence of important phenomena across environments and indeed, if thought to be fundamental, across species (Blundell & Stubbs, 1998).

(2) Power and sensitivity. The preceding discussion of experimental designs illustrates that power tests should be conducted before an experimental manipulation to assess the probability that the experiment is capable of detecting the cause–effect relationships it aims to detect. Furthermore, given the notable tendency of some measurements or experimental designs actually to constrain the capacity of subjects to respond, the investigator has a responsibility to demonstrate that the experimental design is capable of detecting changes in intake.

(3) Demand characteristics. In any experimental circumstances subjects bring with them their past history of eating, beliefs about food and also their beliefs about what they are supposed to do to be a 'good subject' (Blundell, 1995). These beliefs ('demand characteristics') can affect the way a subject behaves during an experiment. Demand characteristics are also influenced by the instructions given to subjects. Thus, the effects of a physiological infusion of glucose on feeding may be differentially influenced by instruction to 'eat as you wish' or 'eat only when you feel hungry'.

(4) The behavioural goals of the subject under the conditions of the experiment. A recent study in our laboratory showed that increasing the sensory variety of nutritionally identical diets led to increased intakes in lean but not overweight men (Johnstone *et al*. 1998*b*). One might reasonably conclude from these data that the lean men were responsive to sensory

cues and that under these conditions the overweight men were controlling their EI more effectively. Closer scrutiny revealed the overweight men to be more restrained eaters than the lean men. The behavioural goals of the subject are not always what they seem and may be far removed from those assumed by the investigator. This may be true even if the results of the experiment actually support the initial hypothesis examined by the experiment. It can only be concluded that the behaviour of the subject supported, and did not prove, the hypothesis. This area should be the subject of a detailed consideration in itself.

(5) The vexed question of regulation. It is often assumed that the 'natural' state of the subject in the laboratory, when not subject to an experimentally-induced manipulation, should be one of energy and nutrient balance regulation. There is no clear reason why this should be the case, especially in short-term experiments. Indeed, given that over 50 % of the adult population is now collectively overweight and obese (Department of Health, 1995) perhaps the assumed 'non--manipulated' state should be characterized by a tendency to overeat! Nevertheless, experiments which claim to address the issue of EB regulation should include a no-treatment control, in which subjects should be in approximate EB. If subjects are in a gross energy imbalance on such a 'control' then the effect of the experiment itself on the 'regulatory' system under investigation should be given serious consideration.

(6) Relevance of questions asked and veridicality of results. Research strategies should be formulated to address key theoretical or practical issues that are important to the study of human feeding behaviour. The research question asked will, in part, influence the interpretation of results. Researchers should point out the limitations of their own experimental designs and avoid over-generalizing results or drawing premature conclusions from individual studies conducted in specific experimental environments, on small numbers of subjects. Since the majority of positive results usually provide indirect support for and never prove an hypothesis, the limitations of that support should be acknowledged.

(7) Bottom up or top down research? It is important to study human feeding by considering the way that it operates as a system within the intact person. However there are a number of mechanistic questions in establishing cause–effect relationships that can never be answered by such investigations. Under these conditions it is most profitable to conduct as near as possible, parallel manipulations (with more invasive investigations) in suitable animal models. Less invasive (and less direct) evidence of similar mechanisms can then be subsequently sought in human subjects.

(8) The time-window of measurement. Many studies on feeding behaviour are of too short a duration to make statements about the effects of outcomes on EB. A number of experiments find results that are counter-intuitive, relative to anecdotally obvious phenomena (e.g. the failure of a subject to alter eating behaviour in response to an exercise manipulation lasting several days). As the time-window of measurement contracts the influence of confounding and constraining variables associated with the experimental design expands. Expanding the sample size may not overcome these problems. There is a shortage of longer-term

studies which assess the effects of a number of manipulations on factors such as EB. Conversely too long a protocol may fatigue subjects and confound results. In studies of FEN intake lasting 10 d or more, analysis of temporal patterns in the data is advisable.

(9) Constraint on the flexibility of subject responses. The experimental environment provides a number of degrees of freedom with which the subject can respond. In general the tighter the control over the manipulation the lower the degrees of freedom or flexibility of subject response (see preceding discussion). Perhaps greater emphasis could be placed in scientific reports on describing the constraints the experimental design places on the subject's response. Most experimental environments, however artificial, are likely to provide useful information provided the measurement of FEN intake is not reported, interpreted or extrapolated out of the experimental context from which it was derived.

Conclusions

Several basic experimental designs have been developed and elaborated to address certain research questions related to FEN intake in the laboratory setting. However, none of them represents the ideal design for measuring FEN intake in the laboratory. Differing designs are appropriate for different research questions. Use of different designs in relation to the same research question is also likely to provide valuable insights into the behaviour of both the subjects and the experimental model in the laboratory environment. Similarly there is no perfect environment in which to measure FEN intakes and the most valuable insights are often obtained by comparing the effect of similar experimental manipulations in different experimental environments. Perhaps there should be more and not less replication of study results, in order to evaluate their robustness, despite the current practice of journals and grant-awarding bodies to give lower priority to studies of this nature. Furthermore, a research issue should be explored by a number of structured and related research protocols rather than individual, experimental attempts to comprehensively assess complex issues pertinent to nutrition and behaviour.

The laboratory measurement of intake is a critical component in our array of experimental tools that are available when attempting to understand the relationship between nutrition and behaviour and its consequences for health and disease. As with most specialized tools this approach is of greatest scientific value if used alongside those other tools (research approaches, experimental environments) which make up the full range of techniques and capabilities available to the investigator. The purpose of scientific investigations is to rotate theoretical models in a number of experimental ways which attempt to disprove the model. By progressing through a series of such negative assaults a model attains the robustness of a theory and scientific progress lurches forward with an additional packet of new knowledge. For that knowledge to be of fundamental importance and applied significance it must be considered in the light of other knowledge obtained elsewhere and often by other means. Only by realizing the constraints and limitations of the measures used to obtain the knowledge can its significance be fully appreciated.

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