PROCEEDINGS OF THE NUTRITION SOCIETY

The Four Hundred and Fortieth Meeting was held, in conjunction with the diamond jubilee of the Dunn Nutrition Unit, at the Babbage Lecture Theatre, University of Cambridge, Cambridge on 16/17 July 1987

SYMPOSIUM ON 'STABLE ISOTOPIC METHODS FOR MEASURING ENERGY EXPENDITURE'

Recent progress in studies on energy expenditure: are the new methods providing answers to the old questions?

By W. P. T. JAMES, P. HAGGARTY and B. A. MCGAW, *Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB2 9SB*

The application of the D_2O^{18} technique for measuring carbon dioxide production comes after a renaissance in interest in the energy metabolism of both small and large animals. Animal nutritionists have, for decades, recognized the importance of accuracy in methods of measurement using direct or indirect calorimeter chambers when monitoring the energy turnover of farm species over periods of days or even weeks. The impetus for these studies came from Blaxter and his colleagues working first at the Hannah in Ayr and then at the Rowett Research Institute in Aberdeen over a period of 30 years from the 1950s. Their studies led to a new approach to the evaluation of the energy available from the feed of ruminants and highlighted the principles for evaluating the feed requirements of farm species (Blaxter, 1962). This, in turn, led to the establishment of feed evaluation units at the Rowett and elsewhere based on the biological evaluation of different crops under standardized feeding trials (Blaxter, 1983).

The contrast between animal and human studies on energy metabolism is striking. The enormous support given to research in animal nutrition was not matched in the human nutrition area so that as the research units in human nutrition dwindled following the Second World War (James, 1986) it seemed highly unlikely that the technical and scientific support for building and maintaining human calorimeter chambers would be forthcoming. Eventually, in Britain, the Medical Research Council, stimulated in part by Durnin and his colleagues (Durnin *et al.* 1973), provided support for Murgatroyd to build calorimeters first in London in Professor Garrow's former unit at the Clinical Research Centre and then at the Dunn Nutrition Unit in Cambridge (Dauncey *et al.* 1978). The value of this approach to studies on energy metabolism soon became apparent and a large number of calorimetry centres are now being established at a time when the D_2O^{18} method is also being introduced. Some might consider this new technique to supersede the technique of whole-body calorimetry. It will, however, become apparent that the techniques needed vary with the questions being asked and both calorimetry and the D_2O^{18} approach are required to resolve some of the important issues.

What then are these important remaining problems in energy studies and are the problems different in animal and human studies? Table 1 presents eight different questions, some of which are interrelated. It is clear that the outstanding problems are remarkably similar in both animal and human nutrition. The techniques which are likely

Table 1. *Persisting questions in energy studies*

		Precision needed (%)	Persisting problems		Technique needed
			Animals	Man	
1.	Measuring differentially the components of total energy expenditure, i.e. RMR, DIT and PA	1–2	√	√	Refined chamber calorimetry
2.	Identifying the genetic component determining body composition	1–2	√	√	Highly-controlled calorimetry
3.	Specifying the nutrient–genetic interaction on body composition	1–2	√	√	Highly-controlled calorimetry
4.	Measuring the metabolizable energy of foods	2–3 (Ruminants)	√	—	Calorimetry
5.	Measuring energy requirements under free-living conditions	5	√	√	D_2O^{18} or $H_2CO^{14}{}_3$ labelling
6.	Differential adaptive changes of BMR, DIT and PA to altered intake	2–5	√	√	Both calorimetry and labelling studies
7.	Studies on the energy cost of growth	2	√	√	Both calorimetry and labelling studies
8.	Energy cost of lactation and lactational output	2	√	√	Both calorimetry and labelling studies

RMR, resting metabolic rate; BMR, basal metabolic rate; DIT, diet-induced thermogenesis; PA, physical activity.

to help depend on their accuracy and precision and how these compare with that demanded for resolving each of the questions.

Differentiating the components of energy expenditure

In farm animals much effort has gone into measuring both fasting metabolism and the thermic response to feeding and into attempts to measure the total energy requirements of the animal under free-living conditions. It rapidly became apparent that highly-controlled studies were required in calorimeters if fasting metabolism was to be distinguished from the thermic response to feed. Unfortunately as soon as animals are confined to the chambers not only is their capacity to exercise limited but their behaviour is also altered as they experience the confined space. Initially the daily rate of energy expenditure is highly variable and only after periods of several weeks' training is it possible to obtain a reproducibility of daily energy expenditure of 2–3%. This limitation has dominated the design and usefulness of chamber studies. Nevertheless, once the behavioural adaptation has occurred, fasting studies are readily applied to simple-stomached animals; the problem of measuring fasting metabolism in ruminants is, however, exaggerated by the continuing metabolic activity of the rumen. By dint of meticulous studies, repeated under standardized conditions, it has been possible to define the fasting metabolism of ruminants and simple-stomached species and then to distinguish the thermic response to feed. This factorial approach to assessing energy

https://doi.org/10.1079/PNS19880036 Published online by Cambridge University Press

requirements can be extended to studies on growth where the energy intake for maintenance of body energy can be measured first and then the energy cost of protein and fat deposition estimated separately. In animal studies this is made possible using carcass analysis and accurate measurements of food intake in addition to chamber calorimetry. This approach has led to repeated revisions of the estimates of energy requirements in both ruminants and in pigs (Close & Fowler, 1983).

By using calorimetric techniques the high thermic response to roughage diets was demonstrated by Blaxter and his colleagues 30 years ago (Blaxter, 1962), but the basis for this difference remains controversial being assigned by some to the energy cost of rumination and digestion and by others to innate differences in the metabolic responses to absorbed nutrients.

Studies on humans may, in many ways, be simpler to conduct because in adults it is possible, with the co-operation of the subject, to obtain accurate measurements of fasting or basal metabolism as the individuals lie, while totally relaxed, under a ventilated hood. Repeated analyses have suggested that there may be a technical error of less than 2% and a variability within subjects amounting to 4% (Bogardus *et al.* 1986), a value which includes the technical error over a short period of measurement in subjects studied at several-month intervals. Studies on a sequential daily basis reveal a coefficient of variation in basal metabolic rate (BMR) of 2% once the subjects are adapted to the procedure, but this 'training' period is very short compared with that needed in animal studies. The accuracy and reproducibility of measurements of the thermic response to food are less clearly documented in man, but the measured variation probably reflects not only subtle differences in the rate of gastric emptying but the problem of ensuring measurements under conditions of total rest for many hours (Zed & James, 1986*a*). Jéquier's group has attempted to overcome this problem by measuring individuals in a calorimetric chamber with Doppler monitoring of movement and simultaneous measurement of energy expenditure; the progressive decline in expenditure with decreased movement has then been extrapolated linearly back to the average energy expenditure in the calorimeter under conditions of total rest. The difference between the intercept and the measured basal metabolic rate is then taken to be a measure of dietary-induced thermogenesis (Schutz *et al.* 1982). This method has the advantage of not requiring subjects to lie down throughout the post-prandial phase, but has the disadvantage of including the energy cost of muscle tone in the sitting or standing position as well as the errors incurred by the extrapolation procedure. The reproducibility of the monitoring of the thermic response to food by indirect calorimetry has rarely been assessed, but it can be very variable (Zed & James, 1986*a*). If, however, one wishes to monitor total energy expenditure, then the reproducibility of 24-h measurements of energy expenditure amounts to only 1·5% (Dallosso *et al.* 1982) when physical activity patterns are rigorously controlled in calorimeters. This value is based on four calorimetry readings conducted over a 4-week period. Similar values have been obtained by Garby *et al.* (1984) and De Boer *et al.* (1987) in comparable studies in large direct or indirect calorimeters.

The accuracy demanded for energy studies on genetic factors governing body composition

One of the intriguing features of studies on farm animals is that despite the huge effort put into establishing the metabolic responses to feeding there is no calorimetric information which allows a distinction between different genotypes of a single species, despite the substantial difference observed in body composition. Thus Fuller, under carefully controlled conditions, was unable to identify any difference in energy

metabolism between Pietrain and Landrace pigs (Fuller *et al.* 1977). This, however, is understandable if one recognizes that any difference in the energy content of the two strains is very small when expressed on a daily basis and constitutes a very small fraction of the normal energy turnover if the appetite of the two strains is the same. In practice, the Pietrain strain tends to eat less *ad lib.* than the Landrace showing that any difference in energy metabolism between different genotypes of pigs cannot be discriminated by standard calorimetric procedures. Even bulls and steers cannot be distinguished during the early stages of growth when their rates of protein and fat accumulation are different, but body compositions are very similar (Webster *et al.* 1977). Obviously once the compositional changes themselves have occurred then there will be a secondary effect on energy metabolism.

These considerations explain much of the emphasis in studies on energy metabolism and energy requirements in animal nutrition (see Table 1). The feeding trial seems crude but if intake is standardized then the comparative-slaughter technique does provide a means of indirectly testing the energetic responses and, if total carcass energy is measured, then an estimate of total energy expenditure can be obtained. It is the recognition of the difficulty of calorimetric discrimination of small but consistent and cumulative effects on metabolic efficiency and total energy expenditure which has maintained the popularity of feeding trials. In vivo estimates of body composition are often too crude for use in either man or animals despite exhaustive efforts to improve the methodology. If, however, the D_2O^{18} technique provided an accuracy equal to that of calorimeters on a daily basis, then an integrated measure over a 2- to 4-week period would be a valuable addition to current techniques.

Confirmation that very accurate measurements are necessary if genetic differences are to be distinguished comes from studies in man. Bogardus *et al.* (1986) have now shown concordance in the resting metabolic rate (RMR) between members of a family so that whereas the mean variability in RMR between families amounted to nearly 2090 kJ (500 kcal) daily, the within-family variability was only 250 kJ (60 kcal). With a methodological variability of 160 kJ (38 kcal) (2% of the average RMR), such an analysis was possible. Fontaine *et al.* (1985) have recently demonstrated a greater concordance between the metabolic rates of monozygotic than dizygotic twins which strongly supports the idea that the familial association in RMR shown by Bogardus *et al.* (1986) is of genetic origin.

Fontaine *et al.* (1985) have emphasized that there may not only be intrinsic differences in energy metabolism of genetic origin but that the genetic programming of metabolism can alter the response to specific nutrients. This genetic–nutritional interaction can readily be seen in experimental studies on rodents with, for example, the *ob/ob* mouse showing a particular propensity to weight gain when the fat content of the diet is raised (Lin *et al.* 1979). In this case the observations were made, as in most feeding trials on farm animals, by carcass analyses on animals fed over periods of weeks, with no attempt being made to discriminate the specific differences in the thermic response to food in the different genotypes.

We have investigated the genetic–nutrient interaction underlying the problem of obesity for many years and have identified not only an abnormally low metabolic response to a mixed diet (James, 1985) but a possible specific nutrient–genotype interaction again related to the response to dietary fat (Zed & James, 1986*b*). Of significance for the present discussion is that the differences were only measurable because of our ability to discriminate a rise in total 24-h energy expenditure in eight obese subjects of only 2·0 (SE 0·7)% from a 6·7 (SE 1·0)% increase in eight lean subjects. Yet even under tightly-controlled conditions with replicate measures on a standardized diet the lowest coefficient of variation yet achieved is about 1·5% (Dallosso *et al.* 1982).

The confidence with which we can discriminate the difference between these two groups depends not only on the inter-individual and constitutional differences related to a propensity to obesity but also on the degree to which total energy expenditure responds to altered dietary intakes. In the obese and lean study the interaction between the 'genotype' and diet proved to be statistically significant ($P<0{\cdot}01$), but this was helped by having duplicate 24-h calorimetry on each dietary phase. It is clear that a methodological variability of less than 2% is required if we are not to impose on research workers a requirement to study very large numbers of subjects under metabolic ward conditions for prolonged periods of time before statistically-significant differences can be identified.

In research in both animal and human nutrition there is a need to be able to measure 'free-living' energy expenditure so that energy requirements may be determined in conditions as close to 'normal' as possible. This is the research area where the D_2O^{18} technique would be particularly appropriate were the method to be reasonably accurate. Both intake and body compositional measurements have appreciable errors so it is important if possible to have a direct as well as an indirect measure of energy expenditure. The D_2O^{18} technique would not, of course, allow one to discriminate physical activity from metabolic effects of diet. If, however, the D_2O^{18} method in the free-living animal or human were combined with episodic measurements in a calorimetric chamber this could allow a distinction to be made between fasting metabolism and dietary thermogenesis measured in the calorimeter and spontaneous physical activity measured by the D_2O^{18} method outside the chamber.

Measurement of energy intake

The D_2O^{18} method would also allow, in free-living conditions, an indirect estimate of energy intake provided studies are conducted under conditions where body compositional changes are being monitored.

Two examples where D_2O^{18} could usefully be applied are the assessment of the energy intakes of free-living animals, e.g. deer on a hillside, and independent evaluation of the information on energy intake in humans in developing countries derived from other methodologies. The analysis of the metabolizable energy (ME) intake of grazing deer is very difficult, but the application of the labelled bicarbonate (Corbett *et al.* 1971) or the D_2O^{18} technique for measuring CO_2 output could allow a reasonable estimate of ME intake to be made. In this case the limitation may prove to be in ascertaining changes in body energy stores rather than monitoring accurately the rate of energy expenditure, but the measurement of water spaces at the start of the D_2O^{18} study would help with this evaluation and increases the accuracy of slaughter studies.

The need for validating measurement of energy intake in man is evident in studies on the energy requirements of children and adults and in an analysis of the metabolic basis of obesity. Table 2 presents information on the supposed energy turnover differences of small and large eaters, and on the energy intake of several groups in developing countries calculated using the dietary-record method. In each case where the intakes seem unusually low a measure of total energy expenditure is much higher and casts doubt on the validity of the intake values. Also, as discussed previously, the intake–balance method is severely limited by the experimenter's ability to discriminate small changes in body composition over the intake measurement period. It could be argued, however, that the difficulty of undertaking expenditure measurements means that the assessment itself interferes with the spontaneous activity of the individuals; the controversy about the validity of the intake rather than the expenditure values cannot be resolved until one has independent measures such as those provided by the D_2O^{18} technique. However,

Table 2. *The supposed habitual energy intakes and measured energy expenditure in different groups of adults*

(Mean values and standard deviations)

Country . . .	Scotland*				England†		England‡									
Sex . . .	Small eaters ♂		Large eaters ♂		Small eaters ♂	Large eaters ♂	Small eaters ♂	Large eaters ♂	England§ ♀		New Guinea‖ ♀		Upper Volta¶ ♂		Upper Volta¶ ♀	
	Mean	SD	Mean	SD	Mean	Mean	Mean	Mean	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Intake (MJ/d)	8·5	1·0	15·3	2·3	10·0	19·4	6·9	15·0	8·2	1·4	5·9	1·5	9·0	2·6	6·2	1·3
Expenditure (MJ/d)	10·6	1·2	10·7	0·5	—	—	—	—	8·9	1·2	7·7	1·1	8·9	1·3	8·1	0·8
Basal metabolic rate (MJ/d)	7·2	0·4	7·4	0·7	8·8	8·8	6·8	6·0	6·4**		—		—		—	

*McNeill *et al.* (1988).
†Rose & Williams (1961).
‡Morgan *et al.* (1982).
§H. L. Davies, A. Crisp, C. Ravenscroft and W. P. T. James (unpublished results).
‖Norgan *et al.* (1974).
¶Bleiberg *et al.* (1981).
**Lying metabolic rate.

such validation is only appropriate when the precision and accuracy of the D_2O^{18} technique are within acceptable limits.

Energy adaptation and energy requirements in man

The ability of humans to adapt to alterations in intake by adjusting their metabolic efficiency or physical activity, or both, has become of increasing importance for two reasons. First, it was widely claimed that the adaptability of normal man was considerable, so that overfed volunteers exhibited only small changes despite substantial increases in energy intake. This prompted the use of calorimetry in studies on obesity to see if the obese had an impaired adaptive capacity (James, 1983). Second, the new approach to energy requirements established by an expert group (Food and Agriculture Organization/World Health Organization/United Nations University, 1985) has implied that energy metabolism does not readily adapt in man without concordant and potentially deleterious changes in body-weight and spontaneous physical activity. Sukhatme & Margen (1982) have challenged this on statistical grounds so successfully that in the *5th World Food Survey* (Food and Agriculture Organization, 1985) there was a special adjustment in the calculation of national energy needs which reduced the maintenance-energy-requirement value to only 1·2 times the estimated basal metabolic needs of the population rather than 1·4 or more times the BMR implied by the expert group. The choice of the lower value as a cut-off point below which a population group was classified as malnourished had the effect of reducing substantially the purported numbers of malnourished in the world. The choice of appropriate energy-requirement values for people working and living normally clearly depends on resolving this issue and an independent measure of spontaneous physical activity in free-living subjects is badly needed.

Energy costs of growth and lactation

The energy cost of growth under different nutritional and environmental circumstances is of immense importance in animal husbandry; the value of knowing the true needs of growing stock is reflected in the recurring revisions of the guidelines by the Agricultural and Food Research Council. In humans there is less concern, except in the neonate and infant where the energy cost of growth is an appreciable part of the requirement of the child. Calorimetry has again been used extensively in animal studies but as stated before, confinement in chambers precludes the measurement of the effects of climatic conditions, activity level, or patterns of feeding etc. on the maintenance-energy requirements of the animal thus confounding calculations of the energy cost of growth.

Calorimetry can be used to assess the response of lactating animals to different nutrient intakes (e.g. Whitelaw *et al.* 1986), but a full analysis of the energy costs depends on obtaining better measures of changes in maternal energy stores. In man it is traditional to assume that the efficiency of milk production from dietary energy amounts, as in the dairy cow, to 80%, but this value is highly suspect when one considers the different efficiencies of carbohydrate utilization through the fermentation route in ruminants rather than by direct glucose absorption in man, and the different energetic efficiency of milk-fat synthesis in the cow synthesizing from acetate and in humans synthesizing from ingested, pre-formed fatty acids.

It is necessary to know the energy cost of lactation so that we may ascribe values to the energy requirements of lactating animals and humans. The D_2O^{18} technique would allow us to measure the energy expenditure of 'free-living' subjects and has the added advantage of being able to trace milk uptake by suckling young (Roberts *et al.* 1986).

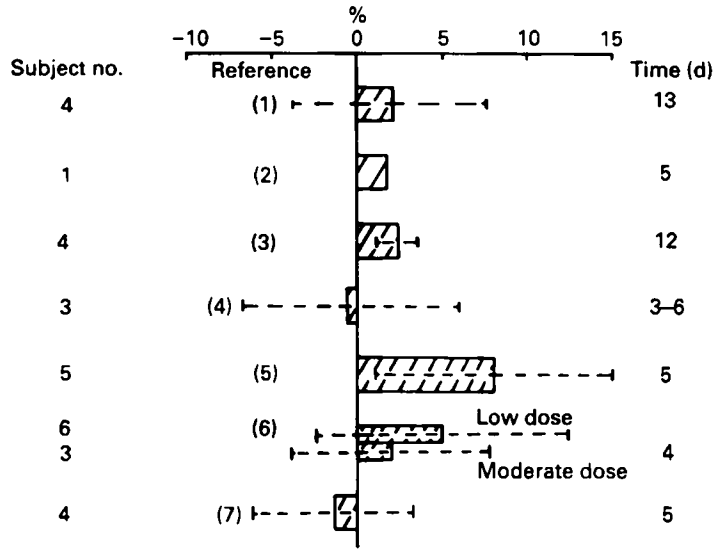

Fig. 1. Summary of information from studies of accuracy of D_2O^{18} technique in man. Mean values, with 2 SEM represented by vertical bars, of difference from reference. Note: different calculations were used for minimizing error.

(1) Schoeller & van Santen (1982), (2) Klein *et al.* (1984), (3) Coward *et al.* (1984), (4) Westerterp *et al.* (1984), (5) Schoeller & Webb (1984), (6) Schoeller *et al.* (1986*b*), (7) Roberts *et al.* (1986).

The precision and accuracy of the D_2O^{18} method

Fig. 1 summarizes information where attempts have been made to establish the accuracy of the technique in man under highly standardized conditions. Schoeller & van Santen (1982) were the first to assess its usefulness; they relied on standardizing the food intake of four volunteers under metabolic-ward conditions with an indirect assessment of changes in body fat by repeating the measurement of the deuterium space at the end of the 12-d study period. The other validation studies shown in Fig. 1 rely on individuals occupying a whole-body calorimeter or wearing a suit. Direct and continuous measures of CO_2 production were obtained by Klein *et al.* (1984), Coward *et al.* (1984), Schoeller's study with Jéquier's group (Schoeller *et al.* 1986*b*) and by Westerterp *et al.* (1984, 1986) and intermittent monitoring of CO_2 production was undertaken by Schoeller & Webb (1984) using the suit method of calorimetry pioneered by Webb. The numbers studied by each group vary from one to four persons and depend, as in Schoeller & van Santen's (1982) original study, on a second measurement of the D distribution space to allow for changes in water space.

In all the studies the average discrepancy from the calorimetric value is small. These results would be very encouraging were it not for three features. First, all the studies were conducted under highly controlled conditions of approximate energy balance and in conditions which aimed at a steady-state. Yet the advantage of the D_2O^{18} technique is that it can be used in free-living subjects where food and water intake are not controlled and the subject has discretionary control over activity level. Second, the results were calculated by each group in a different way, with some workers using a number of variants on one calculation and deciding that the one which gives the best agreement between the D_2O^{18} technique and chamber calorimetry is the appropriate choice (Schoeller *et al.* 1986*b*). Third, we must recognize that in only the information on the

https://doi.org/10.1079/PNS19880036 Published online by Cambridge University Press

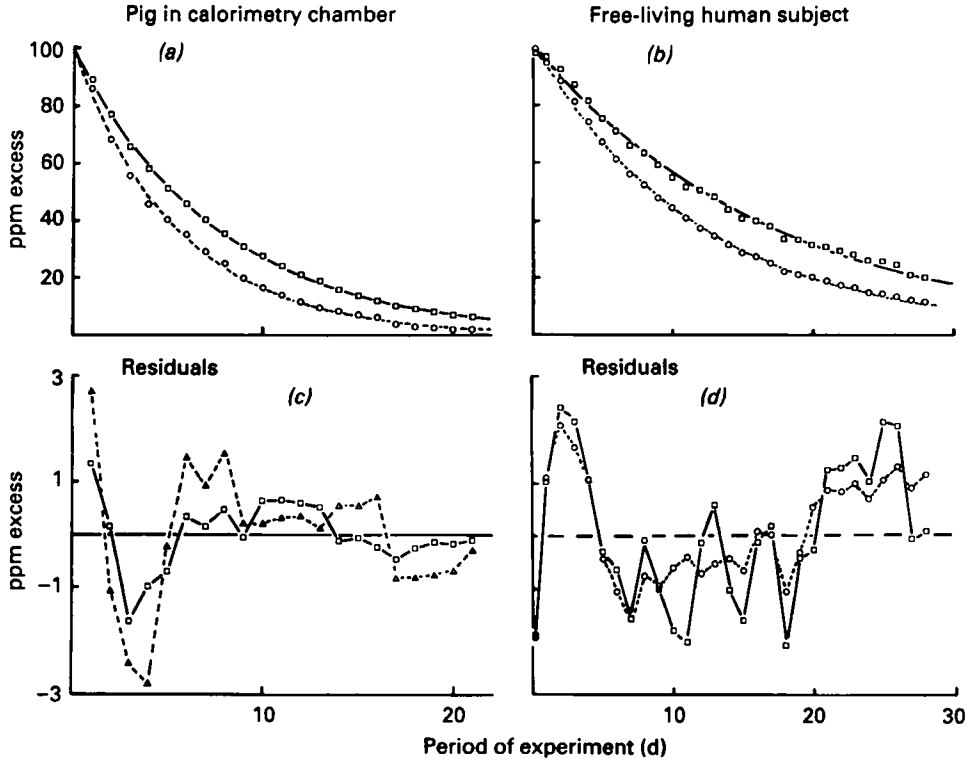

Fig. 2. The decline in isotopic labelling of urine in a pig studied under standardized conditions in a calorimetry chamber and in a free-living young woman. For ease of comparison the values have been normalized to 100 ppm excess at zero time. *(a)*, *(b)*, Values for individual urine measurements; *(c)*, *(d)*, residuals about the calculated single-exponential curve for each label.

four subjects described in limited form by Coward *et al.* (1984) is there a small standard deviation within the group when D_2O^{18} results are compared with indirect calorimetry. In all other studies the values for individuals are often surprisingly discrepant, with standard deviations which amount to about 5%, with an average value which seems to overestimate true CO_2 production by 2–4%.

Sources of error in the D_2O^{18} method

The three principal assumptions noted by Lifson & McClintock (1966) are, that all pool sizes and rates of intake and output remain constant (or the assumption of steady-state), that the proportion of water loss which is evaporative and the isotope-fractionation factors are known, and that there is no isotope incorporation into or release from products, or both, other than CO_2 and water. Violation of these assumptions can affect the calculation of energy expenditure in two ways: by reducing the precision of flux-rate estimation and, more importantly, by introducing bias into the calculation. Before proceeding to apply the technique widely we have sought to assess the magnitude of the errors caused by violation of these assumptions and to find ways of reducing such errors.

The assumption that all rates of intake and output are constant (i.e. steady-state) may not be valid in truly free-living subjects where food and water intake, level of activity etc. are not controlled. In truly steady-state (assuming no measurement error) the isotope-

https://doi.org/10.1079/PNS19880036 Published online by Cambridge University Press

decay curves should be perfect exponentials and any departure from steady-state manifests itself in isotope enrichments which deviate from the simple exponential model. The difference between steady-state and non-steady-state and its effect on the isotope-decay curve are well illustrated by Fig. 2 which shows the decay curve of D_2O^{18} enrichment in urine (normalized to 100 ppm excess at zero time) of a pig in a calorimetry chamber over 21 d and a free-living human subject over 28 d (Ferro-Luzzi *et al.* 1988). In both subjects the decay curves appear superficially to follow simple exponential decay as defined by the fitted lines. However, closer inspection of the deviation of the measured values from the fitted line (shown in the residual plots in Fig. 2) reveals a number of important points. It can be seen that the residuals in the pig values are largely proportional to the absolute enrichment value and this is what we would expect of a subject in a calorimeter chamber on a uniform daily regimen of food and water intake and controlled activity (i.e. steady-state). However, in the free-living human subject with discretionary control over food and water intake and activity we can see that the residuals are not simply proportional to the absolute values of enrichment, but are as large at the end of a 28-d experiment, when enrichment is low, as at the beginning, when enrichment is high. Also, it can be seen that the residuals are not randomly distributed about the fitted line but deviate from it in a systematic manner (i.e. non-steady-state).

This kind of variability has significant consequences when calculating flux-rates from isotope-decay curves using established methods. If the decay is mono-exponential and smooth then transformation of the urine enrichments to natural logs will result in a linear plot *v.* time where the gradient of the line equals the rate-constant and it is this procedure which is applied by most groups using multiple-point analysis (see e.g. Coward & Prentice, 1984; Coward *et al.* 1984; Klein *et al.* 1984; Prentice *et al.* 1985, 1986): Klein *et al.* (1984) used multiple regression on log-transformed values and the other authors used a curve-fitting programme (Feldman, 1977) in which measured values are weighted logarithmically before fitting. These approaches are effectively the same (Coward *et al.* 1988). However, linear transformation of non-linear data (or indeed the assumption of log normal errors in exponential decay curves) may distort the experimental variability, thereby leading to biased estimates for the values of the variables. Using the isotope enrichments given here for the free-living human subject (Fig. 2) we found that the simple procedure of log transformation before variable estimation resulted in a 4·5% underestimate of CO_2 production when compared with calculations based on untransformed values. This is important when put in the context of recently-reported accuracies of ±5% in validation studies comparing the D_2O^{18} technique with the highly-controlled conditions of indirect calorimetry (Fig. 1). It should be kept in mind, however, that this is the most extreme example we have encountered and that in many cases the assumption of log normal error distribution is appropriate, but this highlights the importance to the outcome of D_2O^{18} studies of choosing the appropriate method of calculation. We would caution therefore that residuals from each experimental subject be scrutinized before deciding on the best model to fit the data. The two-point method of calculating CO_2 production (Schoeller *et al.* 1986*b*; Schoeller & Taylor, 1987) is even more sensitive to variability in the isotope-decay curves of truly free-living subjects (Ferro-Luzzi *et al.* 1988; Haggarty *et al.* 1988*c*). Such variability reduces the precision of flux-rate estimation, but not necessarily the accuracy of the technique and this, at least partly, explains the reasonable accuracy but large standard deviations in most of the validation studies performed to date (Fig. 1). It should also be kept in mind that these validations were carried out under conditions which were designed to remove or reduce the effects of precisely those factors which are likely to invalidate the technique in the field, i.e. non-steady-states with fluctuating ambient temperature and humidity, uncertainty about

appropriate values for evaporative loss, etc. This lack of precision within groups is a problem since it may obscure the differences in energy expenditure which have to be discriminated if we are to answer the questions posed in Table 1. Thus, if this problem of variability is not tackled, D_2O^{18} will only be of limited use in, for example, the approximate estimation of the energy expenditure of groups of subjects for planning rather than experimental metabolic purposes. There are two ways of approaching this problem, the first is to identify the causes of variability so that they might be minimized at the planning stage of D_2O^{18} experiments, and the second is to modify our mathematical procedures of flux-rate estimation so that the effects of such variability are reduced.

We have found that variability in the isotope values from free-living subjects can largely be simulated simply from knowledge of daily water intake and CO_2 production (Haggarty *et al.* 1988*b*) and of these the most important is daily water intake (Haggarty & McGaw, 1988). Because water intake affects both D and O^{18} decay there is a large degree of covariance in the decay of both isotopes (Schoeller & Taylor, 1987) and this can be seen in the residual plots in Fig. 2 where both isotopes move above and below the fitted line in a synchronous manner. If, as is currently the practice, rate-constants or pool sizes, or both, are determined for each isotope separately and one flux-rate subtracted from the other, then this synchronous variability contributes to the loss of precision in the calculation of CO_2 production. However, if a curve is fitted directly to the difference between the isotope enrichments this removes that component of variability which acts on both isotopes, thereby increasing the precision of the method (Haggarty *et al.* 1988*a*). Alternatively, experimental protocols may be designed to minimize fluctuations in water intake; a measure which need not interfere with the process of energy expenditure. There is, however, an extra component to the variability in D decay in free-living subjects and this may be due to changes in the enrichment of ingested foods as has been suggested by Schoeller *et al.* (1986*b*). This differential variability is not removed by using difference curves or by standardizing water intake.

More important than loss of precision is the possible bias introduced by violation by free-living subjects of the assumptions underlying the technique. Of these assumptions the most critical is that relating to the estimation of fractionated water loss (see Coward, 1988). Use of incorrect evaporative loss results in bias in the calculation of CO_2 production within a subject, but within a group of subjects this is manifest as a loss of precision and possibly a bias in the calculation of CO_2 production. Even within a homogeneous experimental group, where there has been some attempt to calculate evaporative water loss from independent variables, there can be a large range of values. In pre-term infants the range was 0·5–0·33 (Roberts *et al.* 1986) and in male students Schoeller *et al.* (1986*b*) calculated the difference between water intake and losses in urine and faeces to range from 0·35 to 0·66. Ferro-Luzzi *et al.* (1988), who performed a similar calculation in female laboratory workers, also found large differences between subjects (0·36–0·62). This sort of range would result in an error of calculated CO_2 production within a group of approximately −3% to +3%. This range is large enough to obscure the difference in energy expenditure between control and treatment groups in many studies on energy metabolism (Table 1). There is the additional problem that even if evaporative loss is measured in each subject the route of evaporative water loss (i.e. in the breath, transcutaneous and sweat loss) has to be estimated since each of these routes may be susceptible to different degrees of isotope fractionation (see, for example, Schoeller *et al.* 1986*a*; Haggarty *et al.* 1988*d*) and the resulting calculations involve a number of further assumptions (Haggarty *et al.* 1988*b*). Therefore, when the evaporative loss is not measured directly in each subject the possibility cannot be ruled out that differences in

calculated CO_2 production between or within groups are simply artefacts of the calculation resulting from unobserved differences in evaporative loss. This is probably the most worrying aspect of the assumption or indirect estimation of evaporative loss.

Because of such assumptions and the errors involved in the estimation of evaporative loss, we have developed a method utilizing triply-labelled water which avoids these problems by measuring fractionated evaporative water loss directly in free-living subjects by exploiting the different fractionation factors of the two heavy isotopes of hydrogen (D and tritium). The different fractionation factors give rise to different rates of isotope decay and these may be used to calculate evaporative water loss. The theoretical precision of this method of calculating CO_2 production is ±0·3% (Haggarty *et al.* 1988*b*).

Conclusions

Table 1 highlights the multiplicity of questions which both animal and human nutritionists are attempting to answer. It is apparent that the regulation of energy balance is not only complex but precise. Thus, in both animals and man discrepancies of only 2–3% in energy turnover will, if consistent, lead to substantial changes in body energy. To assess these issues still demands very precise 24-h calorimetry and even here it is very easy to overlook small effects because of the variability in energy expenditure. Clearly for these studies D_2O^{18} is unsuitable given present error estimates, thus making whole-body calorimetry the method of choice.

The D_2O^{18} method is ideally suited to the more general problems of energy studies in free-living animals and man. The earlier validation studies on small animals may not be relevant to larger animals and humans so the attempts to assess the accuracy of the method are indeed appropriate. If the basis of the errors with the technique can be identified, then modified approaches may emerge for obtaining valid information in truly free-living subjects. The attractiveness of the D_2O^{18} technique is evident and it will certainly be widely applied. However, the different methods of calculation and the varying assumptions made in choosing the fractionation and evaporative factors mean that there is a danger of discrepant and misleading results being obtained unless greater effort is made to examine the basis for the errors recorded in current studies.

REFERENCES

Blaxter, K. L. (1962). *The Energy Metabolism of Ruminants*. London: Hutchinson.

Blaxter, K. L. (1983). In *Recent Advances in Animal Nutrition*, pp. 217–232 [W. Haresign, editor]. London: Butterworths.

Bleiberg, F., Brun, T. A., Gothman, S. & Lippman, D. (1981). *British Journal of Nutrition* **45**, 505–515.

Bogardus, C., Lillioja, S., Ravussin, E., Abbott, W., Zawadski, J. K., Young, A., Knowles, W. C., Jacobwitz, R. & Moll, P. P. (1986). *New England Journal of Medicine* **315**, 96–100.

Close, W. H. & Fowler, V. R. (1983). In *Recent Advances in Animal Nutrition*, pp. 159–174 [W. Haresign, editor]. London: Butterworths.

Corbett, J. L., Farrell, D. J., Leng, R. A., McClymont, G. L. & Young, B. A. (1971). *British Journal of Nutrition* **26**, 277–291.

Coward, W. A. (1988). *Proceedings of the Nutrition Society* **47**, 209–218.

Coward, W. A. & Prentice, A. M. (1984). In *Human Energy Metabolism: Physical Activity and Energy Expenditure Measurements in Epidemiological Research Based upon Direct and Indirect Calorimetry. Euro-Nut Report* no. 5, pp. 136–138 [A. J. H. van Es, editor]. Den Haag: CIP-gegevens koninklijke.

Coward, W. A., Prentice, A. M., Murgatroyd, P. R., Davies, H. L., Cole, T. J., Sawyer, M., Goldberg, G. R., Halliday, D. & MacNamara, J. P. (1984). In *Human Energy Metabolism: Physical Activity and Energy Expenditure Measurements in Epidemiological Research Based upon Direct and Indirect Calorimetry. Euro-Nut Report* no. 5, pp. 126–128 [A. J. H. van Es, editor]. Den Haag: CIP-gegevens koninklijke.

Coward, W. A., Roberts, S. B. & Cole, T. J. (1988). *European Journal of Clinical Nutrition* **42**, 207–212.

Dallosso, H. M., Murgatroyd, P. R. & James, W. P. T. (1982). *Human Nutrition: Clinical Nutrition* **36C**, 25–39.
Dauncey, M. J., Murgatroyd, P. R. & Cole, T. J. (1978). *British Journal of Nutrition* **39**, 557–566.
De Boer, J. O., van Es, A. J. H., Vogt, J. E., van Raaij, J. M. A. & Hautvast, J. G. A. J. (1987). *British Journal of Nutrition* **57**, 201–209.
Durnin, J. V. G. A., Edholm, O. G., Miller, D. S. & Waterlow, J. C. (1973). *Nature* **242**, 418.
Food and Agriculture Organization (1985). *5th World Food Survey*. Rome: FAO.
Food and Agriculture Organization/World Health Organization/United Nations University (1985). *Technical Report Series no. 724*. Rome: FAO.
Feldman, H. A. (1977). *American Journal of Physiology* **233**, R1–R7.
Ferro-Luzzi, A., Scaccini, C., Virgili, F., Haggarty, P., McGaw, B. A. & James, W. P. T. (1988). *Proceedings of the Nutrition Society* **47**, 31A.
Fontaine, E., Savard, R., Tremblay, A., Despies, J. P., Poehlman, E. & Bouchard, C. (1985). *Acta Genetica Medica Gemellologiae* **34**, 41–47.
Fuller, M. F., Webster, A. J. F., MacPherson, R. M. & Smith, J. S. (1977). In *Energy Metabolism of Farm Animals, 7th Symposium, Vichy. European Association of Animal Production Publication* no. 19, pp. 177–180 [M. Vermorel, editor]. Clermont Ferrand: De Bussac.
Garby, L., Lammert, O. & Nielsen, E. (1984). *Human Nutrition: Clinical Nutrition* **38C**, 391–394.
Haggarty, P., Franklin, M. & McGaw, B. A. (1988*a*). *Obesity in Europe*, 1 *Proceedings of the First European Congress on Obesity* (In the Press).
Haggarty, P. & McGaw, B. A. (1988). *Proceedings of the Nutrition Society* **47**, 365–374.
Haggarty, P., McGaw, B. A. & Franklin, M. (1988*b*). *Journal of Theoretical Biology* (In the Press).
Haggarty, P., McGaw, B. A., James, W. P. T., Ferro-Luzzi, A., Scaccini, C., Virgili, F. & Franklin, M. (1988*c*). *Proceedings of the Nutrition Society* **47**, 33A.
Haggarty, P., McGaw, B. A., Maughan, R. J., Milne, E. & Gleeson, M. (1988*d*). *Proceedings of the Nutrition Society* **47**, 74A.
James, W. P. T. (1983). *Lancet* **ii**, 386–389.
James, W. P. T. (1985). In *Substrate and Energy Metabolism in Man*, pp. 108–118 [J. S. Garrow and D. Halliday, editors]. London: J. Libbey & Co.
James, W. P. T. (1986). *Nutrition Research* **6**, 1229–1231.
Klein, P. D., James, W. P. T., Wong, W. W., Irving, P. R., Murgatroyd, M., Cabrera, M., Dallosso, H. M., Klein, E. R. & Nichols, B. L. (1984). *Human Nutrition: Clinical Nutrition* **38C**, 95–106.
Lifson, N. & McClintock, R. (1966). *Journal of Theoretical Biology* **12**, 46–74.
Lin, P. Y., Romsos, D. R., Vander Tuig, J. G. & Leveille, G. A. (1979). *Journal of Nutrition* **109**, 1143–1153.
McNeill, G., McBride, A., Smith, J. S. & James, W. P. T. (1988). *Proceedings of the Nutrition Society* **47**, 138A.
Morgan, J. B., York, D. A., Wasilewska, A. & Portman, J. (1982). *British Journal of Nutrition* **47**, 21–32.
Norgan, N. G., Ferro-Luzzi, A. & Durnin, J. V. G. A. (1974). *Philosophical Transactions of the Royal Society Series B* **268**, 309–348.
Prentice, A. M., Black, A. E., Coward, W. A., Davies, H. L., Goldberg, G. R., Murgatroyd, P. R., Ashford, J., Sawyer, M. & Whitehead, R. G. (1986). *British Medical Journal* **292**, 983–987.
Prentice, A. M., Coward, W. A., Murgatroyd, P. R., Davies, H. L., Cole, T. J., Sawyer, M., Goldberg, G. R., Halliday, D. & MacNamara, J. P. (1985). In *Substrate and Energy Metabolism in Man*, p. A18 [J. S. Garrow and D. Halliday, editors]. London: J. Libbey & Co.
Roberts, S. B., Coward, W. A., Schlingenseipen, K.-H., Nohria, V. & Lucas, A. (1986). *American Journal of Clinical Nutrition* **44**, 315–332.
Rose, G. A. & Williams, R. T. (1961). *British Journal of Nutrition* **15**, 1–9.
Schoeller, D. A., Leitch, C. A. & Brown, C. (1986*a*). *American Journal of Physiology* **251**, R1137–R1143.
Schoeller, D. A., Ravussin, E., Schutz, Y., Acheson, K. J., Baertschi, P. & Jéquier, E. (1986*b*). *American Journal of Physiology* **250**, R823–R830.
Schoeller, D. A. & Taylor, P. B. (1987). *Human Nutrition: Clinical Nutrition* **41C**, 215–223.
Schoeller, D. A. & van Santen, E. (1982). *Journal of Applied Physiology* **53**, 955–959.
Schoeller, D. A. & Webb, P. (1984). *American Journal of Clinical Nutrition* **40**, 153–158.
Schutz, Y., Ravussin, E., Diethelm, R. & Jéquier, E. (1982). *International Journal of Obesity* **6**, 23–28.
Sukhatme, P. V. & Margen, S. (1982). *American Journal of Clinical Nutrition* **35**, 355–365.
Webster, A. J. F., Smith, J. S. & Mollison, G. S. (1977). *Animal Production* **24**, 237–244.
Westerterp, K. R., Saris, W. H. M., van Es, M. & ten Hoor, F. (1986). *Journal of Applied Physiology* **61**, 2162–2167.

Westerterp, K. R., Schoffelen, P. F. M., Saris, W. H. M. & ten Hoor, F. (1984). In *Human Energy Metabolism: Physical Activity and Energy Expenditure Measurements in Epidemiological Research Based upon Direct and Indirect Calorimetry. Euro-Nut Report* no. 5, pp. 129–131 [A. J. H. van Es, editor]. Den Haag: CIP-gegevens koninklijke.

Whitelaw, F. G., Milne, J. S., Ørskov, E. R. & Smith, J. S. (1986). *British Journal of Nutrition* **55**, 537–556.

Zed, C. & James, W. P. T. (1986*a*). *International Journal of Obesity* **10**, 375–390.

Zed, C. & James, W. P. T. (1986*b*). *International Journal of Obesity* **10**, 391–405.

Printed in Great Britain