

Non-restrictive methods for measuring energy expenditure

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Knowledge of whole-body energy expenditure is important if we are to make accurate estimates of energy requirements for optimum health and performance in man and optimum health, performance and production in animals. Energy requirements vary with nutritional, genetic and environmental influences and these can interact in complex ways, therefore it is necessary to isolate and identify specific factors which are important in controlling energy expenditure. In order to do that it is useful to reduce whole-body energy expenditure to smaller component parts; e.g. basal metabolic rate (BMR), dietary-induced thermogenesis, the energy cost of activity, growth and lactation, etc. These components are normally measured in a calorimeter. The chambers in which the subjects are housed for the period of measurement are often relatively small; examples at the Rowett Research Institute include four animal chambers each with a floor area of 1.5 m² and two human chambers each with a floor area of 6.5 m². In the highly controlled and defined conditions of such calorimetry chambers it is possible to investigate the effects of nutritional, genetic and environmental influences on each of the components of energy expenditure separately. However, because of the physical restriction necessary in calorimetry chambers and precisely because of the controlled conditions of temperature, activity, food intake, etc. such measurements may not be representative of the true energy expenditure (and hence energy requirements) of free-living animals and man. In order to measure these we need techniques which will allow the measurement of energy expenditure without restriction.

Outside of calorimeter chambers free-living energy expenditure can be estimated in human subjects from recorded food intake and changes in body energy stores. In animal studies changes in body composition can be determined more exactly in serial-slaughter experiments, but it is more difficult to measure energy intake in the field. These techniques are based on the simple relation:

$$\text{energy expenditure} = \text{metabolizable energy intake} - \text{energy retained in the body.}$$

Alternatively energy expenditure may be estimated from calorimetric determination of the energy cost of various activities and, using estimates of the time spent in each of these activities, a value for total free-living energy expenditure can be arrived at. In addition to these approaches are two isotopic techniques, the labelled bicarbonate method and the doubly-labelled-water (DLW) method. Both these techniques measure carbon dioxide production isotopically but the similarity ends there. The former method requires a constant infusion of bicarbonate (labelled with radioactive ¹⁴C or the stable isotope ¹³C) in concert with frequent collection of some body fluid so that the dilution of label and hence bicarbonate production in the body may be calculated. Such experiments typically last for periods of less than 24 h but have the disadvantage that they are invasive. The DLW technique on the other hand gives integrated CO₂ production values over periods of 1–4 weeks, is non-invasive, and requires only infrequent sampling. Therefore, if energy expenditure over long periods is required, as when attempting to estimate energy requirements, or it is important that the subject is disturbed as little as possible, then DLW is the technique of choice. Much has been published on use of the

bicarbonate method in animals (for example, see Young *et al.* 1969; Whitelaw *et al.* 1972) and man (for review, see Elia *et al.* 1988) therefore the present paper deals only with the DLW technique which has lately been the subject of much interest, since reduced isotope costs and increased mass spectrometer precision have made feasible its use in large animals and man.

Theory of the DLW technique

Lifson & McClintock (1966) described this method for measuring energy expenditure from the difference in turnover rate of the hydrogen and oxygen of body water. The turnover rate of the O of body water is greater than that of H because O but not H is lost via respiratory CO₂. The equilibration of CO₂ and water is catalysed by carbonic anhydrase (EC 4.2.1.1). Each enzyme molecule can hydrate 10⁵ molecules CO₂ in 1 s. This is 10⁷ times faster than the uncatalysed reaction and, for the purposes of the DLW method, equilibration can be considered to be instantaneous. When body water is labelled with D₂¹⁸O the difference between the two turnover rates can be measured and employed to calculate the rate of CO₂ production (r_{CO_2}). In its simplest form r_{CO_2} is calculated as the ¹⁸O flux minus the deuterium flux times 0.5 (Coward *et al.* 1985). However, isotope fractionation effects during water and CO₂ loss mean that the apparent flux rates have to be corrected. During evaporation deuterated and ¹⁸O-labelled water molecules are less volatile than those containing protium and ¹⁶O. The equilibrium fractionation factors for deuterium (f_1) and ¹⁸O (f_2) between water vapour and water liquid at 37° are 0.941 and 0.9925 respectively (Dansgaard, 1964; Schoeller *et al.* 1986a). However, there is another fractionation factor relevant to the calculation of r_{CO_2} and that is due to the fractionation which occurs when heavy-water-O equilibrates with CO₂-O (f_3). In this case the heavy species is concentrated in the gaseous phase giving an equilibrium fractionation factor for CO₂ gas to liquid water of 1.039 at 37° (Pflug *et al.* 1979). Assuming that fractionation is an equilibrium process these factors can be used to calculate the water flux (r_w) and hence r_{CO_2} , if the proportion of water which is lost evaporatively (X) is known. This simple model is represented graphically in Fig. 1 and mathematically as follows (D and O are the experimentally-derived flux rates);

$$r_w = D \{ f_1 X + (1-X) \}^{-1}, \quad (1)$$

$$r_{CO_2} = \frac{O - (f_2 X r_w) + (1-X) r_w}{2 f_3}. \quad (2)$$

The D and O flux rates are calculated from the rate-constant of isotope decay times pool size (Shiple & Clark, 1972) and it should be noted that there are differences in the way the rate-constants for isotope decay are calculated; these can be computed from exponential curves fitted to multiple-isotope-enrichment data points (Haggarty *et al.* 1988b), from the gradient of a straight line fitted to log-transformed multiple-isotope enrichment data points (e.g. Klein *et al.* 1984), or by fitting a straight line between two log-transformed data points, one at the beginning and one at the end of the study (Schoeller *et al.* 1986b). A discussion of the relative merits of these approaches has been presented elsewhere (James *et al.* 1988).

In most studies in energy metabolism it is energy expenditure and not r_{CO_2} which is of interest, therefore it is necessary to calculate the O₂ consumption (r_{O_2}) using the respiratory quotient (RQ) of the subject.

The r_{O_2} and r_{CO_2} values can then be substituted in equations such as that proposed by Weir (1949) to derive a value for energy expenditure. Black *et al.* (1986) have shown that the food quotient may be substituted for RQ in adult human subjects when calculating

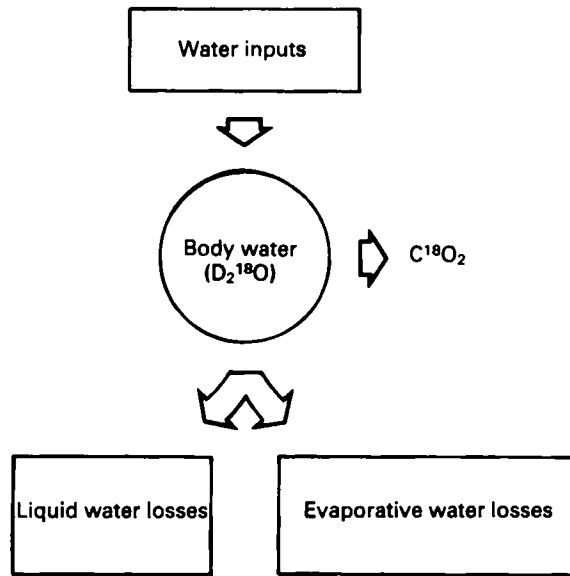


Fig. 1. Simple model for isotope movement in doubly-labelled-water studies.

r_{O_2} and energy expenditure, and that any error incurred in this way will 'usually be negligible and should never exceed $\pm 2\%$ '. However, in human infants and growing production animals the RQ may deviate substantially from that found in adult weight-stable humans and estimation of the correct value may prove difficult. This remains one of the largest problems in both the DLW and bicarbonate methods of measuring energy expenditure during growth. A number of laboratories are presently evaluating methods of estimating RQ in growing animals and one isotopic approach has already been suggested (Speakman & Racey, 1987).

Factors affecting the accuracy and precision of the DLW technique

Lifson & McClintock (1966) outlined a number of assumptions which have to be fulfilled before the technique can be applied with confidence. The four main assumptions (illustrated in Fig. 2) are: (1) that all pool sizes and rates of intake and output remain constant (or the assumption of steady-state); (2) that the proportion of water loss which is evaporative and the isotope fractionation factors are known; (3) that no isotope re-enters the body; (4) that there is no isotope incorporation into or release from products other than CO_2 and water.

Assumption 3 which refers to rebreathing of expired isotope is only a problem in very confined spaces where the expired gases can build up sufficiently. Such a situation is only likely to occur during prolonged confinement in calorimetry chambers, for example, therefore this is a reasonable assumption in free-living subjects. In the light of recent work we can now begin to assess the validity of the other three assumptions in animals and man.

(1) *The assumption of steady state.* The basic unit of time for DLW studies, in humans at least, is 1 d; urine samples are normally used to measure isotope enrichment and these are usually taken as the first voiding of the bladder in the morning. Thus variability in isotope decay relating to non-steady-state should be considered on a daily basis. As with RQ the daily variability in evaporative water loss (X), and the fractionation factors (f_1 , f_2 and f_3) are of little importance if the average value for these variables over the

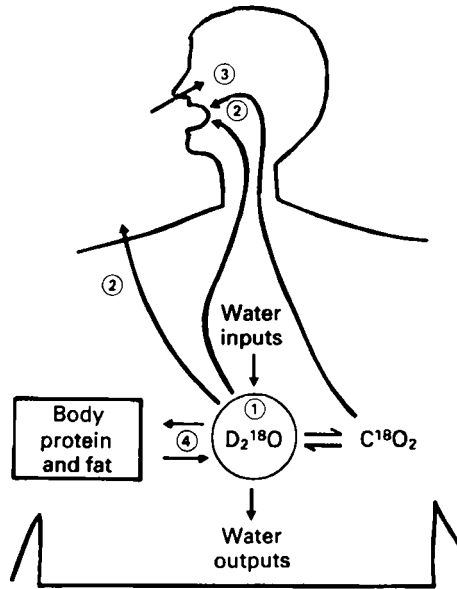


Fig. 2. Assumptions inherent in the doubly-labelled-water technique: (1) that all pool sizes and rates of intake and output remain constant (or the assumption of steady-state); (2) that the proportion of water loss which is evaporative and the isotope fractionation factors are known; (3) that no isotope re-enters the body; (4) that there is no isotope incorporation into or release from products other than CO_2 and water.

experimental period is known. Where deviation from steady-state is most important is in the determination of deuterium and ^{18}O flux-rates from isotope decay values. The flux-rates (D and O) are calculated from the rate-constant of isotope decay times, the isotope distribution space or pool size, and variability in either the pool size or rate of isotope decay will reduce the precision of the computed flux-rates.

It is known that pool sizes can change during the course of a DLW experiment (typically 1–4 weeks), but if the mean of the initial and final pool sizes is used in the equation very little error arises in the calculation of CO_2 production, at least in adult humans where percentage changes in the pool sizes are not large. However, in rapidly growing immature animals and in human infants the percentage change is larger and may not be linear. It is therefore necessary to know the form of change (e.g. exponential) in such subjects before an average pool size can be calculated from initial and final values. The form of change in pool sizes can be estimated with reasonable accuracy simply from body-weight changes throughout the experiment. However, the assumption that all rates of intake and output are constant in truly free-living subjects where food and water intake, level of activity, etc. are not controlled is more problematical. As illustrated in Fig. 1 it is r_w and r_{CO_2} which are the main determinants of the rate of isotope decay, and consequently it is daily variability in these variables which is likely to result in variability in the isotope decay values. We have shown that the observed variability in decay values from free-living subjects can largely be explained by daily variation in water intake (Haggarty *et al.* 1988a). The lesser effect of r_{CO_2} on variability in isotope decay can be

deduced from observed flux-rates; e.g. in one sedentary female subject we measured a H_2^{18}O flux-rate of 2.828 litres/d and a D_2O flux-rate of 2.219 litres/d, thus only 21.5% of H_2^{18}O flux was due to r_{CO_2} . Also, when it is considered that approximately 65% of r_{CO_2} is the product of basal metabolic rate in sedentary female subjects (Food and Agriculture Organization/World Health Organization/United Nations University, 1985), only 35% of CO_2 production is under discretionary control and therefore variable. This means that less than 8% of H_2^{18}O flux, and none of the D_2O flux, is susceptible to daily variation in r_{CO_2} . The significant degree of covariance in deuterium and ^{18}O decay (Schoeller & Taylor, 1987; Haggarty *et al.* 1988a) support the proposal that it is mainly water intake which gives rise to variability in the decay curves.

(2) *Isotope fractionation and evaporative water loss.* As can be seen from eqns (1) and (2) the calculation of r_{CO_2} is dependent on the value used for the proportional evaporative water loss (X). In some studies the authors have attempted to estimate evaporative loss in each subject (e.g. Schoeller *et al.* 1986b; Westerterp *et al.* 1986; Ferro-Luzzi *et al.* 1988), whilst in others one estimate is taken for all the subjects (Schoeller & Webb, 1984; Prentice *et al.* 1985, 1986; Fancy *et al.* 1986). However, within an experimental group, where there has been some attempt to calculate evaporative water loss from independent variables, there can be a large spread of values. In pre-term infants for example the range was 0.05–0.33 (Roberts *et al.* 1986) and in male students Schoeller *et al.* (1986a) calculated the difference between water intake and losses in urine and faeces (assumed to be evaporative loss) to range from 0.35 to 0.66. Thus even in a homogeneous group such as male students there can be a twofold range in evaporative water loss. This has a significant effect on the calculation of r_{CO_2} ; if an evaporative water loss of 0.5 had been assumed for all these subjects the error on r_{CO_2} would be approximately –3% in the subject with the lowest evaporative loss and +3% in the subject with the highest evaporative loss. An additional problem is that the fractionated evaporative water loss (X) to be used in eqns (1) and (2) is not the same as the proportion of water lost as vapour (Haggarty *et al.* 1988a) and it is this latter variable which is measured in water balance studies (e.g. Schoeller *et al.* 1986b) and in chamber studies where water vapour loss is quantified (Klein *et al.* 1984). Schoeller *et al.* (1986b) have suggested a way round this by separating water vapour into that lost from the breath and that lost from the skin, then further dividing skin loss into fractionated and unfractionated components. However, this approach involves a number of further assumptions about the amount of water lost per litre of CO_2 in the breath, the amount of fractionated water lost from the skin, etc. Because of such assumptions we have suggested a method which measures evaporative water loss directly in free-living subjects by exploiting the different fractionation factors of the two heavy isotopes of O (^{18}O and ^{17}O) and the two heavy isotopes of H (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 (for full discussion, see Haggarty *et al.* 1988a).

(3) *Isotope sequestration and release.* Label movement into and out of products other than CO_2 and water may be reduced to two distinct processes:

(3a) *Exchange of water isotopes with labile H and O.* The exchange phenomenon is well documented and its most obvious manifestation is found in the difference between D and ^{18}O distribution spaces. This phenomenon arises because D can exchange freely with labile H in body solids thus resulting in a distribution space which is greater than body water. On the other hand, ^{18}O exchange with body solids is much less frequent and water labelled with this isotope is a better marker for body-water space. The differences in distribution space are dealt with by Coward *et al.* (1985) by determining the D and ^{18}O distribution spaces separately whereas the original Lifson & McClintock (1966) equation

assumes that the spaces are the same. Schoeller *et al.* (1986a) have modified the equation proposed by Lifson & McClintock (1966) to take account of the different distribution spaces by assuming that the D distribution space is always 1.03 times the ^{18}O space. The relative merits of each approach have recently been discussed in detail (Roberts *et al.* 1987; Wong *et al.* 1987). There is of course also exchange of water-O with bicarbonate, indeed the DLW technique is based on this fact. Thus the ^{18}O distribution space, like the D space, will be greater than the body-water space. Taking values derived by Irving *et al.* (1984) for the bicarbonate pool size in man (11306 $\mu\text{mol/kg}$ body-weight), the assumption that ^{18}O is in equilibrium with this pool and that 1 mol water is equivalent to 3 mol bicarbonate it follows that the bicarbonate pool is equivalent to 0.6105 g water/kg body-weight. Thus in a 50 kg subject with a body-water pool size of 28 kg the ^{18}O distribution space will overestimate body water by 30.525 g or 0.1%. This factor is forty times smaller than that for D where the body water is overestimated by approximately 4%.

(3b) *Incorporation of water isotopes into and release from non-labile positions.* Synthesis of palmitic acid results in the incorporation of 13.3 H atoms derived from water (Windmueller & Spaeth, 1966) whilst no water-O is incorporated. During synthesis of protein from amino acids one water-H is incorporated per peptide bond and no water-O is incorporated. This phenomenon of label sequestration is well documented and indeed H-isotope incorporation from water into protein and fat has been used as a method of measuring turnover rates in vivo (see, for example, Jungas, 1968; Humphrey & Davies, 1975). Such processes will result in increased D flux, thus causing r_w to be overestimated and r_{CO_2} to be underestimated; but are rates of synthesis in vivo sufficient appreciably to affect the D flux-rate? Taking the extreme example of the genetically obese Zucker rat, which has a very high rate of lipogenesis relative to body size, we have calculated that fatty acid synthesis would result in an underestimate of r_{CO_2} of approximately 4%. This value was calculated knowing that the rate of fatty acid synthesis in 25-d-old obese rats is 2731 $\mu\text{mol/d}$ (Haggarty *et al.* 1986) and D flux is approximately 1906 mmol/d (Bell & Stern, 1977). Synthesis of 1 molecule of C_{16} fatty acid results in the incorporation of 13.3 atoms of heavy H (Windmueller & Spaeth, 1966). Thus, if we assume that C_{16} is the mean chain length, synthesis of 2731 μmol fatty acid will result in the incorporation of 36.32 mmol H derived from water; this is equivalent to 18.16 mmol water since there are two atoms of H/molecule of water. This will cause r_w to be overestimated by 1% and r_{CO_2} to be underestimated by 4%. The magnitude of the effect in other species will depend on the rate of reductive biosynthesis relative to r_{CO_2} , therefore generalizations cannot be made for all species in all physiological states.

On the other hand, because of isotope discrimination effects, any body fat synthesized before the isotope dose is given will be depleted in D relative to body water, and water derived from this source during a DLW experiment would cause the body water-D enrichment to fall. Simultaneous incorporation and release would introduce additional variability into the isotope decay values thereby making the determination of flux-rates less precise. This problem has already been discussed from the point of view of the enrichment of ingested food with respect to that of ingested water (Schoeller *et al.* 1986a). Because of the same isotope discrimination effects the D enrichment of food is generally less than that of drinking water, therefore any metabolic water derived from oxidized foodstuffs will be depleted in H thereby causing r_w to be overestimated and r_{CO_2} to be underestimated. However, fat, protein and carbohydrate are depleted by different amounts (Schoeller *et al.* 1986a) and changes in the composition of food ingested throughout a heavy-water experiment may result in variability in the isotope decay values. Schoeller *et al.* (1986a) have calculated that changing baseline isotope enrichment

due to a change in diet can result in errors in the calculation of CO_2 production ranging from -6% to $+8\%$.

A realistic model for isotope movement in DLW studies

Considering these assumptions and their validity we come to a model for label movement in heavy-water studies (Fig. 3) which is more complex than Fig. 1 but also more realistic. Each of these additional processes can affect the calculation of r_{CO_2} in two ways: by reducing the precision of flux-rate estimation and, more importantly, by introducing bias into the calculation. Daily variability in the composition of food eaten, total water intake and body-water pool size will introduce variability into isotope decay values, as will isotope incorporation into and release from non-labile positions in body solids. All these processes will reduce the precision of the determination of flux-rates and hence r_{CO_2} . However, if any of these processes were to occur systematically then this could introduce bias; for example, if there were net incorporation of D into fat during the course of a DLW experiment. Potentially the most serious source of bias in the DLW technique is the estimation of the proportion of water loss which is evaporative. As discussed already there can be large differences in evaporative loss even within a homogeneous group of subjects, therefore when evaporative loss is not measured in each subject the possibility cannot be ruled out that calculated differences in r_{CO_2} between or within groups may simply be artefacts due to undetected differences in evaporative loss.

All the additional processes illustrated in Fig. 3 can, however, be dealt with at the planning stage of DLW experiments so that their impact on the calculation of r_{CO_2} is

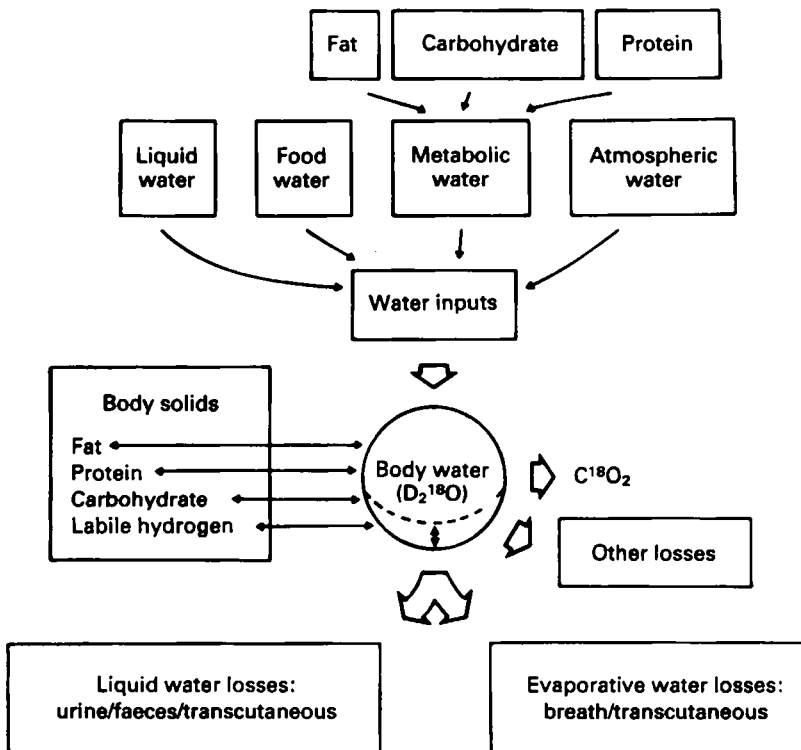


Fig. 3. A realistic model for isotope movement in doubly-labelled-water studies.

minimized. For example, it may be possible to standardize daily water intake without interfering with free-living energy expenditure or, in dietary intervention studies, it may be advantageous to control the source and hence enrichment of the various components of the diet. In addition, methods of indirect estimation (Schoeller *et al.* 1986b) and direct measurement (Haggarty *et al.* 1988a) of proportional evaporative water loss have been proposed and, if applied to each subject, may eliminate this the largest source of error.

Application of the DLW technique

Despite the potential problems the DLW method has already been used to great effect in a number of studies in human subjects. As stated previously one of the main uses of DLW is in the estimation of free-living energy requirements. At present energy requirements are calculated as some multiple of BMR (computed from body-weight and height), the magnitude of which depends on an arbitrary classification of the subject's activity level. It is therefore interesting to compare energy requirements determined using DLW with present best estimates from the Department of Health and Social Security (1979) and Food and Agriculture Organization/World Health Organization/United Nations University (1985) prediction equations. Prentice *et al.* (1985, 1986) have observed that both lean and obese women in Britain had lower free-living energy expenditures than the lowest estimate from the Department of Health and Social Security (1979) and Food and Agriculture Organization/World Health Organization/United Nations University (1985) prediction equations. Similarly, we have observed that women classified as sedentary in Italy had free-living energy expenditures lower than the minimum Food and Agriculture Organization/World Health Organization/United Nations University (1985) estimates (Ferro-Luzzi *et al.* 1988). At the other end of the physiological spectrum we have found the energy expenditure of a group of elite female athletes in Britain to be greater than the highest estimate given by the Department of Health and Social Security (1979) for very active individuals and the Food and Agriculture Organization/World Health Organization/United Nations University (1985) for females engaged in heavy work (Haggarty *et al.* 1988c). Westerterp *et al.* (1986) have also observed that the energy expenditure of athletes during the Tour de France cycle race greatly exceeds Food and Agriculture Organization/World Health Organization/United Nations University (1985) estimates for men engaged in heavy work. These examples illustrate the potential of DLW to refine and improve estimates of the energy requirements of specific groups. However, it should be kept in mind that the DLW technique is not an alternative to chamber calorimetry, but its natural complement. The most powerful use of the heavy-water technique is in conjunction with controlled chamber calorimetry, where it is possible to measure, for example, BMR, the thermic response to feeding, and the energy cost of set activities in the chamber, and then to determine the combined effect of all the energy-expending processes in a free-living DLW experiment where the subject has discretionary control over food intake and physical activity and is exposed to the elements.

In the studies listed previously it was sufficient that energy expenditure be estimated within relatively large tolerances. However, studies which require more exact values, to enable discrimination of small differences in energy expenditure, demand greater precision and accuracy. A number of laboratories are presently working on the assumptions and problems outlined here to eliminate potential bias and further increase the precision of the DLW method for measuring energy expenditure in free-living subjects.

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