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Stable isotopes in nutritional science and the study of energy metabolism

By Andrew M. Prentice

ABSTRACT

The presence in nature of stable isotopic forms of almost all the elements in organic molecules, and of many minerals of nutritional interest, offers enormous possibilities for their application in human nutrition research. This review summarises the range of applications and discusses the reasons why some have proved more productive than others. Discoveries stemming from the use of the doubly-labelled water ($^2H_2{}^{18}O$) method for assessing energy expenditure are considered in depth in order to illustrate the great potential of isotope techniques. An analysis of the reasons for this success concludes that there is a need for the development of world-wide consensus on standardised procedures for stable isotope methods. The development of such protocols, together with strong partnerships between mass spectrometrists and biologists, will unlock the great potential of other stable isotope methods.

Applications of stable isotopes

The occurrence in nature of stable isotopes of certain elements which are chemically indistinguishable, but which can be separated and measured on the basis of their different mass has been exploited in biological research for over 50 years (1). Many of these applications have been in the field of nutrition where there is both qualitative interest in the nature of biochemical pathways and quantitative interest in nutrient flux and turnover.

An abbreviated list of some of the stable isotopes most commonly exploited by nutrition scientists appears as Table 1. A key feature of this table is the existence of multiple isotopes of some elements which allow double-labelled experiments of greater sophistication than could be achieved with only a single isotope. The existence of stable isotopes of the chief elements of organic molecules (C, H, O and N) also permits multiple labelling of water and organic compounds. Precise positional labelling of compounds such as sugars and fatty acids further expands the experimental repertoire of stable isotopes.Table 2 lists the extensive range of applications which have been applied throughout a broad spectrum of animals, and over all stages of the life course. There is no single source of information on all of these applications, so the interested reader will need to search the literature. However, the following texts cover many of the basic principles and a range of applications in some detail (2-9).

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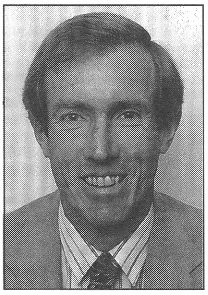


Limiting factors

The long list of applications in Table 2 conceals the regrettable fact that most of these possibilities have only been exploited by a handful of laboratories around the world, and that there remains an enormous untapped potential. The following sections will present one of the domains (in energy expenditure research) where stable isotopes have excelled, and will demonstrate what can be achieved when a technique is refined in a number of laboratories, and applied using rigorously validated and standardised procedures. First it is worth considering what have been the barriers to progress in some of the other fields.

Perhaps the biggest limitation is the high capital costs of mass spectrometers (which are frequently in the range \$100,000 to \$250,000) and the need to maintain a dedicated technical support team who understand both the mechanical operation of the equipment and the bio-mathematics of tracer techniques. The cost of the isotopes themselves can also be extremely high especially when required in relatively large quantities as in the doubly-labelled water method (see below), or when they have to be specially synthesised for a particular application. Although cost and expertise are real barriers to progress, particularly in developing countries, experience with the doubly-labelled water method has shown that it can often be easier to generate funding for costly techniques which promise real advance than for older, but cheaper, technologies which perform less well.

Another generic problem relates to the need to find an appropriate balance between excess simplicity (which can render answers meaningless) and excess complexity (which intimidates potential users or generates mathematical answers which are physiologically uninterpretable). Some breath test techniques fall into the former category, and many multi-compartment kinetic models fall into the latter. It is only when the theoretical purists and biological pragmatists can find common ground that a technique will succeed.

Lack of agreed standardisation between users has also been a major problem with respect to some applications. For instance, in the field of protein turnover a potential new user is faced with a bewildering choice of possible methodological variants each of which provide a different answer (^{13}C or ^{15}N labelling; glycine, essential amino acids or labelled protein as tracers; oral or intravenous dosing; bolus dose, constant infusion, or flooding dose; plasma or urine samples; stochastic or compartmental modelling; etc). In this respect the early establishment of consensus recommendations for doubly-labelled water applications was an important stimulus to the progress outlined below (10). The International Atomic Energy Agency (IAEA) is currently pursuing this theme by setting up standardised stable isotope methods through their Co-ordinated Research Programmes (11).

Perhaps the most important barrier to progress has been a failure to put together appropriate teams of people with complementary expertise ranging from biologists or clinicians with burning hypotheses to be tested, to mass spectrometrists and mathematical theoreticians capable of supplying a solution. It is a very rare individual who has the intellect, time and drive to cover all of these aspects so the establishment of a cohesive stable isotope team is of prime importance. Such teams can be created within large institutes or, with sufficient encouragement, can span the globe. IAEA is also assisting in this respect with Technical Co-operation Grants which link established centres of excellence with each other and with developing country partners (IAEA 1997).

The following sections describe our own laboratory's experience with stable isotope methods for assessing human energy expenditure (EE) and will use the example of the doubly-labelled water ($^{2}H_2{}^{18}O$) method to show what can be achieved.

Stable isotope use

A detailed knowledge of the energy requirements of animals and man forms one of the cornerstones of nutritional science, since it is these requirements which drive an animal's intake of food and hence of other nutrients. The history of energy metabolism as a precise science spans two centuries with its origins in the work of *Antoine Lavoisier* who was the first to recognise the true role of oxygen and combustion in the generation of heat and mechanical work by biological organisms (12). For most of these two centuries measurements have been made by direct calorimetry (measurement of heat loss) or by indirect calorimetry (measurement of respiratory gas exchange and subsequent calculation of heat production), but in the 1940's Dr *Nathan Lifson* at the University of Minnesota made a conceptual breakthrough which developed into the doubly-labelled water (DLW) method as we now know it (13). He realised that the liberation of carbon dioxide could be traced by following the disappearance rate of an oral dose of water labelled with oxygen-18 (assessed from the ^{18}O concentration in serial urine samples). The need for the use of deuterium (^{2}H) as a double label is to correct for the fact that the primary route of oxygen disappearance is in the form of water, and this must be accounted for in order to compute carbon dioxide production. The doubly-labelled water method provides an accurate means of assessing the integrated free-living energy expenditure of people over periods ranging from 10-20 days in adults. The 40 year delay between discovery of the method and its first applications in humans was simply due to cost. In the 1940s it would have cost about $5000 to make a single measurement in man, but by the early 1980s the precision of isotope ratio mass spectrometers had improved to such an extent that the requirement for isotope was decreased by a factor of 10 and the method became affordable (though still expensive). *Schoeller* was the first to restimulate interest in DLW and published the first validation in man (14).

A somewhat analogous method is termed the labelled bicarbonate technique. This requires administration of a constant infusion of a solution of $NaH^{13}CO_2$ given subcutaneously by mini-pump (15,16). The need for a constant infusion is an obvious drawback, but the method is applicable to shorter time frames than DLW and is particularly suited to clinical studies in which relatively short-term changes in energy expenditure are anticipated (e.g. during trauma and recovery, surgical interventions, intensive care, febrile illness, etc). For some reason the labelled bicarbonate method has not yet entered widespread usage, so the investigative examples given below are all derived from DLW. These are preceded by a brief summary of the principle of the method and its chief advantages and disadvantages.

Table 1. Examples of stable isotopes commonly used in nutritional metabolic studies.

Elements and stable isotopes (% natural abundance in brackets)

Hydrogen	*Carbon*	*Nitrogen*	*Oxygen*	*Calcium*	*Iron*	*Zinc*
^{1}H (99.985)	^{12}C (98.892)	^{14}N (99.630)	^{16}O (99.759)	^{40}Ca (96.947)	^{54}Fe (5.82)	^{64}Zn (48.89)
^{2}H (0.015)	^{13}C (1.108)	^{15}N (0.370)	^{17}O (0.037)	^{42}Ca (0.646)	^{56}Fe (91.66)	^{66}Zn (27.81)
			^{18}O (0.204)	^{43}Ca (0.135)	^{57}Fe (2.19)	^{67}Zn (4.11)
				^{44}Ca (2.083)	^{58}Fe (0.33)	^{68}Zn (18.57)
				^{46}Ca (0.0035)		^{70}Zn (0.62)
				^{48}Ca (0.186)		

Table 2. The range of potential applications of stable isotopes in human nutrition.

Category	Examples
• Food analysis	Food composition, adulteration of foods
• Diet type	Prehistoric diets, position of animals on food chain
• Nutrient intake	Cross-validation of methods
• Nutrient stores	Whole-body vitamin stores, body composition
• Nutrient absorption	Ca, Fe, Zn, etc by double-isotope methods, intestinal uptake of fatty acids
• Nutrient transfer	Breast-milk intake, placental perfusion, AV studies across muscle and fat depots
• Nutrient conversion	Amino acid inter-conversions, intermediary metabolism
• Nutrient recycling	Urea salvage, glucose/glycogen recycling
• Nutrient flux	Protein turnover, fatty acid cycling
• Energy expenditure	Doubly-labelled water, labelled bicarbonate
• Nutrient-dependent anabolism	Haemoglobin synthesis rate, lipogenesis
• Cell turnover	Lymphocytes, erythrocytes
• Physiological parameters	Gastric emptying, bowel colonisation
• Clinical applications	Triglyceride malabsorption, helicobacter pylori diagnosis

Figure 1. Principle of the doubly-labelled water method. K_H and K_O represent the rate constants for the disappearance of deuterium and oxygen-18.

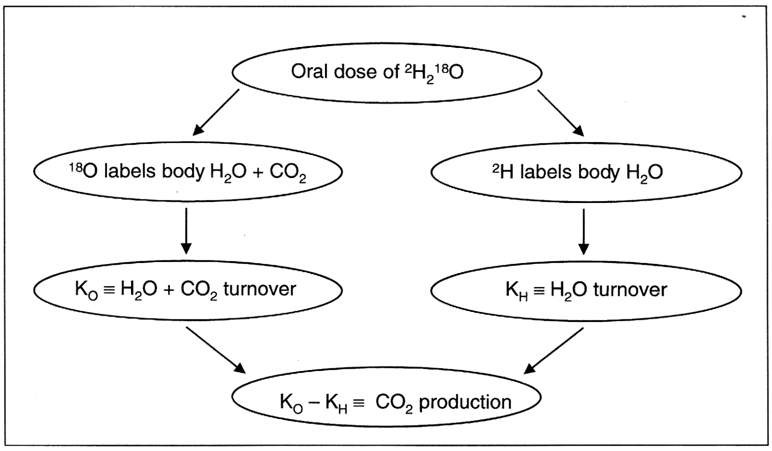


Figure 2. Example of isotope disappearance curves from an adult human.

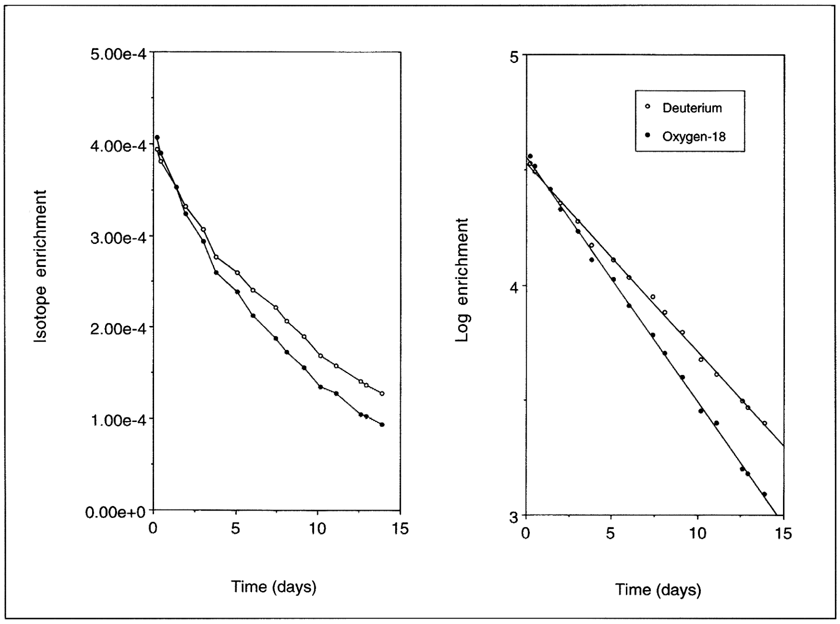


Table 3. Some advantages and disadvantages of the doubly-labelled water ($^2H_2{}^{18}O$) technique.

Advantages

• *Accuracy* The key feature of any isotopic technique is its ability to provide the correct answer. For the DLW method this has been widely established through cross-validation studies against continuous indirect calorimetry performed in animals and humans. In all mammalian species so far studied there has been no significant difference between DLW and reference methods (10).

• *Physiological validity* DLW provides the first means of estimating total energy expenditure (TEE) in free-living people. Combination with other techniques based within a metabolic unit (eg indirect calorimetry) yields a particularly powerful research tool. For instance, whole-body calorimetry provides minute-by-minute estimates of a subject's underlying physiological influences on energy expenditure and fuel selection, while DLW provides a 'real-life' estimate of both the physiological and behavioural influences on TEE. Additional advantages of DLW are that: a) it provides a measure of the energy cost of physical activity (computed as TEE-BMR); b) it integrates TEE over many days thus increasing the likelihood that the result reflects true habitual expenditure; and c) it provides a measure of total body water (from the initial isotope dilution) which can be used to calculate body composition.

• *Internal estimates of precision* Another advantage of DLW is that it can provide an internal estimate of validity and precision for each measurement, derived from the statistical fit of the data. This is an important bonus which could be extended to many isotope methods involving the fitting of data to kinetic or steady-state models.

• *Safety and subject acceptability* The safety of stable isotopes is now well accepted by ethical committees in most countries, and this has allowed the method to be successfully applied in the most sensitive of physiological states, namely pregnancy, in which measurements have even been made in the periconceptual period. From the subjects' point of view the technique is simple and non-invasive since it only requires them to take a drink of water and collect aliquots of urine for the next 14 days.

• *Versatility & robustness* DLW has now been applied in humans over the entire age span (from premature babies to the very elderly) and over the extremes of physical activity (from bed-bound patients to the world's fittest athletes). The robustness of the technique is best illustrated by its ability to measure TEE at the highest extremes of human endeavour in Antarctic explorers, Everest mountaineers, round-the-world yachtsmen, and in Tour De France cyclists.

• *Objective measurement* Another key advantage of DLW is that it provides an objective measure of TEE which is minimally vulnerable to interference from the subject being measured.

Disadvantages

• *Technical complexity* There is no doubt that the mass spectrometric and mathematical analysis necessary for DLW require a high level of training and expertise which is only available in a limited number of centres. However, manuals are available (6,10) and most of the existing centres are generous in offering training. Another solution is to collaborate.

• *Time* Although automated mass spectrometric procedures have greatly improved the maximal rate of sample throughput, the process is still time-consuming and the turnaround time for analyses is usually in the order of weeks or months in busy laboratories. It is not a technique that lends itself to next day delivery of answers.

• *Costs* Isotope ratio mass spectrometers cost in excess of US$150,000 and each human dose of isotope costs $200-1000 depending on body size. Add to this the costs of consumables and staff, and it is readily apparent that isotope methods are not cheap. However, funding bodies usually appreciate the value of using innovative techniques which provide an accurate and appropriate answer, and in general it has not proved difficult to obtain support for DLW studies addressing important hypotheses.

Theory and practice of the DLW

The principle of DLW is summarised in Figure 1. In brief, an oral loading dose of a mixture of ^2H_2O and $H_2{}^{18}O$ is used to enrich the body water pool with ^2H and ^{18}O. The plateau concentration of the isotopes measured 3-4h after dosing provides an estimate of the water pool which is used both in the DLW calculation, and as a measure of the fat-mass to lean-mass ratio of the individual. The ^2H equilibrates with body water and the rate constant for its disappearance is proportional to water turnover. The ^{18}O equilibrates with both water and the bicarbonate pool which is generated from the carbon dioxide appearing as the end product of oxidative metabolism. These are in rapid equilibrium through the carbonic anhydrase reaction, and thus the rate constant for ^{18}O disappearance is proportional to the sum of water and bicarbonate turnover. The difference between the two rate constants therefore gives a measure of carbon dioxide production from which energy expenditure can be calculated using classical indirect calorimetric equations. The two rate constants are derived from the slopes of log-transformed plots of the decline in ^2H and ^{18}O measured in serial samples of urine (or saliva or plasma) (Figure 2).

This simple exposition of the method overlooks a series of isotope fractionation corrections and other assumptions which need to be made, and a list of qualifying conditions which must be more or less met in order for the method to be valid (6,13, 17,18). The complexities of the mathematics and the assumptions inherent in DLW can appear daunting to the novice, and have led to some scepticism as to the validity of the method. However, in 1988 an expert consensus meeting thoroughly explored the limits of errors in the method and formulated recommendations for its application in man (10). This was a key step in establishing the validity of DLW, and ensuring cross-comparability between different laboratories.

The key advantage of the DLW method is that, for the first time, it provides a non-invasive method for assessing peoples' free-living energy expenditure as they live their normal lives. Some of the additional advantages and disadvantages are summarised in Table 3. In spite of its complexities and cost, over the past decade the method has contributed to some major advances in the understanding of human energy requirements in health and disease, as illustrated below.

Energy mechanisms in obesity

In the early 1980s obesity research was dominated by a search for energy-sparing defects which might explain the fact that obese people appeared to reach their obese state and then maintain it on a similar energy intake to their lean counterparts (19). This was an obvious first target for the doubly-labelled water method. We applied the technique to groups of lean and obese women studied in tandem by whole-calorimetry and DLW, and in 1986 we published a paper which, in spite of its simplicity, had a profound impact on the future direction of obesity research in our own and many other laboratories (20). The whole-body calorimetry showed that obese women had significantly *higher* basal metabolic rates and 24h energy requirements than lean women when measured under standard conditions (Figure 3). This finding was not new and it is known that this is due to the increased lean body mass and higher energy costs of activity in obese people.

However, having evaluated the physiological differences using calorimetry (which intentionally eliminates behavioural noise by the use of highly standardised activity protocols) the key question then became whether obese people are behaviourally less active than lean people in their everyday life. A number of ingenious attempts to address this question had been tried previously (such as the use of covert observation, time-lapse photography, radar, actometers, pedometers, etc) but none had been truly quantitative. Doubly-labelled water was thus ideal, and the results of the first study are illustrated in Figure 3 alongside the calorimetry data. It emerged that the obese women also had significantly higher levels of energy expenditure in their free-living state. The total energy expenditure (TEE) could then be compartmentalised into BMR (measured by the calorimetry) and the residual (TEE – BMR) which we term activity-plus-thermogenesis (A&T). Using the most appropriáte denominators to adjust these values for differences in body mass between the two groups (lean-body mass for BMR and total body mass for A&T). There were no detectable differences in either compartment between the lean and obese women (19,20). We have since realised that total body mass over-corrects the energy cost of activity (21), but this actually strengthens the conclusion that the obese women were not less active than the lean. There have been numerous subsequent DLW measurements in obese people in a number of laboratories world-wide. The results are summarised in Figure 4 which confirms that energy expenditure rises consistently as body weight increases. This data shows that substantially overweight men and women must be maintaining very high energy intakes in order to sustain their high body weight.

These energy expenditure results had to be reconciled with the mass of data suggesting that obese people consumed similar amounts of food to the lean, or perhaps even less. How could this be true if they were consistently using up more energy? The paradox is resolved by reference to the arrows on Figure 3. These indicate the self-recorded energy intake of the subjects measured at the same time as the DLW. In the lean subjects there was excellent agreement between intake and output, as would be expected from adults in energy balance. In contrast, the obese group under-recorded their food intake by over 3.5 MJ/d, or over one third. The reasons for this are complex and have been discussed in detail elsewhere (22). In the present context the important message is that the accuracy and lack of an observer effect of DLW had allowed us to cross-check food intake records (which have themselves turned out to be highly susceptible to observer effects) and demonstrate massive bias in the latter. In the early days of DLW applications there were many sceptics who preferred to believe the food intake records than the DLW. However, we and others have scrupulously analysed the limits of possible errors in DLW by a propagation of errors analysis using worst case assumptions (10,17), and have shown that all other techniques corroborate the DLW data rather than the food records (23-28).

This demonstration that obese people seriously under-report their habitual food intake has subsequently been replicated in every study that has examined the issue and has turned out to be one of the most robust bio-psychological phenomena ever uncovered. It had a major impact on re-directing much of the obesity research effort away from the search for metabolic defects on the expenditure side of the balance equation and towards the current emphasis on metabolic, neurological, psychological, social and genetic influences on the regulation of food intake (29).

Doubly-labelled water has also been used in over- and under-feeding experiments designed to test for putative adaptive thermogenic mechanisms for auto-regulatory body weight maintenance (30). Here its power has been in its ability to integrate all possible components of altered energy expenditure in the real-life setting. In our own detailed experiments on this question we could detect no evidence of active adaptive mechanisms designed to dissipate excess energy as heat as had been hypothesised since the turn of the century (30). These experiments illustrate another feature of the recent applications of DLW in man, namely that the method has not necessarily generated new hypotheses,

Figure 3. Comparison of energy expenditures in lean and obese women. Solid areas represent basal metabolic rate measured in the calorimeter. Open and hatched areas represent activity-plus-thermogenesis. Arrows show self-recorded food intake. Data from *Prentice* et al (20).

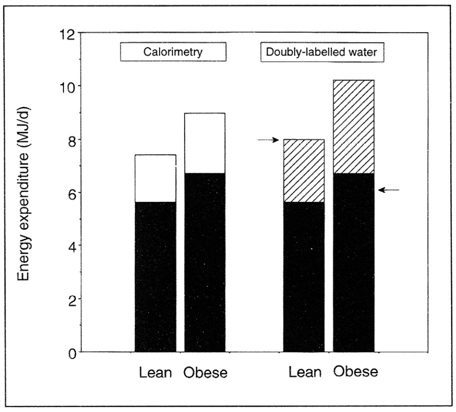
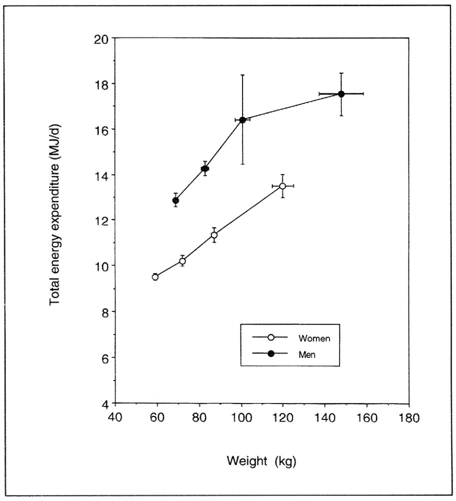


Figure 4. Total energy expenditure in relation to body weight. Data from *Prentice* et al. (59).



but has allowed age-old questions to be answered with some authority.

Hypermetabolism in wasting

A similar perception that food intake was 'normal' in various clinical wasting syndromes generated an analogous, though reversed, hypothesis; namely that progressive weight loss must be caused by a metabolic and/or behavioural hypermetabolism. This view was encouraged by numerous reports of raised BMR in certain acute clinical conditions such as burns and major trauma (31), but prior to the advent of DLW it had not been possible to assess *total* daily expenditure.

Elderly hospitalised Alzheimer's patients frequently show rapid weight loss of up to 30-50% over 2 years and, quite apart from its effects on quality of life, nutritional debilitation of this degree has been shown to be an important and reversible correlate of mortality (32). We studied this problem with DLW in a long-stay hospital

Figure 5. Energy expenditure in elderly Alzheimer's patients compared to younger adults. Data for younger women from *Black* et al. (60) and Alzheimer's patients from *Prentice* et al. (33)

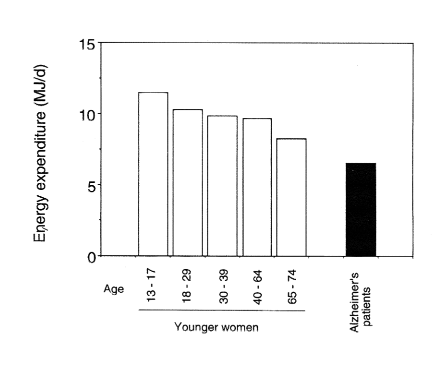
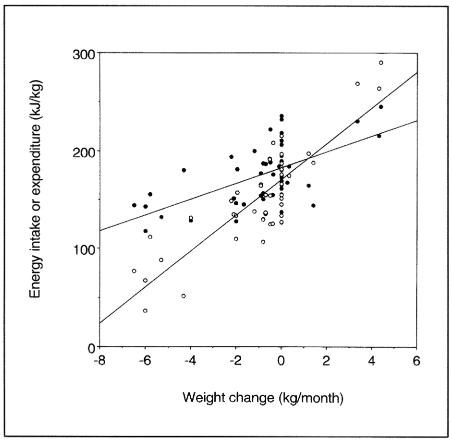


Figure 6. Energy expenditure and intake in AIDS patients. Solid circles show estimates of total energy expenditure (per kg per day) measured in 44 AIDS patients. Open circles show simultaneous measures of energy intake (per kg per day). Data from *Macallan* et al. (34).



in which dietary assessments had appeared satisfactory thus leading to the hypothesis of hypermetabolism. In fact our DLW measurements revealed the lowest average energy expenditures that have been measured in any group of adults (33) and strongly refuted the hypermetabolism theory (Figure 5). This prompted a more thorough examination of the entire feeding and care procedures which indicated major short-comings (33). There were implications of this work for long-stay institutions for the elderly and infirm far beyond the particular hospital studied (32), and the results corroborated and strengthened observational studies which had indicted low staff-patient ratios and the use of poorly qualified staff as part of the problem. In this context the most important feature of the DLW method was that it requires minimal subject compliance and hence could be used in severely demented patients.

The second study addressed the wasting of AIDS patients, and was designed to make simultaneous measures of energy intake and expenditure in patients in various phases of HIV infection (34). The timing of the measurements was designed to cover periods of weight loss, weight stability and weight regain. The key findings are shown in Figure 6. They show that, contrary to the initial hypothesis that periods of weight loss would be associated with periods of elevated energy needs, energy expenditure was in fact lower in the weight-losing patients. The reason that weight loss was occurring was because their appetite and energy intake had been depressed even further. Similarly, weight gain occurred in people with high energy expenditures because their food intake was even higher. Again this data had implications beyond the setting in which it was obtained. It removed the excuse of hypermetabolism as a cause of weight loss and focussed attention firmly on the nutritional care of HIV patients. The particular role of DLW was to show that simple measures of resting metabolic rate (RMR) can be highly misleading because they fail to account for the fact that, although RMR may be raised in acute illness, the patient is generally moribund, possibly bedbound, and thus conserves energy from physical activity. The converse is true in periods of recovery since the reduction in RMR is more than offset by an increase in physical activity.

Energy needs for child growth

Ever since expert committees started formulating recommendations for energy and nutrient intakes of population groups they have had to rely greatly on the assumption that the observed intakes of healthy (and in the case of children, adequately growing) people can be extrapolated and used as recommended intakes for others. The expert committee responsible for the 1985 FAO/WHO/UNU recommendations for energy and protein intake realised that this carried a number of drawbacks and recommended that new values should be based on measures of energy expenditure wherever possible (35). This proved to be a prescient move at the dawn of the DLW era.

By 1988 there had been a number of studies of the energy expenditure of infants and young children performed by different laboratories in the UK, US, Peru and Gambia. We combined these in a single analysis, added allowances for normal growth, and generated new estimates of the average energy needs for infants (36). There was a remarkable agreement between all of the new DLW studies and the final calculations indicated significantly lower requirements than had hitherto been in place suggesting that existing values might be a 'prescription for overfeeding' (36). In older children DLW has also been used to cross-check dietary reference values, and in this case there was remarkable congruence between the old and the new estimates (37).

A re-evaluation of dietary requirements was not the driving force behind many of these studies in children. They were initially designed to test a variety of hypotheses concerning the causes of growth failure in The Gambia (38,39), the costs of catch-up growth in Peru (40,41), and the causes of obesity in the UK and US (42, 43). There is still considerable controversy within the last of these topics (44), but DLW continues to be used to explore the issue further and important new publications will appear in the coming months. The main residual problem is no longer how to measure energy expenditure, but is a conceptual and statistical one of how to make appropriate adjustments for differences in body weight and composition (21).

Energetic adaptations

Another field in which the doubly-labelled water method has excelled is in enhancing our understanding of how women accommodate the extra energy needs of pregnancy and lactation on widely different planes of nutrition. This has been an area in which there was once again a serious mismatch between the theoretical requirements (in this case of reproduction) and the apparent changes in energy intake (45). DLW has now been applied extensively in both pregnant and lactating women, often applied in tandem with indirect calorimetry, and in our laboratory with whole-body calorimetry (46-48). DLW was approved by ethical committees for use in the peri-conceptual period and repeatedly during pregnancy and lactation. The acceptability of the method to subjects was generally very good except in cases where there was a misunderstanding about the distinction between stable and radioactive isotopes.

The ability to make such accurate and extensive measurements of all components of the energy budget advanced the experimental paradigms from large-scale cross-sectional studies, in which the chief aim was to document *average* energy needs, to detailed studies focussing on alterations in the metabolism and behaviour of individual women. From our perspective the most interesting outcome of these new studies has been the finding that human pregnancy is characterised by adaptive responses which are strongly determined by a woman's pre-conceptual fat stores and closely related to weight gain (46,49,50). Well-nourished women tend to show energy-profligate increases in basal and total energy needs in gestation, whereas poorly-nourished women exhibit an energy-sparing suppression in metabolism which can last well into the third trimester (48,50,51). These energy-responsive changes can be detected both by comparing populations of affluent versus poor women and by comparing thin versus fat women within different nutritional settings (45), and go a long way towards explaining the remarkable efficiency of human reproduction amongst the poorest peoples of the world. Once again DLW excelled in its applicability within diverse communities such as in our own compare-and-contrast parallel studies in The Gambia and UK.

EE at extremes of human endurance

The above examples have all been within the field of human health research, though studies such as those in pregnancy have dimensions related to basic physiology. The doubly-labelled water method has also been widely applied in the fields of sport and military research to examine levels of energy turnover at the extremes of human endurance. A selection of these results is illustrated in Table 4. which shows TEE expressed as the physical activity level (PAL = TEE/BMR) in order to adjust for differences in body size. The highest expenditures have been observed in Tour de France cyclists during the actual competition itself (52) and in the Antarctic explorers (53). Such levels of energy flux are probably unsustainable in the long term because it is simply not possible to consume and digest the requisite intake, and in the case of the cyclists they have to receive nocturnal tube feeding to replete glycogen and energy stores. The Antarctic explorers also found it impossible to maintain energy balance, and lost all of their mobilisable adipose tissue and significant quantities of lean body mass (53). The Antarctic explorers were also able to measure protein turnover rates using ^{15}N-glycine (54). These new measurements with doubly labelled water corroborate the old data on heavy manual labourers collected using time and motion studies combined with indirect calorimetry. Nowadays such heavy manual work is becoming rare in industrialised, mechanised societies and these extremes of endurance are generally associated with voluntary pursuits.

In this respect it is also noteworthy that DLW played an important role in highlighting the extremely low levels of energy expenditure associated with modern sedentary lifestyles (55) in marked contrast to traditional lifestyles (56). This confirmed what was intuitively obvious, but added a greater emphasis by quantifying the extent of the decline in daily requirements, and thus encouraged a new era of interest in the effects of inactivity on susceptibility to obesity and other chronic diseases.

Validation of dietary intake

The revelation that obesity-prone individuals seriously under-record their food intake (see above) focussed a spotlight on the validity of other dietary records. For the first time it was possible to use a completely independent non-invasive method to cross-check the accuracy of diet records. One of the first attempts to do this uncovered disturbing evidence of under-recording in non-obese adults randomly selected from the population (57). It was shown that men and women divided into tertiles according to their recorded food intake actually had very similar average levels of energy expenditure showing that much of the variance in energy intake records was due to under-reporting. This was later extended to younger age groups where it was found that the phenomenon of negative bias started to appear in early teenage years, especially in girls (58).

These findings generated considerable antagonism among a number of nutrition researchers since they threatened the very basis of much nutritional epidemiology which assumed that errors in food records were likely to be randomly distributed about the null point. Our results suggested that this was far from true, and that the personal attributes which caused people to under-record their dietary intake were also those which could be considered to be negative lifestyle factors in the causation of disease (eg low socio-economic status, poor educational attainment, smoking, etc). The defensive response from epidemiologists was joined by people who were initially sceptical about the validity of DLW. It was therefore necessary for us to create other proofs, based on physiological first principles, to show that it was the DLW data rather than the intake records which were most accurate. This was possible using data from various sources (especially whole-body calorimetry) to generate minimum cut-off levels for the energy expenditure compatible with normal life (23,24). Use of these new cut-offs demonstrated that many of the largest and most expensive epidemiological datasets ever collected contain a very high degree of negative bias. This in turn has brought into question the validity of a number of diet-health associations which have been based on inaccurate epidemiological data. Not surprisingly it was difficult to persuade investigators who had invested enormous effort into collecting such data that it was seriously flawed. However, addition of new evidence from many other research groups around the world gradually created an incontrovertible case (22,25) and there have now been a number of international symposia directed solely to the issue of dietary under-reporting and how to cope with it within epidemiological datasets.

Table 4. Extremes of human energy expenditure assessed by doubly-labelled water. Physical activity level (PAL) = total energy expenditure (TEE)/basal metabolic rate (BMR). Data from various sources (52,54,61,62).

	PAL	SD
Men		
Swimmers	2.1	0.38
Soldiers	2.4	0.48
Mountaineers	2.5	0.28
Nordic skiers	3.4	0.55
Antarctic walkers	4.4	0.10
T-de-F cyclists	4.7	0.25
Women		
Swimmers	1.7	0.35
Mountaineers	1.9	0.08
Runners	2.0	0.15
Triathletes	2.2	0.30
Nordic skiers	2.6	0.15

Future perspectives

The above examples provide a very personal perspective on how a single stable isotopic method has transformed an entire area of nutritional research. It has done this at the level of basic science by clarifying concepts of energy homeostasis, as well as at the public health level in the fields of child growth, obesity, wasting diseases, reproduction and in the epidemiology of diet-health relationships. The new method was a quantum leap in technology, but it did not replace the classical methodology of indirect calorimetry. In fact the most important data have been obtained through the judicious combination of old and new techniques. These achievements were reached in spite of the high capital and consumables expenses of the doubly-labelled water method and the fact that its use caused periodic world shortages of oxygen-18. The method was initially an elite tool available to only a few laboratories. Its use is now more widespread, but still remains confined to a very limited number of expert centres. This is probably an asset to the long-term credibility of the data since competency and quality control are still at a premium in what remains a complex technique.

A question arises as to why DLW has been so much more successful than many of the other stable isotope methods listed in Table 2. There are many answers to this, but perhaps a key element has been the collaboration within individual research centres (between the mass spectrometrists and mathematical modellers on the one hand, and the biological empiricists on the other), and between research centres worldwide in order to set out consensus recommendations on how best to use the technique in humans (10). The development of similar alliances may be an important way of releasing the undoubted potential that exists for other applications of stable isotopes in nutritional science.

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