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Validation of habitual energy intake

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Abstract

Objective: To provide a framework for use of the doubly labelled water method to measure energy expenditure in order to validate dietary instruments for the assessment of energy.
Design: Review and description of the use of doubly labelled water method for use as a biomarker for habitual energy intake.
Results: The doubly labelled water method has a relative accuracy of 1% and within-subject precision of 5 to 8%. Comparison of self-reported energy intake with energy expenditure demonstrated that over one-third of individuals may underreport energy intake by more than 25%.
Conclusions: The doubly labelled water method, although expensive and dependent on non-routine laboratory instrumentation, is an excellent biomarker of energy intake.

Keywords
Energy metabolism
Doubly labelled water
Stable isotopes
Nutritional epidemiology

The assessment of dietary intake plays a vital role in many aspects of nutritional science and, not surprisingly, a variety of dietary assessment instruments have been developed; including weighed food records, diet histories, 24-hour recalls and food-frequency questionnaires, each with many variations to suit particular investigative situations[1]. As these survey instruments have been developed and modified, investigators have tested their validity. These have included measurements of repeatability and accuracy. The latter, however, have usually involved comparison with a second survey instrument that has a known history of use and an assumed level of accuracy. Thus, while the testing procedure provided estimates of precision, the accuracy was not absolute, but rather a relative accuracy that could not detect biases that might be inherent in both methods. Such biases could include errors in the methodology used to convert a food to its nutrient values or systematic reporting bias by the participant.

There have, however, been a few attempts to validate accuracy using more objective measures of dietary intake. As reviewed by Bingham[2], urinary nitrogen has been used as a biomarker to test the accuracy of self-reported protein intake for over 75 years. Although some dietary instruments, primarily diet histories, have demonstrated good agreement between reported protein intake and urinary nitrogen, most validations have demonstrated modest underreporting. Some validations, however, have reported large discrepancies, with the largest underreport found amongst obese women, who underreported their protein intake by 50%[3]. Other validations have been performed using direct observation as the criterion method for validating intake instruments. Krall and Dwyer[4] asked participants to complete 3-day diaries and a 1-week food-frequency questionnaire (FFQ) during an in-patient period in which the food provided was monitored carefully and found that energy, macronutrients and vitamins A and C were underreported on the FFQ. Very recently, Schaefer *et al.*[5] had subjects complete an FFQ during trials in which participants were provided with high- or low-fat diets. The FFQ was found to underestimate fat intake on the high-fat diet and overestimate fat intake on the low-fat diet. Although these validation studies using objective criteria have demonstrated reporting errors for dietary intake instruments, there have not been many attempts to duplicate or extend these studies because the measurement methods are quite cumbersome or artificial for the participant.

Validation against energy expenditure

The number of validations against an objective criterion method has increased recently as investigators have begun to use energy expenditure as a criterion for validation of dietary instruments measuring energy intake[6,7]. The use of energy expenditure as a criterion method for validating energy intake is based on the principle of energy balance. Because energy can be neither created nor destroyed, metabolisable energy intake must equal energy expenditure unless there is a change in body energy stores. When using energy expenditure as a criterion for validation of energy intake, it is important to distinguish between habitual energy intake and current energy intake. Habitual energy intake is the average energy intake consumed by an individual to meet energy requirements for expenditure and normal growth. Except during the first year of life and the periods of pregnancy and lactation, the energy requirements for growth are small compared with energy

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Table 1 Potential differences between energy intake and energy expenditure resulting from changes in body composition

Condition	Delta weight	Energy value (kcal day^{-1})	Error (%)	
			Vs. habitual energy intake	Vs. actual energy intake
Infancy (1 month of age)	5.8 g kg^{-1} per day	100	25	25
Infancy (6 months of age)	3.2 g kg^{-1} per day	45	7	7
Pregnancy	13 kg per 9 months	230	9	9
Lactation	Milk production	650	26	20
Slimming diet	200 g day^{-1}	1500	50	100

expenditure and energy expenditure is almost equal to habitual energy intake (Table 1). Actual energy intake, even when averaged over a week or two, may be quite different from energy expenditure due to changes in body energy stores. Acute energy restriction for weight loss, either voluntarily or due to illness, can introduce differences of over 50% between actual energy intake and energy expenditure (Table 1). These energy intakes, however, are not habitual and under these conditions energy expenditure is a better measure of habitual energy intake than intake itself. Habitual weight gains such as those associated with growth or gradual excessive weight gain do introduce a bias into the estimate of habitual energy intake determined from energy expenditure. The bias can be corrected for by adding the energy equivalent of the average daily tissue accrual. However, this is typically quite small. For example, an adult who gains even 2 kg in a year with a typical energy density of 7800 kcal kg^{-1} averages a habitual energy intake that is only 43 kcal day^{-1} (2 kg year^{-1} × 7800 kcal kg^{-1}/365 days year^{-1}) greater than energy expenditure.

The use of doubly labelled water (which measures energy expenditure) as a criterion has identified major biases and errors in dietary intake instruments beginning with the first report by Prentice *et al.*[8]. They found that while non-obese British women accurately reported energy intake as a group, obese women underreported their energy intake by an average of 35%. An even greater discrepancy was reported by Bandini *et al.*[9], who compared a 2-week food diary against doubly labelled water and found that non-obese adolescents underreported energy intake by 19% and obese adolescents underreported intake by 41%.

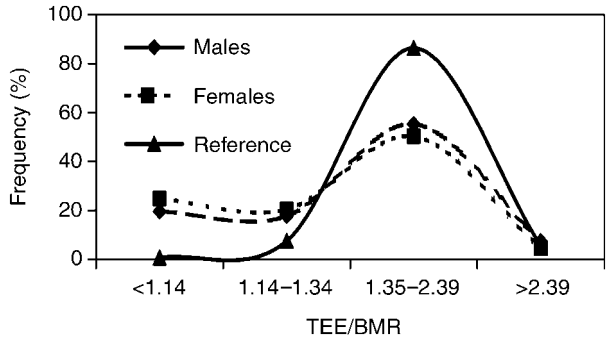

Fig. 1 Frequency distribution of self-reported energy intake (dashed lines) from a national sample of Scandinavian adults[11] compared with energy expenditure measured by doubly labelled water from a historic reference population of adults living in industrialised countries[10]. Results are expressed as the ratio to resting metabolic rate (TEE – total energy expenditure; BMR – basal metabolic rate)

To date, comparisons against doubly labelled water have mostly been performed on small groups of individuals[6,7]. Recently, however, some large diet studies have begun to compare reported energy intake against basal metabolic rate[10]. At least one of these has published data in a format that permits an indirect comparison with average energy expenditure as assessed in a different group by doubly labelled water. In a study of 3020 Scandinavian adults, energy intake from an FFQ was compared against calculated basal metabolic rate[11]. Because basal metabolic rate comprises 50 to 80% of total energy expenditure, it was not surprising that most individuals reported energy intakes exceeding their basal metabolic rate (Fig. 1). However, when the frequency distribution of the ratio of intake over basal metabolic rate is compared against a historic control of the ratio of expenditure from doubly labelled water to basal metabolic rate[10], it is apparent that over one-third of participants severely underreported their habitual energy intake (Fig. 1). These and other studies have demonstrated that underreporting of dietary intake is quantitatively significant both in terms of the proportion of individuals who underreport and the degree to which an individual may underreport.

Doubly labelled water

The development of the doubly labelled water method for measuring energy expenditure in man has recently provided an excellent means of validating dietary instruments for energy intake[6,7]. The doubly labelled water technique can be used to measure total energy expenditure over a period of about two weeks. The principle of the method is that after a loading dose of water labelled with deuterium, a stable isotope of hydrogen, and the stable isotope ^{18}O, these tracers quickly equilibrate in

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body water. The deuterium is eliminated from the body as water and the elimination rate is thus proportional to water turnover. ^{18}O is eliminated as water and carbon dioxide, and thus its elimination is proportional to the sum of water turnover and carbon dioxide production. The difference between these two elimination rates is, therefore, proportional to carbon dioxide production. Total energy expenditure can be calculated from carbon dioxide production using common indirect calorimetric equations[12].

The major advantages of the doubly labelled water method are its objectivity, its minimal interference with the subject's daily activities, its accuracy and its precision. It is objective because the body water acts as a metabolic recorder and thus the method does not require the subject to keep logs or report a history. The tracer elimination rates are determined from spot urine samples collected on the day the tracer is given by mouth and again at the end of the period, so there is little disruption of the participant's daily activities. The doubly labelled water method has been validated against near-continuous respiratory gas exchange and also against weighed food intake and has an accuracy of 1% as determined in multiple laboratories[13].

In order to detect individual reporting errors, individual precision is equal in importance to accuracy when the doubly labelled water method is used as a criterion method. Precision (defined as the within-individual coefficient of variation (CV) or the CV for the validation of energy expenditure from doubly labelled water against near-continuous respiratory gas exchange), unfortunately, has proved to be variable in inter-laboratory studies. The doubly labelled water method involves taking the difference between the deuterium and ^{18}O elimination rates. Because this difference is small (15 to 25%) compared with the individual tracer elimination rates, the method is quite sensitive to small analytical errors and precision can suffer. Although published validation studies against highly precise near-continuous calorimetry have demonstrated that doubly labelled water can attain a precision of 4 to 5%[14–16], an inter-laboratory comparison using standard water and urine specimens indicated that less than half of the laboratories involved in doubly labelled water studies 10 years ago could attain this level of analytical precision[17,18]. Of great concern was the indication that some laboratories (presumably the least experienced) might have precision as low as 35%.

Because the precision of the doubly labelled water method is laboratory-specific, it is important that any study using this method as a criterion for validation of energy intake instruments includes a measure of the reproducibility of the doubly labelled water method. Test–retest reproducibility within a subset of the participants is usually considered the best evidence of precision because this includes not only the analytical precision, but also the within-individual variation in energy expenditure. Because of the expense and shortage of ^{18}O, however, it may be impractical to include a test–retest sub-study and thus historic evidence must often be relied upon. Published studies have demonstrated test–retest precision of 9 to 12%[19]. When relying on historic data, however, it is vital to document analytical precision through the inclusion of blinded repeat analyses of specimens from a subset of six to 12 participants in any validation study.

Doubly labelled water procedures

The doubly labelled water method is safe for use in all participants. The tracers are stable isotopes and thus pose no radiation hazard. Indeed, there are no known hazards associated with the ^{18}O isotope[20]. At the doses typically employed, there are also no hazards associated with deuterium, but at doses that are 40 times those typically used for energy expenditure, deuterium can cause temporary vertigo and it is toxic at doses that are 1000 times those used for energy expenditure studies[20].

Participant exclusions include individuals who have travelled within 2 weeks before or after dose administration because this can cause error due to changes in deuterium and ^{18}O background abundance[21]. Travel is defined as an overnight trip of more than 200 miles in most areas and 100 miles for inland trips in coastal areas where the geographic isotopic gradients are larger[22]. Similarly, administration of intravenous fluids during this same period is also a cause for exclusion. Individuals with malabsorption should also be excluded from energy intake validation studies because this might reduce the metabolisable energy value of foods.

Participants should present after a fast of at least six hours, so as to maximise absorption of the tracers. A baseline urine specimen is collected and the doubly labelled water is administered orally. If the analytical precision is better than 0.15‰ for ^{18}O and 1‰ for deuterium, the tracers can be dosed at 0.18 and 0.12 g kg^{-1} total body water for ^{18}O hydride and deuterium oxide, respectively. If the analytical precision is worse, then a proportionately larger dose should be used. The dosing container should be washed with 50 ml of water and the participant should drink the water to ensure complete administration of the isotopes. If the plateau method[23] is used, then additional urine specimens should be collected at 2, 3 and 4 hours after the dose because two to three voids are required before urine reaches isotopic equilibrium[24]. For subjects with post-void urinary retention or for those over 60 years of age where urinary retention is not uncommon, a blood serum sample should be collected. When the modified two-point method is used, two final urine specimens should be collected 14 days later at about the same time of day as the post-dose urine specimens[25]. These urine specimens should be collected at least one hour apart. In tropical locales, the 14-day interval should be reduced to 7 days because high water turnover can result in excessive tracer elimination. A large number

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of other sampling protocols have been developed and shown to provide equally valid results. This particular protocol is the least cumbersome for the participants because all specimens can be collected in a supervised environment with only two clinic visits of 4 to 6 hours and 2 hours, respectively.

Urine specimens should exceed 25 ml and be capped after collection to avoid evaporation or contamination. A 4 ml aliquot should be transferred to an O-ring sealed plastic tube and frozen at −10 °C. If freezing is not an immediate option, specimens can be refrigerated for several weeks or even stored at room temperature. Specimens are usually shipped without freezing. However, it is recommended that they be packed with sealed, frozen gel coolants to keep them cool.

Isotopic analysis

At this time, isotope ratio mass spectrometry is the only method that provides sufficient precision at the low enrichments of tracer used for doubly labelled water analysis. As indicated above, the minimal dose requirements are dependent on the precision of the isotope analysis. To use the minimal dose, precision requirements are 0.15 and 1‰ (3 and 0.15 ppm) for ^{18}O and deuterium, respectively. Better precision does not allow much further dose reduction because natural variations in background isotope abundance become limiting[21]. Isotope ratio mass spectrometers are generally priced in excess of several hundred thousand dollars (US), but price reductions may be possible in the future if manufactures can redesign the instrumentation with the goal of developing specific task instruments. Recent improvements in design, particularly of on-line water analysis systems, have dramatically improved sample throughput from several samples per hour to a sample every 10 minutes[26]. It has generally been observed that one to three years of experience is required to obtain optimal laboratory performance, although new technicians working in an existing laboratory can learn to use the modern instruments for routine analyses within weeks.

Calculations

During the 1980s, there was considerable controversy regarding the calculation of energy expenditure from a doubly labelled water study[27]. With regard to studies in adult humans under all but the most extreme environmental conditions or illness, this controversy is almost resolved. Most investigators use equation (1) for analysis of the isotope dilution spaces[23]:

$$N = (WA/18.02a)[(E_a - E_W)/(E_s - E_p)], \qquad (1)$$

where N is the isotope dilution space (mol), W is the weight of water (g) used to dilute a sample of the dose water to make the calibrating dilution, A is the weight of the dose (g) given to the participant, a is the weight of dose (g) used to make the calibrating dilution, and E is the isotope abundance measured in the calibrating dilution (subscript a), the dilution water (subscript W), the sample of equilibrated body water (subscript s) and the pre-dose sample of body water (subscript p). Total body water is then calculated as the average of the deuterium dilution space divided by 1.041 and the ^{18}O dilution space divided by 1.007[28]. Average CO_2 production (r_{CO_2}, mol day^{-1}) is calculated using equation (2) or a similar equation[28]:

$$r_{CO_2} = (TBW/2.078)(1.007k_o - 1.041k_d) - 0.0246r_{Gf}, \qquad (2)$$

where TBW is the total body water (mol), k_o is the oxygen elimination rate (day^{-1}), k_d is the deuterium elimination rate (day^{-1}) and r_{Gf} is the rate of fractionated gas loss, which is estimated to be 1.05 TBW$(1.007k_o - 1.041k_d)$.

Total energy expenditure (TEE) is calculated from CO_2 production using standard relationships from indirect calorimetry. We typically use the modified Weir equation[12]:

$$TEE = 22.4r_{CO_2}(1.11 + 3.94/R), \qquad (3)$$

where R is the respiratory ratio. Use of equation (3) thus requires an estimate of the composition of energy substrates that have been oxidised because the energy value of a mole of CO_2 varies between substrate[12]. The uncertainty in the estimate of substrate composition is the ultimate limit on the precision and accuracy of the doubly labelled water method[29]. On first inspection this limitation appears severe; however, when it is realised that substrate composition is averaged over a period of a week or two, then the error is usually estimated to be 3% or less. The substrate composition is approximated by the diet macronutrient composition with adjustment for change in body composition when there is a significant change in weight[29].

Criteria for acceptance of a result from a doubly labelled water study vary between laboratories. In our laboratory, we require that the ratio of deuterium to ^{18}O dilution space be between 1.00 and 1.07, and that CO_2 production calculated for the period defined by the second post-dose urine and the first endpoint urine and that calculated from the third post-dose urine and the second endpoint urine be within 8%. We also test for equilibration of the second and third post-dose urine by comparing the total body water estimates from the two urine specimens. If the difference is larger than 5%, the urine is likely to be unequilibrated with body water and the result is not considered valid unless there is also a blood sample from which total body water can be determined[30]. If the difference is between 2 and 5%, then we consider that the second post-dose urine is not quite equilibrated and use only the third post-dose urine for calculation of total body water. We have observed differences of 5% or more in 10% of non-institutionalised individuals over 70 years old, an

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age group subject to post-void urine retention, and less than 2% of individuals less than 70 years old.

Conclusions

Energy expenditure has been used as a biomarker of metabolisable energy intake. Numerous investigators have identified significant bias in self-reported energy intake, which in the worst cases have exceeded 50%[31]. The most accurate and precise method for measuring energy expenditure is the doubly labelled water method. Doubly labelled water has a relative accuracy of 1%, a laboratory-dependent analytical precision of 3% or greater, and a within-subject repeatability of 5 to 8%. The method, however, is expensive due to the high cost of ^{18}O. Moreover, the analyses of deuterium and ^{18}O require highly specialised, expensive equipment. As such, the method cannot be considered routine. However, the method is widely available and is currently being applied in dietary instrument validations with sample sizes ranging from 20 to 500 participants. These uses of accurate biomarkers will help define, and hopefully eliminate, biases in dietary assessment instruments.

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