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Chapter 10

TOTAL ENERGY EXPENDITURE OF FREE-LIVING HUMANS CAN BE ESTIMATED WITH THE DOUBLY LABELED WATER METHOD

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I. INTRODUCTION

Energy is required for muscular activity, growth, reproduction, and synthesis of metabolites such as proteins, fatty acids, nucleic acids, and steroids, which are essential to maintain basal metabolic functions as well as optimal growth and development. Numerous methods such as the food record, [^{13}C]-bicarbonate infusion, and indirect calorimetry have been used to estimate energy expenditure in humans.

The food record seldom reflects the true caloric content of ethnic foods, and this procedure does not work well with children. It is also well documented that overweight individuals often underreport their food intake.

The [^{13}C]-bicarbonate infusion method is invasive and of short duration (<24 hr). Activity of the subject is restricted during the infusion. Therefore,

energy expenditure measured by this method is not representative of the true daily energy expenditure of the free-living subject.

Whole room calorimetry is considered the gold standard for measuring energy expenditure in humans. Although the subject is free to move around in the calorimetric chamber, spontaneous physical activity is greatly reduced. Furthermore, the measurement is carried out under strictly controlled, artificial environmental conditions and often is of short duration (<24 hr). However, indirect calorimetry does provide important information about the basal metabolic rate, respiratory quotient, sedentary energy expenditure, and sleeping metabolic rate.

The doubly labeled water method ($^2H_2{}^{18}O$) yields an average caloric expenditure for a period of 5–14 days. The procedure is noninvasive, nonrestrictive, and reflective of actual caloric expenditure under free-living conditions.

The purpose of this article is to describe the theory of the $^2H_2{}^{18}O$ method, its assumptions, the analytical procedures and equations used in its calculation, and its validity compared to the energy balance method and indirect calorimetry when measuring the total energy expenditure (TEE) of humans.

II. THEORY OF THE $^2H_2{}^{18}O$ METHOD

The possibility of using $^2H_2{}^{18}O$ to estimate TEE was first recognized by Lifson *et al.* (1949) and subsequently demonstrated and validated in small animal studies (Lifson and McClintock, 1966). Following oral ingestion of $^2H_2{}^{18}O$, the isotopes are distributed rapidly in body water (Fig. 1).

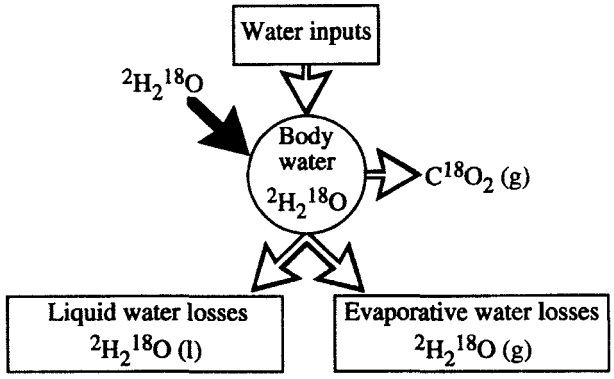

FIG. 1. A schematic diagram showing the introduction of $^2H_2{}^{18}O$ into the body water and the distribution of ^2H and ^{18}O in water losses and ^{18}O in the expired CO_2.

With carbonic anhydrase, oxygen-18 (^{18}O) in body water also reaches isotopic equilibrium rapidly with the bicarbonate or carbon dioxide (CO_2) in the body. The rate of disappearance of deuterium (^{2}H) from the body therefore reflects water turnover, whereas the rate of disappearance of ^{18}O represents water turnover as well as carbon dioxide production (rCO_2).

Mathematically, water turnover (rH_2O) and rCO_2 can be presented as

$$rH_2O = N \times k_H \tag{1}$$

$$rH_2O + 2 \times rCO_2 = N \times k_O, \tag{2}$$

where N is total body water and k_H and k_O are the fractional turnover rates of ^{2}H and ^{18}O as measured in the body fluid, respectively. Substituting rH_2O from Eq. (1) into Eq. (2) and solving for rCO_2 yielded the classical Lifson equation for calculating rCO_2 from the rates at which the two isotopes, ^{2}H and ^{18}O, are eliminated from the body,

$$rCO_2 = 0.5 \times N \times (k_O - k_H). \tag{3}$$

A. ASSUMPTION OF THE $^{2}H_2{}^{18}O$ METHOD

1. Constant Body Water Pool Sizes

During the study period, the $^{2}H_2{}^{18}O$ method assumes no changes in body water pool sizes. This assumption might be appropriate for adults of stable weight, but would not be appropriate for premature infants.

2. Constant H_2O and CO_2 Fluxes

In weight-stable adults and healthy subjects, the H_2O and CO_2 fluxes are relatively constant. However, in diseased subjects, in subjects recovering from surgery, or in subjects undergoing exhaustive exercise, the assumption could be violated.

3. No Sequestration of ^{2}H and ^{18}O in Metabolites Other Than H_2O and CO_2

Sequestration of ^{18}O is negligible. However, ^{2}H is known to be incorporated into cholesterol and fat during biosynthesis. Therefore, the assumption of no sequestration of ^{2}H into metabolites other than H_2O and CO_2 is not valid. However, the amount of ^{2}H incorporation into metabolites during

biosynthesis is very small in humans and hence its effect on the accuracy of the $^2H_2{}^{18}O$ method is minimal.

4. *H_2O and CO_2 Leaving the Body Are Isotopically Identical to Body Water*

Because more energy is required to change liquid H_2O containing ^2H and ^{18}O to H_2O vapor than liquid H_2O containing ^1H and ^{16}O, at equilibrium, H_2O vapor therefore contains 5.5% fewer ^2H and 1% fewer ^{18}O than liquid H_2O (Halliday and Miller, 1977; Pflug *et al.*, 1979; Schoeller *et al.*, 1986; Wong *et al.*, 1988). When CO_2 is allowed to come in contact with liquid H_2O, isotope exchange between the oxygen atoms of the CO_2 and H_2O will take place. If the reaction is allowed to finish, the CO_2 will end up with 3.9% more ^{18}O than the liquid H_2O (Halliday and Miller, 1977; Pflug *et al.*, 1979; Schoeller *et al.*, 1986; Wong *et al.*, 1988). Therefore, H_2O vapor and CO_2 leaving the body are not isotopically identical to body water. However, Eq. (3) can be modified to correct for the isotope discrimination which takes place during evaporation and during isotope exchange between CO_2 and H_2O.

5. *No Reentry of the Labeled H_2O and CO_2 into the Body*

Reentry of the labeled H_2O and CO_2 into the body is possible in small animals living in small burrows. However, in humans, the possibility of reentry is minimal and its effect on the accuracy of the $^2H_2{}^{18}O$ method for estimation of TEE therefore is negligible.

B. EQUATIONS USED IN THE $^2H_2{}^{18}O$ METHOD

Because of the many violations of the assumptions associated with the $^2H_2{}^{18}O$ method, the classical Lifson Eq. (3) subsequently has been modified to account for the differences in isotope dilution spaces of ^2H (N_H) and ^{18}O (N_O), the changes in body pool sizes during growth, and isotope discrimination during evaporation and equilibration. With these corrections, rCO_2 is calculated as

$$rCO_2 \text{ (mol/day)} = \alpha \, (k_O \times N_O - k_H \times N_H), \tag{4}$$

where α is the correction factor for isotope fractionation and insensible water loss. The constant α has a value of 0.4556 for infants and 0.4584 for adolescents and adults.

The isotope dilution spaces (N_H, N_O) are calculated from the dose mixture of $^2H_2{}^{18}O$ (days) in grams given to the subject and the rise in ^2H or ^{18}O abundance in the physiological fluid (E_d) at time zero using the extrapolation method or at equilibration using the plateau method as

$$N_H \text{ or } No \text{ (mol)} = (d \times A \times E_a)/(a \times E_d \times k), \quad (5)$$

where A is the amount of laboratory water in grams used in the dilution of "a" grams of the dose mixture, E_a is the rise in ^2H or ^{18}O abundance in the laboratory water after the addition of the dose mixture, E_d is the rise in ^2H or ^{18}O abundance in the physiological fluid at time zero or at equilibration, and k is a constant to convert grams of H_2O to moles and has a value of 18.02.

The rCO_2 is converted to TEE using the Weir equation (de V. Wier, 1949),

$$\text{TEE (kcal/day)} = 22.4 \times (1.106 \times rCO_2 + 3.941 \times rO_2), \quad (6)$$

where rO_2 is the oxygen consumption in mol/day and is calculated from the respiratory quotient (RQ) using the relationship

$$RQ = rCO_2/rO_2. \quad (7)$$

The RQ can be measured by indirect calorimetry or a population RQ can be used. Otherwise, a food quotient (Black *et al.*, 1986) calculated from dietary intakes, with appropriate corrections for changes in body composition, can be used in place of RQ.

III. ANALYTICAL METHODS

A. ISOTOPES

Deuterium oxide at 99.8 at. % ^2H and $H_2{}^{18}O$ at 10 at. % ^{18}O can be purchased from Isotec, Inc. (3858 Benner Road, Miamisburg, OH 45342), Enritech Enrichment Technologies Ltd. (P.O. Box 2449, Rehovot 76123, Israel), or Cambridge Isotope Laboratories (50 Fontage Road, Andover, MA 01810).

The ^2H and ^{18}O are nonradioactive isotopes and have natural abundances of 0.01 and 0.20%, respectively. Both isotopes are found in the human body and in the food and water we consume every day (Table I).

TABLE I
DAILY INTAKES AND BODY CONTENT OF ^2H AND ^{18}O

Isotopes	Intake (mg/kg/day)	Body content[a] (g)
^2H	6.9	1.5
^{18}O	133.4	68.6

[a] Amounts of ^2H and ^{18}O in a 50-kg adult.

In most human studies, the isotopes are taken orally. For intravenous administration, the isotopic water can be ultrafiltered for the removal of pyrogen and bacterial contamination (Wong *et al.*, 1991). The $H_2{}^{18}O$ at 10 at. % ^{18}O is suitable for studies in older infants, children, and adults. For premature infants and newborns, $H_2{}^{18}O$ at 50 or 95 at. % ^{18}O is preferred in order to minimize the volume of isotopic water administered.

It is crucial to know the exact quantity of $^2H_2{}^{18}O$ given to the volunteer. For oral administration, the bottle containing the isotopic water should be rinsed twice with drinking water, formula, breast milk, or suitable dietary fluids. For intravenous doses, the exact weight of the syringe before and after administration of the isotopic water must be known or the syringe can be flushed with saline solution to ensure complete administration.

B. MASS SPECTROMETRIC ANALYSES

1. Sample Collection

Any physiological fluid such as plasma, saliva, urine, or breath water vapor can be collected for the determination of fractional turnover rates of ^2H and ^{18}O and N_H and N_O. In most human studies, urine is the preferred sample because it is noninvasive and the easiest to collect. In infants, urine can be collected using cotton balls (Wong *et al.*, 1993a). The urine absorbed by the cotton balls can be expressed with a plastic syringe into an appropriate sample vial. If the samples are not processed immediately for isotope ratio measurements, they should be stored at −20°C until ready for analysis. The sample collection apparatus must be free of moisture in order to avoid dilution of the samples, particularly when only small quantities are available.

2. Sample Preparation for ^2H Abundance Measurements

Water in 10 μl of the sample is reduced to H_2 with 200 mg of zinc turning (Biogeochemical Laboratory, Dept. of Geological Sciences, Indiana

University, Bloomington, IN 47405) at 500°C for 30 min in an evacuated reduction vessel (Wong *et al.*, 1992). Upon cooling to room temperature, the H_2 is introduced into a Finnigan Delta E gas–isotope ratio mass spectrometer (Finnigan MAT, San Jose, CA, 95134) for ^2H abundance measurement (Wong *et al.*, 1987).

3. *Sample Preparation for ^{18}O Abundance Measurements*

A total of 100 μl of the sample is allowed to equilibrate with 300 mbar of CO_2 of known ^{18}O content for 10 hr at 25°C in a VG ISOPREP–18 H_2O–CO_2 equilibration system (Fisons Instruments, Inc., 32 Commerce Center, Danvers, MA 01923). At the end of the equilibration, the CO_2 is allowed to expand into a VG PRISM gas–isotope–ratio mass spectrometer system (Fisons Instruments, Inc.) for ^{18}O abundance measurement (Wong *et al.*, 1987).

4. *Isotope Abundance Measurements*

Gas–isotope–ratio mass spectrometry is used to measure the ^2H and ^{18}O abundances in the H_2 and CO_2 samples, respectively. The instrumentation is known as gas–isotope–ratio mass spectrometry because all samples entering the ion source of the mass spectrometer must be in gaseous forms such as H_2 for ^2H abundance measurements and CO_2 for ^{18}O abundance measurements. Upon entry into the ion source of the mass spectrometer, the H_2 or the CO_2 gas is ionized by electrons to form positively charged ions of $^1H^1H^+$ and $^1H^2H^+$ for H_2 or $C^{16}O_2^+$ and $C^{16}O\,^{18}O^+$ for CO_2. Because of the difference in ionic masses between these positively charged ions, they are separated into two ion beams through a magnetic field. The amounts of ^1H and ^2H in the H_2 or ^{16}O and ^{18}O in the CO_2 are directly proportional to the amplified ion beam intensities of the $^1H^1H^+$ and $^1H^2H^+$ or $C^{16}O_2^+$ and $C^{16}O\,^{18}O^+$ as measured by the detectors of the mass spectrometer. These amplified signals are compared to those of the laboratory H_2 or CO_2 standard and are expressed as the isotope ratios of $^1H^2H/^1H^1H$ or $C^{16}O^{18}O/C^{16}O_2$.

5. *Units*

The ^2H and ^{18}O abundances in the H_2 and CO_2, respectively, are expressed in delta (δ) per mille (°/oo) units which are defined as

$$\delta\ (°/oo) = (R_x/R_s - 1) \times 10^3, \tag{8}$$

where R_X and R_S are the $^1H^2H/^1H^1H$ or $C^{16}O^{18}O/C^{16}O_2$ of the sample (x)

and standard (s), respectively. The δ (°/oo) values are normalized against two international water standards, Vienna-Standard Mean Ocean Water (V-SMOW) and Standard Light Antarctic Precipitation (SLAP) as follows (Gonfiantini, 1984),

$$\delta^2\text{H or } \delta^{18}\text{O (°/oo)} = (\delta_{X\text{-WS}} - \delta_{\text{V-SMOW-WS}})/(\delta_{\text{SLAP-WS}} - \delta_{\text{V-SMOW-WS}}) \times \delta_0, \quad (9)$$

where $\delta_{\text{X-WS}}$, $\delta_{\text{V-SMOW-WS}}$, and $\delta_{\text{SLAP-WS}}$ are the δ^2H or δ^{18}O values of the sample (x), V-SMOW, and SLAP measured against the laboratory working standard (ws), respectively. The δ_0 has an accepted value of −55.5 °/oo for ^{18}O abundance measurements and an accepted value of −428 °/oo for ^{2}H abundance measurements. These relative δ values can be converted to absolute atom percent values using the procedures described by Wong and Klein (1986).

IV. VALIDATIONS OF THE $^2H_2{}^{18}O$ METHOD

The $^2H_2{}^{18}O$ method assumes no changes in body water pool sizes and H_2O and CO_2 fluxes during the study period. In healthy weight stable adults, TEE estimated by the isotope method has been shown to be within 2% of the calorimetric values (Klein *et al.*, 1984; Coward *et al.*, 1984; Schoeller, 1988). With appropriate corrections for changes in body water pool sizes, TEE of 1- and 4-month-old formula-fed infants (Wong *et al.*, 1990) and of premature infants (Roberts *et al.*, 1986; Jensen *et al.*, 1992) estimated by the $^2H_2{}^{18}O$ method has been shown to agree within 1% of the energy balance values in the 1- and 4-month-old infants and by indirect calorimetry in the premature infants. Under conditions when H_2O and CO_2 fluxes are not constant, such as recovery from surgery (Jones *et al.*, 1988) and heavy exercise (Stein *et al.*, 1987), the isotope method still agrees within 10% of the calorimetric values.

It is well known that ^{2}H in body water is incorporated into cholesterol (Wong *et al.*, 1993b) and fat during biosynthesis. Except under conditions of excessive lipogenesis or a high rate of weight gain (>100 g/day), isotope sequestration in humans results in less than 1% error in the TEE estimate.

Reentry of the labeled H_2O and CO_2 is most likely when premature infants are confined in incubators. However, TEE of premature infants estimated by the isotope method has been shown to be within 1% of the calorimetric estimates (Roberts *et al.*, 1986; Jensen *et al.*, 1992). Therefore, reentry of the labeled H_2O and CO_2 in humans is not likely to affect the accuracy of the isotope method for estimation of TEE in free-living subjects.

V. CONCLUSION

The $^2H_2{}^{18}O$ method has long been validated as an accurate method for estimation of TEE in small mammals and birds (Lifson and McClintock, 1966). In spite of the violation of many of the assumptions associated with the isotope method, with proper corrections, TEE estimated by the $^2H_2{}^{18}O$ method has proven accurate against TEE estimated by energy balance and indirect calorimetry in infants, children, and adults.

Because the isotope method is noninvasive, nonrestrictive, and does not expose the subjects to radiation, the $^2H_2{}^{18}O$ method is considered the method of choice by the nutrition community for the estimation of energy requirements during infancy, growth, pregnancy, and lactation.

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