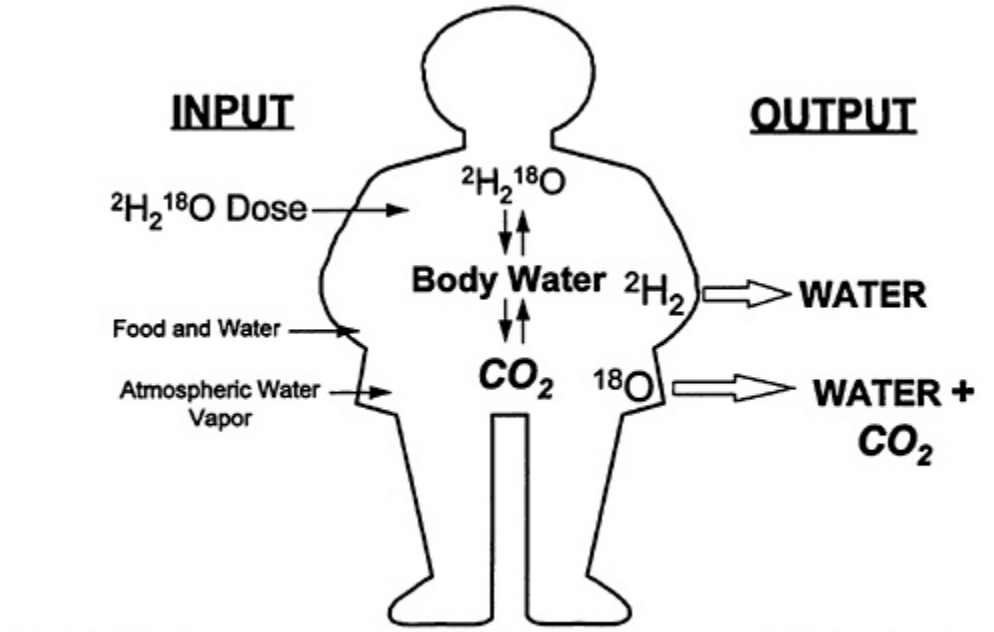
Energy Expenditure using Doubly Labeled Water



^{18}O elimination (water + CO_2) - $^2H_2$ elimination (water) = CO_2 Production

Metabolic Solutions, Inc.
460 Amherst Street
Nashua, NH 03063
603-598-6960

Measuring Energy Expenditure Using the Doubly Labeled Water Method

Introduction

Traditional measurements of daily energy expenditure make use of semicontinuous monitoring of respiratory gas exchanges by monitoring for five 1-hour periods per 24 hours or using “spot" measurements with a metabolic measuring cart. Use of these techniques will give good estimates of resting energy expenditure (REE). However, these methodologies do not necessarily provide accurate measurements of total daily energy expenditures because of the need to integrate the spot or semicontinuous measurements over 24 hours where activity and clinical state will vary. Furthermore, an “activity" factor must be multiplied by the REE to estimate daily energy expenditure.

Experiments with Stable Isotopes

Stable isotopes of hydrogen and oxygen have been recently used to measure energy expenditure in free-living humans. The doubly labeled water method using these isotopes is a form of indirect calorimetry which has been extensively validated in animals and humans. The method is completely safe, requires only periodic sampling of body fluids, is non-restrictive, and is ideally suited for measurement of energy expenditure in free-living or hospitalized patients.

Development of the Doubly-Labeled Water Method

The development of the doubly labeled water method originated from a study by Lifson et al. in 1949. Using the stable isotope of oxygen, Lifson et al. administered ^{18}O-labeled water to animals and showed that the ^{18}O-label appeared in expired CO_2. This demonstrated that expired CO_2 was derived from body water. It is now known that a rapid isotopic equilibration occurs between the oxygen atoms of body water and CO_2 through the action of the enzyme carbonic anhydrase.

Further experiments by Lifson, Gordon and McClintock showed that total daily CO_2 production could be measured from the differential elimination of water labeled with stable isotopes of hydrogen and oxygen. After the administration of doubly labeled water ($^2H_2{}^{18}O$), the labeled hydrogen ($^2H_2$) would be eliminated as water (^2H_2O), corresponding to water output, whereas the oxygen isotope would be eliminated as water ($H_2{}^{18}O$) and as expired carbon dioxide ($C^{18}O_2$). By measuring the difference between the elimination rates of labeled oxygen and hydrogen, the carbon dioxide production rate can be calculated. The carbon dioxide production rate is converted into energy expenditure by knowing the respiratory quotient (RQ) of the food ingested during the observation period.

Model Assumptions

The model of Lifson assumes that the total body water pool (N) is a homogeneous compartment that remains constant during observation. Further assumptions implicit in the model are that the tracer isotopes of hydrogen and oxygen exit the body only as water and carbon dioxide and that dietary and atmospheric sources of water and oxygen do not change the background levels of isotopes. The basic mathematical equation relating carbon dioxide production to the isotope elimination rates is given in equation 1:

$$rCO_2 = (N/2)\ (k_{18} - k_2) \qquad (1)$$

where N is the total body water pool, k_{18} is the rate of disappearance of ^{18}O and k_2 is the rate of ^{2}H disappearance.

The practical application of the method required the incorporation of isotopic fractionation factors to account for fractionation (non-equal equilibration) of the isotopes of water and carbon dioxide during changes in state. It had been recognized that isotopically labeled water and carbon dioxide would leave the body at different rates depending on its chemical state, either gas or liquid. Measured isotope fractionation factors for deuterium and ^{18}O indicate that breath water, non-sweat water vapor and expired CO_2 are isotopically fractionated relative to body water. With this correction, the equation describing the model becomes:

$$rCO_2 = (N/2\ f_3)\ (k_{18} - k_2) - rH_2O_G\ (f_2 - f_1)/2\ f_3 \qquad (2)$$

where f_1 is the deuterium fractionation factor between water and water vapor, f_2 is the ^{18}O fractionation factor between water and water vapor, f_3 is the ^{18}O fractionation between water and carbon dioxide and rH_2O_G is the rate of water loss via isotopically fractionated routes. Appropriate choices for the fractionation factors in infants and adults have been the subject of numerous papers. The isotope fractionation factors currently used are:

$f_1 = 0.941$ $^{2}H_2O$ (gas) / $^{2}H_2O$ (liquid)
$f_2 = 0.992$ $H_2{}^{18}O$ (gas) / $H_2{}^{18}O$ (liquid)
$f3 = 1.039$ $C^{18}O_2$ (gas) / $H_2{}^{18}O$ (liquid)

Typical Study Protocol

A typical study protocol using the doubly labeled water method starts with a urine collection before the dose to determine baseline values for the hydrogen and oxygen isotopes. The subject is given a single oral bolus dose of heavy water ($^2H_2{}^{18}O$). Generally, adults are given a dose consisting of 0.15 g $H_2{}^{18}O$/kg body weight and 0.06 g ^2H_2O/kg body weight. Children and neonates are given higher doses due to their faster water turnover rates. A dose of heavy water to adults costs between $500 and $700.

Following the administration of the dose, urine will be collected during the observation period. Two study designs have been validated to measure energy expenditure. One protocol design extensively tested by Schoeller et al. is the two-point method, a urine at the beginning and end of the observation period, is used to determine the isotope elimination rates. Another protocol reported by Klein et al. and Coward and Prentice, calculates the isotopic elimination rates from regression analysis of multiple samples collected periodically throughout the metabolic period rather than from just the initial and final samples. Recently, Coward has reported that both methods give equivalent energy expenditure measurements.

Regardless of the protocol design, a urine or saliva sample is collected within the first six hours to determine total body water (TBW). The following morning, 24 hours later, the urine voided marks the beginning of the measured energy expenditure period. The study period ends after 7 to 21 days when a urine sample is collected to close the energy expenditure period. The optimal metabolic period for observation in a doubly labeled period is predicted to be between 0.5 and 3 biological half-lives of water. Between the initial and final samples, the subject is free to engage in normal activities. A second dose of doubly labeled water is administered at the end of the study period and urine or saliva collected after 3 to 6 hours for a second determination of TBW. This second determination of TBW is used to measure any changes in the total body water pool during the observation period.

Determining Energy Expenditure

The daily food intake is noted during the observation period and the RQ calculated based on the daily intake. Generally, the diet remains the same during the observation period for the most accurate results. The rate of carbon dioxide production is used in conjunction with the Weir equation to estimate energy expenditure Q over the period in which body water samples are collected (22). The Weir equation (Equation 3) uses measured values for the respiratory quotient (RQ) and the urinary nitrogen production rate (UN):

$$Q = 3.941\ (rCO_2 / RQ) + 1.106\ rCO_2 - 2.17\ UN \qquad (3)$$

Assumptions of the Model

The doubly label water model developed by Lifson incorporated many assumptions about the water pool, water and CO_2 flux, and the isotope exchanges with the body pools. These assumptions are estimates which have been shown to be reasonable in much testing.

Constant Water Pool

The model assumes a constant water pool volume during the metabolic period. The pool volume will change with eating and drinking but over a 24 hour period, these changes are quantitatively insignificant with respect to the total pool size. However, the application of the method to a growing premature infant, which increases total body water by 20% in the period of 1 week, requires a linear growth model to calculate the water pool sizes. Two point and multipoint regression models have dealt with the steady-state kinetics of the water and CO_2 fluxes. The water and CO_2 fluxes do change episodically. However, these models have shown that they can estimate the average flux over the metabolic period to a high degree of accuracy.

Exchange of Isotopes with Water and CO_2 Pools

The most important controversy of the doubly labeled water method has been the assumption that the isotopes are only exchanged with the body water and CO_2 pools. It is now well known that the hydrogen dilution space, estimated from the extrapolation of the elimination curves back to time zero, has been observed to be between 2 and 6% larger than the body water pool as determined by desiccation. Furthermore, the hydrogen dilution space is consistently larger than the oxygen dilution space. This implies that the hydrogen isotope exchanges with other pools in the body. It has been suggested that the hydrogen exchanges with acidic amino acids in proteins. The oxygen dilution space appears to overestimate the body water pools by about 1%. The oxygen isotope can exchange with inorganic compounds in the body.

There is not total agreement on how to correct the estimates of the water and CO_2 fluxes due to these overestimates. Schoeller et al. has used an average relationship, based on all his human data, between the isotope dilution spaces and the total body water pool size: hydrogen dilution space/1.04 = oxygen dilution space/1.01 = total body water. Roberts, Coward and Lucas have used individually determined dilution spaces in their model. It is very important to determine the dilution spaces accurately because small errors are magnified 3- to 5-fold in the calculation of the CO_2 production rate.

Validation of the Model

Although controversies about the values to use for the water pool size exist, the doubly labeled water method has been validated in humans against continuous respiratory gas exchange measurements. Schoeller et al. and Westerterp et al. have used the two point method for their validations. Schoeller has completed validations in 33 subjects ranging from adults, to infants, to total parenteral nutrition patients. The mean difference from the respiratory gas exchange method was 0.6% with a standard deviation of 6%. Other laboratories have validated the doubly labeled water method using the multipoint method and achieved roughly the same level of precision.

Procedures for Measuring Energy Expenditure

Theoretically, any body fluid can be sampled for measurement of the water isotopes. Thus, blood, saliva and urine can be sampled. However, urine samples are most often the choice used by investigators because of the ease of collection and availability of the fluid. The total amount of fluid necessary for both analyses is about 2.5 ml per time point. Therefore, we will discuss urine collections in our methodology.

Protocol

Following an overnight fast (about 8 hours), urine or plasma specimens are collected before the administration of the isotope dose. This will serve as the baseline isotope measurement. A double-labeled dose of water is orally administered to each subject. A mixed $^{2}H_{2}{}^{18}O$ dose containing 0.15 gm/kg body weight of $H_{2}{}^{18}O$, 99 atom % excess ^{18}O, (or 1.5 gm/kg body weight of $H_{2}{}^{18}O$, 10 atom % excess ^{18}O), and 0.06 gm/kg BW $^{2}H_{2}O$, >99 atom % excess, is given orally and then followed with 100 ml of tap water. The first urine or plasma collection is four hours following the dose. This is used to determine the isotope dilution space and total body water. The next urine or plasma collection is 24 hours from the isotope dose. Thereafter, a minimum of two samples, at the beginning and end of the study period, are necessary to determine energy expenditure during the study period. However, we recommend at least three or greater urine or plasma collections per week for most accurate results. Although many have argued that the two point method gives equal accuracy, our experience has suggested that a linear regression of more than two points gives the best accuracy.

Storage of Specimens

All urine samples should be collected in non-acidified plastic bottles. The urine should be aliquoted immediately into smaller plastic tubes (about 5 ml urine) and stored frozen (-20 °C or lower) until analysis. Save two or three aliquots. It is preferable that plastic tubes (cryogenic tubes) which have been specifically designed for storage at low temperatures be used. Blood samples (2 ml) should be collected in sodium or lithium heparin (Vacutainer tubes with Green Tops). Plasma (about 1 ml) should be transferred to cryogenic tubes and stored frozen (-20 °C or lower) until analysis.

Diets

A complete diet record for the study period is necessary to calculate energy expenditure. The respiratory quotient of the diet will be used in the Weir equation for determining energy expenditure. Black et al. has predicted resipiratory quotients from food composition data (Human Nutr. Clin. Nutr. 40C:381-391, 1986. Alternatively, respiratory quotients can be measured directly by respiratory gas exchange measurements.

Calculation of Energy Expenditure

Measurements

The delta deuterium and oxygen-18 values for the pre-dose (δ_{pre}) and post-dose samples (δ_{pos}) are determined. The doubly labeled dose is diluted with tap water. The amount of dose diluted and water used is recorded. The deuterium and oxygen-18 content of the tap water (δ_{tap}) and diluted dose (δ_{dose}) are measured.

Treatment of Mass Spectrometric Data

The unprocessed isotopic data is expressed as a fraction of the initial dose given as suggested by the consensus report by the International: Dietary Energy Consultancy Group at the 1990 Vienna Austria Meeting (AM Prentice. The Doubly -Labelled Water Method for Measuring Energy Expenditure: Technical Recommendations for Use in Humans. Vienna: Nahres-4, International Atomic Energy Agency; 1990. This is achieved using the formula:

$$X = \frac{(\delta_{post} - \delta_{pre})}{(\delta_{dose} - \delta_{tap})} \times \frac{18.02a}{WA}$$

where W = Amount of water (grams) used to dilute the dose, A = Amount of dose (grams) administered to subject, a = amount of dose (grams) diluted for analysis.

Linear regression is used to calculate the slope and intercept of the linear relationship between the time in days and the normalized data for each isotope. The pool sizes N_D and N_O are derived as the reciprocal of the intercept (or plateau value). The intercept of the regression line is the ratio of the pool size spaces N_D/N_O. The multipoint data is plotted to inspect for any outliers. Any outliers are re-analyzed. The rate constants k_D and k_O are represented by the slope of the regression line. N_D/N_O ratios lying outside the range of 1.015 and 1.06 are treated as suspect and samples will be re-analyzed.

Calculation of Daily CO_2 Production

The mean daily CO_2 production (rCO_2, mol/day) is calculated according to the revised equations of Speakman, Nair and Goran (Am. J. Physiol. 264: E912-E917, 1993):

$rCO_2 = (N/2.196) \times (k_O - 1.0427k_D)$

where $N = [(N_o) + (N_D/1.0427)]/2$.

Calculation of Energy Expenditure

The estimate of energy expenditure is calculated from the carbon dioxide production assuming 127.5 kcal/mol carbon dioxide (a typical Western diet will produce a respiratory quotient of 0.85, with 15% of energy from protein oxidation, as suggested by Elia in the IDECG concensus report, Vienna 1990 Meeting). The use of a general value for the conversion of CO_2 to energy expenditure for a "western" type diet was found to predict to within 5% the energy expenditure of 63 randomly-selected individuals.