On estimating intrasubject variability in doubly labeled water experiments

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The doubly labeled water technique is a useful way to measure energy expenditure, but very little has appeared about the variability of estimated energy expenditure. By estimating the energy expenditure at each day of the study period, the average daily carbon dioxide production per unit of time can be estimated by the sample mean, and the intrasubject variability can be estimated by the standard error of the mean. An example in which 14 daily isotopic enrichments of oxygen and hydrogen are measured illustrates the methodology of estimating energy expenditure and the intrasubject variability. (J. Nutr. Biochem. 5:39–42, 1994)

Keywords: intrasubject variability; energy expenditure; doubly labeled water studies

Introduction

The doubly labeled water technique of measuring energy expenditure was first described by Lifson et al.,[1] and since that time a great deal of excitement has been generated. There have been many modifications and revisions of the original protocol, and Wolfe[2] provides an overall review of the topic. Very little has appeared in the way of measuring the intrasubject variability, and the problem may be approached from several directions. Intrasubject variability means the variability of repeated determinations of rCO_2 on the same subject. First, one may measure the within-subject variability experimentally by replication, where the energy expenditure of a subject is measured several times under identical conditions. This is very difficult to do because of time and cost constraints. Another approach is mathematical. For example, Cole, Franklin, and Coward[3] developed an analytical approximation to the variance of the carbon dioxide production rate, while Wolfe[2] and Broemeling and Wolfe[4] employed a statistical resampling scheme called the jackknife. Other studies that investigate the precision of the doubly labeled water technique include Goran et al.,[5] Schoeller et al.,[6] and Delaney et al.[7] The Goran study is quite informative because it is an experimental study that replicates the energy expenditure of each subject three times under carefully controlled conditions. The purpose of this article is to propose a new technique that simplifies the estimation of intrasubject variability.

A somewhat different approach is now taken, where the carbon dioxide production rate rCO_2 is estimated at each time (usually daily) that isotopic enrichments of oxygen and hydrogen are measured. Then, energy expenditure is estimated by the sample mean of the n estimated rCO_2 values, where n is the number of days of the study period, and the intrasubject variability is estimated as the standard error of the mean. Thus, the problem is transformed to the standard problem of estimating a population mean (the unknown average rCO_2) by a sample mean and the variability of a sample mean (the intrasubject variability) by the standard error of the mean. This approach is quite familiar and is easily implemented, and allows one to estimate the sample size in future experiments.

In what is to follow, the doubly labeled water technique is briefly reviewed, the proposed method of estimating energy expenditure and intrasubject variance is described, and the methodology is illustrated with a human subject in a study of Prentice et al.[8]

Methods and materials

The doubly labeled water technique

The fundamental equation for carbon dioxide turnover, ignoring fractionization, is shown in Equation 1.

Supported by Shriners Hospitals grants 15870 and 15849, and NIH grant 6M41089.

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Received March 10, 1993; accepted July 1, 1993.

$$rCO_2 = (K_o{*}N_o - K_d{*}N_d)/2 \qquad (1)$$

The oxygen and hydrogen decay rates are K_o and K_d, and the corresponding pool sizes are N_o and N_d. These four unknown parameters are assumed to be constant over the study period of n days, thus the carbon dioxide production is assumed to be constant. Of course, the assumption that the decay rates and distribution volumes are constant is somewhat unrealistic. Due to nonsteady-state conditions, it would be more accurate to assume that the average distribution volumes and rates of elimination that are estimated from the protocol are representative of the 'true' mean distribution volumes and rates of tracer elimination. The assumption of constants in Equation 1 is necessary for the regression analysis that is described below. The study period consists of measuring the isotopic enrichment of oxygen and hydrogen on each of n days, where the enrichment follows the two-pool model as shown in Equation 2.

$$E_o(t) = I_o{*}\exp(-K_o{*}t) \text{ and} \qquad (2)$$
$$E_d(t) = I_d{*}\exp(-K_d{*}t),$$

$E_o(t)$ is the t-th enrichment of oxygen, and I_o is the zero-time enrichment of oxygen. Least squares estimates of the four parameters are computed by regressing the log of the isotopic enrichments on time, then the pool sizes are estimated from the Equation 3.

$$N_o = [\text{dose } {}^{18}O{*}18.02]/[20.02{*}.002005{*}I_o] \text{ and} \qquad (3)$$
$$N_d = [\text{dose } {}^{2}H{*}18.02]/[20.02{*}.00015576{*}I_d],$$

If the doses are expressed in grams and the two pool sizes above are each scaled by 1000/18, then rCO_2 is given in moles per unit time. An equivalent way to estimate the elimination rates and pool sizes is to follow Prentice et al.[8] and use the normalized enrichments in Equation 4, where

$$NE_o(t) = \exp(-K_o{*}t)/N_o \text{ and} \qquad (4)$$
$$NE_d(t) - \exp(-K_d{*}t)/N_d.$$

$NE_o(t)$ is the normalized enrichment of ^{18}O at time t, and the pool sizes are the reciprocal of the intercepts. The carbon dioxide production is estimated by substituting estimates of the flux rates and pool sizes into Equation 1. How confident are we of this estimate?

Carbon dioxide production

The solution to estimating energy expenditure and intrasubject variation is approached by transforming the problem to one of estimating the average rCO_2, and for this an estimate of rCO_2 at each time of the study period is required. Considering the two-pool model (Equation 4), then this system can be solved to yield the CO_2 rate at time t ($t = 1,2, \ldots, n$), as in Equation 5.

$$rCO_2(t) = [K_o{*}\exp(-K_o{*}t)/NE_o(t) - K_d{*}\exp(-K_d{*}t)/NE_d(t)]/2 \qquad (5)$$

The derivation is in the appendix. Note that in Equation 5, the exponential function decreases, but so do the normalized enrichments, with the result that the average carbon dioxide production tends to remain constant. After substituting the estimated elimination rates into Equation 5, there are n estimates of carbon dioxide production. The decay rates are estimated by regression using either the original enrichments (0/00) or the normalized enrichments.

If the n estimates of rCO_2 are assumed to be a random sample of size n from a population with unknown mean rCO_2, the mean is estimated by the sample mean in Equation 6.

$$rCO_2^{-} = \Sigma_t rCO_2(t)/n \qquad (6)$$

The sum is overall t. The intrasubject variability is estimated by the estimated standard error of the mean[9] as in Equation 7.

$$SEM = S/\sqrt{n} \qquad (7)$$

S is the sample standard deviation of the n rCO_2 values $rCO_2(t)$, $t = 1, 2, \ldots, n$. If in addition the sample is from a normal population (a test for normality should be performed), a $1 - \alpha$ $(1 < \alpha < 1)$ confidence interval[9] for rCO_2 is Equation 8.

$$rCO_2^{-} \pm t(1 - \alpha, n - 1){*}SEM \qquad (8)$$

The $1 - \alpha$ estimation error is Equation 9.

$$E = t(1 - \alpha, n - 1){*}SEM \qquad (9)$$

$t(1 - \alpha, n - 1)$ is the upper $1 - \alpha$ point of the t distribution with $n - 1$ degrees of freedom. The last statement is interpreted to mean that one is $1 - \alpha$ confident that the estimate in Equation 6 is within E moles/day of the 'true' rCO_2. The SEM from Equation 7 will be used to estimate the sample size in future experiments, and this will be discussed in the example.

The methodology

The protocol for estimating energy expenditure and intrasubject variation is summarized as: (A) measure n pairs of isotopic enrichments (0/00) of oxygen and hydrogen on each of n days of the study period; (B) estimate the flux rates by regressing the log of the normalized enrichments on time; (C) substitute the estimated flux rates (/day) into Equation 5; (D) compute the sample mean rCO_2^{-} (moles/day) from Equation 6 to determine the estimate of energy expenditure; (E) compute the sample standard deviation S and the SEM (moles/day) with Equation 7, which gives the estimated intrasubject variation; (F) compute the $1 - \alpha$ estimation error E from Equation 9.

Results

The above methodology is illustrated from the study of Prentice et al.[8] We use the calculations from that study, and all of our calculations agree with theirs. The 14 daily enrichments of oxygen and hydrogen for subject 3 are plotted in *Figures 1 and 2*, while *Table 1* provides

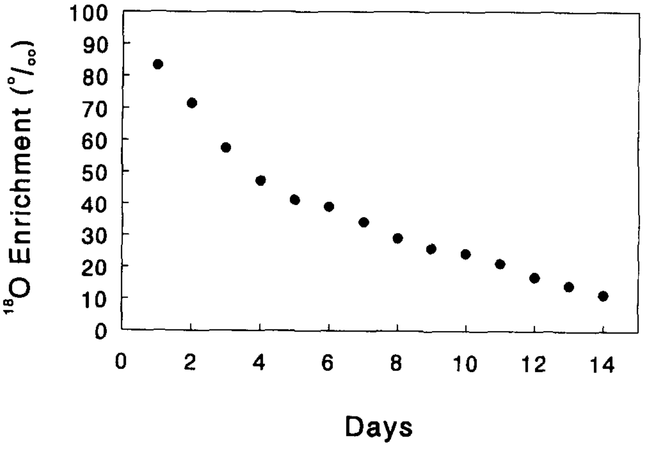

Figure 1 Daily oxygen enrichments

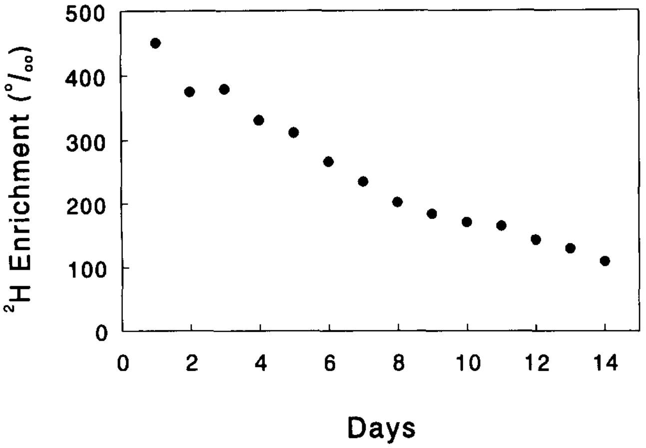

Figure 2 Daily hydrogen enrichments

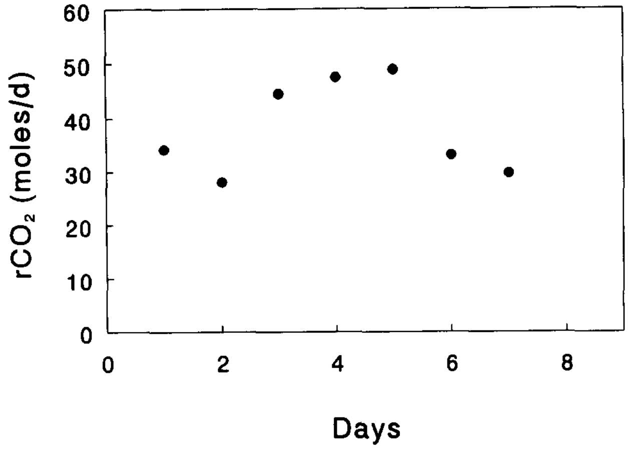

Figure 3 Daily CO$_2$ production

Table 1 Daily enrichments, normalized enrichments, and CO$_2$ production

Day	18_{O}	2_{H}	$N^{18}O$	$N^{2}H$	rCO_2
1	83.25	450.41	0.000456	0.000504	26.51
2	71.53	375.00	0.000470	0.000426	21.55
3	57.51	379.192	0.000380	0.000431	38.79
4	47.27	331.12	0.000314	0.000381	43.00
5	41.19	312.05	0.000275	0.000362	45.82
6	39.08	266.52	0.000261	0.000315	31.84
7	34.12	234.99	0.000229	0.000282	29.77
8	29.04	202.11	0.000197	0.000249	28.74
9	25.91	184.01	0.000177	0.000230	26.77
10	24.31	170.58	0.000166	0.000216	21.01
11	21.11	164.74	0.000146	0.000210	25.82
12	16.88	143.24	0.000118	0.000188	32.00
13	14.24	129.42	0.000101	0.000174	35.26
14	11.43	109.28	0.000083	0.000153	39.64

Data from Prentice et al.[8] 0/00 = del above background.

the times, normalized enrichments, and the estimated rCO$_2$ values that were computed from Equation 5. The estimated rCO$_2$ values are also plotted in *Figure 3*. The flux rates were estimated as:

$K_o = 0.13415$ (/day) and
$K_d = 0.0892$ (/day).

These values were substituted into Equation 5, which gave the estimated rCO$_2$ values. From the 14 estimated rCO$_2$ values, the sample mean is found in Equation 10.

$$rCO_2^{\sim} = 31.897 \text{ moles/day} \tag{10}$$

The sample standard deviation is $S = 7.687$ moles/day. The estimated energy expenditure of 31.897 agreed very well with the 31.77 moles/day estimated directly by Equation 1 from Prentice et al.[8] The standard error of the mean estimates the intrasubject variability and is computed from Equation 7 as Equation 11.

$$SEM = 7.687/\sqrt{14} = 2.05 \text{ moles/day.} \tag{11}$$

Assuming the sample is from a normal population, it can be confirmed that the 95% estimation error is $E = 2.16*2.05 = 4.43$ moles/day, and one is 95% confident that the estimate of 31.897 is within 4.43 moles per day of the true rCO$_2$. An advantage of the proposed approach is that Equation 7 can be used to estimate sample sizes.

Sample size strategies

From Equation 7, one may solve Equation 12.

$$\sqrt{n} = S/SEM, \tag{12}$$

S is the sample standard deviation. From the present experiment the estimated population standard deviation is $S = 7.687$ and $SEM = 2.05$. Suppose a future experiment is performed with subject 3, but the desired intrasubject variability is to be reduced to 1.75 moles per day.

How many daily enrichments are required? Substituting $SEM = 1.75$ into Equation 12 gives $n = 19$ days, or 5 additional days, a value that is usually too large for doubly labeled water experiments. On the other hand, suppose one would be satisfied with an intrasubject variability of only SEM = 3 moles/day. How many days are needed? Again from Equation 12, n = 7 days.

Increased precision demands more measurements and decreased precision requires less. It should be noted that a reliable estimated S of the population standard deviation leads to a reliable estimate of the sample size, but what should be done if little or no previous information is available?

If little prior information is available, one could take a preliminary sample of k (> 5) daily enrichments and estimate SEM, then if the intrasubject variability is too high use Equation 11 to estimate the additional enrichments that are necessary to achieve a target value for SEM. Suppose that this strategy was employed with subject 3 with 7 daily enrichments as the preliminary sample. *Table 2* gives the estimated rCO_2 values for the first 7 days.

These values were computed by substituting the estimated flux rates $K_o = 0.1464115$ and $K_d = 0.0899$ (which were calculated from the first seven enrichments of oxygen and hydrogen) into Equation 5, which in turn leads to a SEM = 3.28 moles/day and S = 8.68. Suppose the desired intrasubject variability is to be 2.5, then n is estimated from $\sqrt{n} = 8.68/2.5$ or n = 12 days.

Table 3 gives the estimated sample sizes n and additional days n-k required to achieve a given level of intrasubject variability SEM, based on the initial k = 7 measurements of isotopic enrichments.

Discussion

The proposed method is easy to apply, and has the advantage that standard statistical procedures of estimating the mean and its standard error are used to estimate the energy expenditure and intrasubject variability in doubly labeled water experiments. In addition, sample size strategies are easily implemented with the proposed procedure.

It should be noted that the proposed method rests on the assumption that the estimated energy expenditures are assumed to be a random sample from the population with the unknown rCO_2 as the mean of the population. This implies that the estimated rCO_2 values in Equation 5 are independent, and that the average value of rCO_2 remains constant over the n days of the study period. We might expect some degree of positive autocorrelation between the estimated rCO_2 values, which would lead to an underestimate of the intrasubject variability; however, the study period is usually too short for time series techniques to be applied.[10]

Table 2 Estimated rCO_2 values for the initial 7 days

Day	rCO_2
1	34.24
2	28.07
3	44.47
4	47.47
5	48.78
6	33.14
7	29.71

Calculated from Equation 5.

Table 3 Sample size strategies

SEM:	3.28	3.00	2.75	2.5	2.25	2.0
n:	7	8	10	12	15	19
n − k:	0	1	3	5	8	12

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Appendix

Daily carbon dioxide production

Invert both parts of Equation 4 and multiply each by their respective flux rates. This yields

$$K_o/NE_o(t) = K_o{}^*N_o{}^*\exp(K_o{}^*t) \text{ and}$$

$$K_d/NE_d(t) = K_d{}^*N_d{}^*\exp(K_d{}^*t)$$

thus

$$K_o{}^*N_o = K_o{}^*\exp(-K_o{}^*t)/NE_o(t) \text{ and}$$

$$K_d{}^*N_d = K_d{}^*\exp(-K_d{}^*t)/NE_d(t).$$

Subtracting the second equation from the first and dividing by 2 yields Equation 5, the rCO_2 value at time t.