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Review

Estimating free-living human energy expenditure: Practical aspects of the doubly labeled water method and its applications

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ABSTRACT: The accuracy and noninvasive nature of the doubly labeled water (DLW) method makes it ideal for the study of human energy metabolism in free-living conditions. However, the DLW method is not always practical in many developing and Asian countries because of the high costs of isotopes and equipment for isotope analysis as well as the expertise required for analysis. This review provides information about the theoretical background and practical aspects of the DLW method, including optimal dose, basic protocols of two- and multiple-point approaches, experimental procedures, and isotopic analysis. We also introduce applications of DLW data, such as determining the equations of estimated energy requirement and validation studies of energy intake.

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INTRODUCTION

Changing body composition or reducing body weight requires modifications to energy balance, i.e., the balance between energy intake (EI) and energy expenditure (EE): body weight can be reduced when EI is lower than EE over a given period. Thus, measuring EE precisely is important for determining daily food intake targets.

Direct calorimetry measures and records the heat released from a subject in a thermally-isolated chamber. However, direct calorimetry is often impractical. Therefore, indirect calorimetry, which uses CO_2 production and/or oxygen consumption measured from subjects, is generally used [1]. However, it is difficult to assess EE in a daily free-living state using indirect calorimetry methods without any interference. Instead, non-calorimetric techniques such as questionnaires, heart rate monitors, motion sensors, and the doubly labeled water (DLW) method are used to obtain various indices of daily physical activities in free-living populations [2]. However, the greatest problems with the use of these non-calorimetric techniques in humans are the errors associated with determining total EE (TEE) and physical activity level (PAL,TEE/basal metabolic rate) in the free-living state. Among the non-calorimetric techniques, the DLW method using two stable isotopes, ^2H and ^{18}O, has become the gold standard for measuring TEE under free-living conditions because of its precision and accuracy [3-5].

Accurately assessing TEE is critical for understanding the estimated energy requirement (EER) of populations [6]. This could help prevent and/or reduce the high prevalence of obesity, which is associated with diseases such as cardiovascular disease, diabetes, and hypertension [7,8]. To derive a proper EER equation, the TEE of individuals who maintain an energy balance must be measured accurately. EER is usually determined by assessing sex, age, weight, height, and physical activity status. PAL has been calculated from data taken from a pooled analysis of DLW studies to determine physical activity coefficients used in EER equations [9]. On the other hand, assessing TEE by the DLW method is challenging because of the high costs of isotopes and equipment for isotope analysis as well as the expertise required for analysis. The EER equations for Western populations have already been derived using the DLW method [9]. However, it remains unclear whether these equations are appropriate for Asian populations or populations in developing countries, who have different lifestyles from those of Western populations. Furthermore, information about the practical aspects of performing the DLW method is limited.

This review provides information about the theoretical background and practical aspects of the DLW method. We also introduce applications of DLW data, including the determination of EER equations and validation studies of EI.

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Principles of the DLW method

The DLW method was introduced for human use approximately 30 years ago [10]. This method provides information on TEE in free-living individuals over a period of 4-20 days. The principle of the method is as follows. Subjects receive a loading dose of water labeled with the stable ^2H and ^{18}O isotopes, and these isotopes mix with the hydrogen and oxygen in body water within a few hours. As energy is expended, CO_2 and water are excreted. The CO_2 is lost from the body only via the breath, while the water (including both ^2H and ^{18}O) is lost not only via the breath but also in urine, sweat, and through other means such as evaporation. As ^{18}O is contained in both CO_2 and water, it is lost from the body more rapidly than ^2H, which is contained in water but not in CO_2 (Fig. 1). Lifson [11] observed that the isotope ratio of O_2 in water is rapid and complete isotopic equilibrium with the O_2 in CO_2. Therefore, a water molecule labeled with ^{18}O will not only mix with body water and exit the body the same way as ^2H-labeled water, but will mix with CO_2 and exit the body as CO_2. During a period of 4-20 days, the difference between the rate of loss of ^{18}O and ^2H from the body reflects the rate at which CO_2 is produced, which in turn can be used to estimate EE by using a modified Weir's formula [12] based on the CO_2 production rate (rCO_2) and respiratory quotient (RQ).

Dose of DLW

The dose of DLW is based on body size to match the required body water enrichment. In practice, given that the dose should be prescribed per unit of total body water (TBW), investigators must estimate TBW for each subject. However, because measuring TBW is inconvenient, most researchers use the assumption that TBW represents 60% of body weight in non-obese adults. In our previous studies, we used a single dose of approximately 0.06 g $^2H_2O\cdot kg^{-1}$ body weight (99.8 atom %, Cambridge Isotope Laboratories, MA, USA) and 1.4 g $H_2{}^{18}O\cdot kg^{-1}$ body weight (10.0 atom %, Taiyo Nippon Sanso, Tokyo, Japan) [13,14]. The 99 atom % ^2H and 10 atom % ^{18}O levels are the most commonly used enrichment levels of labeled water available in the market. Although DLW doses are not always the same among studies, most studies follow the recommendation of the International Atomic Energy Agency (IAEA), which suggests optimal doses for a typical DLW study in adults with 0.12 $g\cdot kg^{-1}$ body water of 99 atom % ^2H-labeled water and 1.8 $g\cdot kg^{-1}$ body water of 10 atom % ^{18}O water [15].

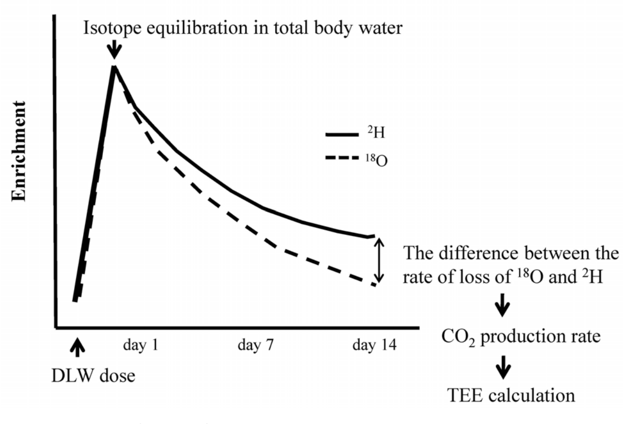

Fig. 1. Decline of ^2H and ^{18}O levels in total body water during a doubly labeled water experiment. TEE, total energy expenditure; FQ, food quotient.

During DLW preparation, the dose must be sterilized by performing distillation or filtration through a 0.22-μm filtering system. The weight of the water should be recorded to four significant digits to allow the determination of the amount of labeled water each subject is administered. The administration container, which should be easy to drink from, should be washed with 50-100 mL tap water after the dose is consumed, and that water should also be consumed; this ensures that the entire dose is received (most containers will not deliver the total dose without washing). For children and the elderly, a straw can be used in order to prevent water loss during administration.

Two basic DLW protocols: two-point and multiple-point approaches

The original DLW method evaluated isotope elimination curves after taking only two samples (from blood, urine or saliva), an initial and a final measurement. This approach allows subjects to continue their normal lives without any restriction between the two time points, providing a valuable technique for quantifying the energy demands of free-living animals and humans [16-18]. The first DLW study in human subjects employed the two-point sampling methodology that had previously been used in animal studies [10]. After Schoeller and Van Santen's study was published [10], Klein et al. [19] tested the approach of taking samples at much more frequent intervals and reconstructed a time course for isotope elimination from the body by using a fitted regression curve. They suggested that their multiple-sample method could better determine the production rate of CO_2 from multiple samples rather than from measurements at only two points of time. On the other hand, some studies found that there was little bias when simultaneously applying the two-point and multiple-point methods [20]. Below, we summarize the general methods for performing two-point and multiple-point DLW protocols and try to compare the relative precision of the two-point and multiple-point methods.

Experimental procedure for the two-point method

Fig. 2-A shows the time points of urine sampling and log-linear plot of isotope elimination in a two-point DLW protocol [15]. The Experimental procedure for the two-point method is described in detail in the IAEA Human Health [15]. A brief description of the experimental procedure for two-point method follows. The most common protocol for the two-point approach employs morning dosing and sampling. The participant's body weight and height are measured before the start of the experimental period. After an overnight fast and collection of the baseline specimen, the participant drinks the DLW preparation. Importantly, the participant should void and empty their bladder 1 h after the dose. This voided urine need not be collected for analysis, but the time of the void should be recorded. The participant may consume a small meal and should consume fluid to maintain a hydrated state for TBW determination. Additional urine samples should be collected at 3 and 4 h after the dose. The participant should not drink or eat

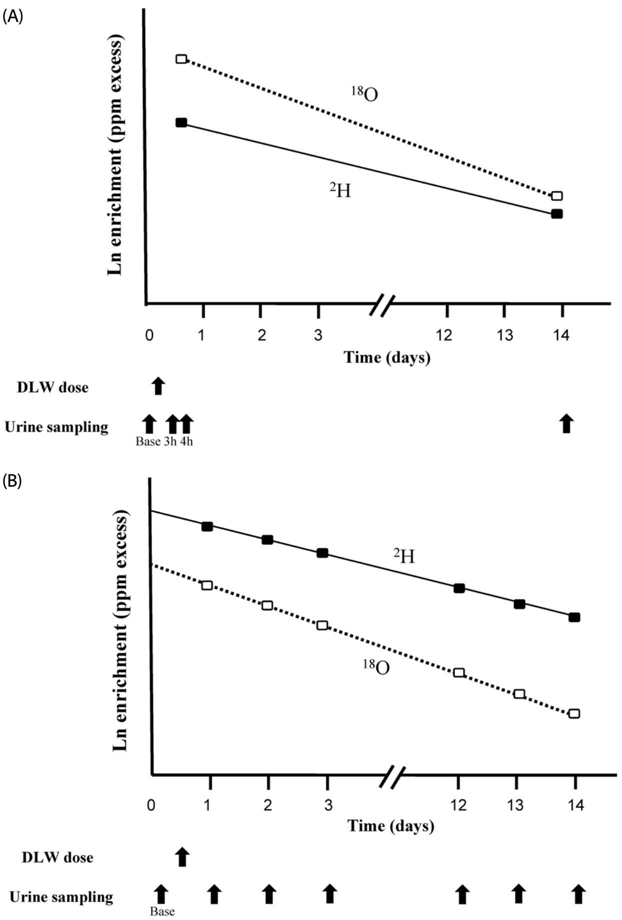

Fig. 2. **The time points of urine sampling and log-linear plot of isotope elimination in a two-point (A) and multiple-point (B) doubly labeled water (DLW) protocol.** (A) On day 0, the $^{2}H_2{}^{18}O$ (DLW) dose was given orally to each subject after collecting a baseline urine sample. Additional urine samples should be collected at 3 and 4 h after the dose on day 0. On the final day of experimental period, the participant should provide the one more urine sample. The isotope elimination rates (k_x) are calculated from the gradient of the isotope elimination curve. (B) On day 0, the $^{2}H_2{}^{18}O$ (DLW) dose is given orally to each subject after collecting baseline urine sample. After administration of this dose, the participants are requested to collect urine samples on the following day and at two additional sampling points at the same time of the day during the study period. In the two-point protocol (A), $k_x = \ln(E_2 / E_1) / (t_2 - t_1)$ where E is the enrichment calculated as $\text{abundance}_x - \text{abundance}_{\text{baseline}}$ and t is the time interval after the dose administration. The subscripts 1 and 2 refer to the specimen, where 1 is the post-dose specimen and 2 is the final specimen. In the multi-point protocol (B), k_x is the gradient of the linear regression line through the isotope elimination data.

between the 3- and 4-h urine specimens to minimize any short-term effects of water intake on urine enrichment. On the final day of the experimental period, the participant should provide urine samples. Elimination rates of ^{2}H and ^{18}O are determined by using urine samples collected at the two time-points. The typical interval between dosing and final urine collection is 7 or 14 days because a week includes both weekdays and weekends. Because the elimination rate of ^{18}O is dependent of both metabolic rates and water elimination, the final urine collection should be performed within day 7 in case of children and athletes.

TEE calculation by using the two-point method

The principle of the TEE calculation is described in detail in the IAEA Human Health Series [15]. To help understating how TEE is calculated by using the two-point method, this section is described based on reports from Saitoh's group in Japan [21]. Saitoh and colleagues have calculated TEE by using the two-point protocol and have published approximately 12 articles on this method pertaining to Japanese populations. Notably, there is only one DLW study that has been published for the Korean population, which was conducted in soccer players [22]; this study used the method of Saitoh's group. Ebine et al. [21] used saliva samples (3 and 4 hours after the DLW dose) for the measurement of TBW, whereas the elimination rates of ^{2}H and ^{18}O were determined by using urine samples collected according to the two-point method (Fig. 2-A). The dilution space was determined from saliva ^{2}H enrichment by using the following equation:

$$N = [WA\ (\delta a - \delta t)] / [18.02a\ (\delta s - \delta p)]$$

where N (moles) is the dilution space of ^{2}H, W (g) is the amount of tap water in which the dose is diluted for analysis, A (g) is the given dose, a (g) is the amount of the dose diluted with tap water for analysis, and δ (%) is the enrichment of the diluted dose (a), tap water (t), saliva sample after dosing (s), and saliva sample at baseline (p). TBW (moles) was calculated as $N/1.041$ [23]. Note that the Saitoh's group used only ^{2}H dilution space when calculating N space, however, N space could be calculated by the average dilution space of ^{2}H and ^{18}O to reduce the measurement error of ^{2}H [15].

$$\text{TBW (mol)} = \text{N(mol)} / 1.041$$

rCO_2 was calculated as follows:

$$rCO_2\ \text{(mol/day)} = 0.4554 \times \text{TBW}\ (1.007\ k_O - 1.041\ k_H)$$

where rCO_2 (mol/day) is the rate of CO_2 production, TBW is the total body water in moles, and k_O and k_H are the rates of ^{18}O and ^{2}H elimination, respectively. TEE is determined from the rate of CO_2 production and the food quotient (FQ), which is derived from food consumption data [24], using the following modified Weir's formula [12]:

$$\text{TEE (kcal/day)} = 22.4\ \{3.9\ (rCO_2/\text{FQ}) + 1.1\ (rCO2)\} \times 4.184 / 1000$$

Black et al. [24] suggested that the RQ is quite similar to the FQ and that the former can be predicted from the latter. They also demonstrated that the error in calculating energy expenditure from the FQ is less than 3% in most situations.

Experimental procedure for the multiple-point method

Fig. 2-B shows the time points of urine sampling and log-linear plot of isotope elimination in multiple-point DLW protocol [15]. The Experimental procedure for the multiple-point method is described in detail in the IAEA Human Health Series [15]. A brief description of the experimental procedure for multiple-point method differed from the two-point method follows. After the administration of this dose, the participants are asked to collect urine samples on the following day and at multiple time-points. Samples should be collected at the same time of the day during the study period. The ^{2}H and ^{18}O zero-time

intercepts and elimination rates (k_H and k_O) are calculated by using a least-squares linear regression method based on the isotope concentrations in multiple specimens.

TEE calculation by using the multiple-point protocol

The following section describes how TEE is calculated by using the multiple-point method based on reports from the National Institute of Health and Nutrition (NIHN) in Japan [25]. The ^2H and ^{18}O zero-time intercepts and elimination rates (k_H and k_O) are calculated by using least-squares linear regression of the natural logarithm of the isotope concentration as a function of the elapsed time from dose administration (Fig. 2-B). The zero-time intercepts are used to determine the isotope pool sizes. TBW is calculated from the mean value of the isotope pool size of ^2H divided by 1.041 and that of ^{18}O divided by 1.007. The equation for calculating rCO_2 is that used for the two-point method. The TEE calculation is also performed by using a modified Weir's formula [12] based on the rCO_2 and the FQ, but a modified equation is used, as follows:

$$\text{TEE (kcal/day)} = 1.1\ rCO_2 + 3.9\ rCO_2 / \text{FQ}$$

Relative precision of the two-point and multiple-point methods

Two previous studies have compared in detail the data generated by the simultaneous application of the two-point and multiple-point methodologies [26,27]. The studies found that there was little bias when using the two-point method, probably because systematic temporal variation in metabolism in the subjects offset any benefit of the multiple-point approach. However, theoretically, if such systematic temporal variation in energy demands were absent, the multiple-sampling approach would provide a more precise result [28-30]. Djafarian *et al.* [20] also compared data generated by using the two-point and multiple-point methodologies in children and suggested that the using the two-point method may be most appropriate for population-based comparisons while using the multi-point method may be best suited for exploring individual variation in TEE. Thus, using the multiple-sample approach may be more suited to situations where maximum precision is required. In the studies to compare the two-point and multiple-point methodologies [20,26], timed samples for multi-point methods were collected on a daily basis for the 10 or 14 days. However, it should be noted that human DLW studies using multiple-point methodology does not always analyze everyday samples, but analyzes some samples at the points of the first, middle, and end during the study period [13,14]. In addition, with urine samples, there is concern about whether water stored in the bladder is incomplete equilibrium with the body water. Especially in the elderly, voiding may be incomplete because of urine retention. Blanc *et al.* [31] suggested that 24 hours would be necessary for the urine to precisely calculate dilution spaces and the intercept method in multiple-point method should reduce the problem of delay in isotope equilibration. Because urine samples are generally collected by each subject personally at their residence, it could be that sample collection occurs at the wrong time or that subjects do not discard the first urine samples voided after waking up (the urine in such samples may be concentrated). Furthermore, urine samples may have leaked from containers during transit. Thus, the greatest advantage of the multiple-point method is that it minimizes the impact of imprecise analyses. It must be remembered that the multiple-point sampling method and curve-fitting approach involves substantially more isotope analysis, and consequently greater cost. However, the extra cost associated with the multiple-point method could be offset by the benefits of the resultant estimates of energy demands being more accurate than estimates based on the two-point method [29,32].

Isotopic analysis

Here, we summarize isotopic analysis procedures by using a general isotopes ratio mass spectrometer (IRMS) based on the method introduced by the IAEA [15]. Before analyzing urine samples with the IRMS, the urine samples must be equilibrated to gas samples, because the IRMS system can analyze only gas materials (sample preparation before the analysis). The gas for equilibration of ^{18}O is CO_2 [33], and that for ^2H is H_2 [34]. A platinum, chrome or zinc catalyst is used for equilibration of ^2H. An IRMS system has a magnetic sector mass spectrometer with multiple internal detectors. The sample must be introduced into the mass spectrometer as an ion source in the form of pure gas molecules (CO_2 or H_2). Sample gas is ionized by the impact of electrons emitted from a hot filament within a high vacuum. The ions are separated in a magnetic field according to molecular weight. Each sample is compared with a reference gas of known composition. In the case of the NIHN in Japan, isotopic analyses are conducted using the DELTA Plus device (Thermo Electron Corporation, Bremen, Germany) calibrated using Vienna Standard Mean Ocean Water, 302B and the Greenland Ice Sheet Precipitation standard provided by the IAEA [25]. There are two kinds of IRMS systems categorized according to their inlet systems: dual inlet gas IRMS and continuous flow IRMS systems. Dual inlet gas IRMS is the classical technique. Briefly, dual inlet gas IRMS has superior precision, while continuous flow IRMS systems have the advantages of better automation, higher throughput, and reduced sample sizes. Each sample and the corresponding reference are analyzed in duplicate or triplicate. Ishikawa-Takata et al. [25] reported the average standard deviations through the analyses performed by using the dual inlet gas IRMS system are 0.5% for ^2H and 0.03% for ^{18}O.

Applications of the DLW data Determining EER

For many decades, energy requirements were based on estimates of EI. However, in 1985, the World Health Organization (WHO) proposed using TEE for more accurate estimates of energy requirements. Around this time, reports on the DLW method for measuring TEE in free-living humans began to be published in Western countries [10,35]. Therefore, the DLW data were based on subsequent estimates of energy requirements [9].

As an example of daily recommended intake (DRI) determination for energy using DLW data in the United States, Black *et al.* [36] compiled the DLW data of 574 subjects and found the recommended EI levels for American infants and children are high, whereas those for adolescents and adults are low. Approximately 10 years later, the DRI for energy in the United States was determined on the basis of pooled DLW data [9] and energy requirements were determined on the basis of TEE calculated by the following formula:

EER for men (kcal) = 662 - 9.53 × age + PA [15.91 × body wt. (kg) + 539.6 × height (m)]

PA (physical activity coefficient) = 1.0 (sedentary), 1.11 (low active), 1.25 (active), 1.48 (very active)

EER for women (kcal) = 354 - 6.91 × age + PA [9.36 × body wt. (kg) + 726 × height (m)]

PA = 1.0 (sedentary), 1.12 (low active), 1.27 (active), 1.45 (very active)

When the PAL is 1.00 to 1.39, 1.40 to 1.59, 1.60 to 1.89, and 1.90 to 2.50, PA is categorized as "sedentary," "low active," "active," and "very active," respectively. After the EER equations were determined using the DLW data, further DLW studies were performed to assess the accuracy of these equations in various subject groups [37,38].

In the study determining the PAL of healthy Japanese adults (n = 150, age 20-59 years), the mean PAL values of men and women were 1.72 ± 0.22 and 1.72 ± 0.27, respectively. The study also compared PAL values obtained using the DLW method according to categories used for DRI for Japanese people (Table 1). The distribution of four categories across sex and age groups was uniform. Categories III (light heavy) and IV (heavy) had relatively higher PAL compared with categories I (light) and II (moderate). When they combined categories III and IV together (n = 10, PAL = 1.88 ± 0.29) because of their small number, this category had significantly higher PAL than category I ($P = 0.036$). The DLW data of Ishikawa-Takata *et al.* [25] have been used for the determination of the EER for Japanese people. In determining the EER for other age groups including infants, children, and the elderly, they used PAL data from DLW studies performed in other countries [39]. In China, researchers have measured TEE values for Chinese people using the DLW method, in order to determine if the current levels of nutrient intake are appropriate. Yao et al. [40] measured the TEE of 73 Chinese adults aged 35-49 years from a wide variety of occupations. Their results suggested that current energy requirement recommendations slightly underestimate the energy needs of women in occupations requiring light physical activity, but were accurate for men and women engaged in occupations requiring moderate and heavy physical activity. Zhuo *et al.* [41] recently reported that the TEE of young Chinese men measured by using the DLW method is approximately 10% lower than the current diet energy-based recommended nutrient intake, suggesting that the EER equation for Chinese men may be overestimated. In Korea, the Korean Nutrition Society determined energy requirements on the basis of the WHO equation, which predicts TEE as resting metabolic rate multiplied by PAL with the DRI for Koreans in 1995 and 2000 [42,43]. However, the Korean Nutrition Society determined energy requirements from the DRI for Koreans in 2005 using the same method used to determine the DRI for Americans using the DLW method [9,44].

Application in validating EI

Before the development of the DLW method, self-reported habitual EI was often used as a proxy measure of TEE. It has become clear that TEE obtained by the DLW method is being used as a biomarker of EI [5]. Energy balance is the principle used to validate EI measures determined using EE measures obtained by the DLW method. Energy balance occurs when EI equals EE under the conditions of stable body weight during an experimental period. Thus, this principle of energy metabolism enables the determination of the energy requirements of certain populations from either EI or EE.

The many available methods for obtaining information regarding dietary intake can be divided into three general categories: (1) recall of foods eaten, (2) diet histories or retrospective questionnaires, and (3) diet records [45]. Studies published from the late 1980s using the DLW method indicate underreporting is a common problem associated with the self-assessment of dietary EI. Numerous subsequent studies comparing self-reported EI and EE values assessed by the DLW method have been published [45,46], and the problem of misreporting, particularly underreporting, by participants has been noted [45].

Okubo *et al.* [47] found 11% underreporting in 158 Japanese adults aged 20-59 years on a dietary assessment questionnaire compared with TEE measured by the DLW method. Similarly, Redman *et al.* [48] studied 217 healthy American adults aged 21-50 years and found 15% underreporting of EI assessed by self-report in the DLW period. Furthermore, Scagliusi *et al.* [49] found underreporting in 65 Brazilian adult women aged 18-57 years in a cross-sectional study; the authors assessed EI by 24-h recalls, a 3-day food record, and a food frequency questionnaire (FFQ) and assessed TEE by DLW. Using these 3 methods, they found a bias of > 20% towards the underreporting of food intake; these values are similar to those reported in other DLW studies of adults using various methods to assess EI [50,51].

Misreporting is also prominent in children because of their

Table 1. PAL among categories according to Dietary Reference Intake in Japan

		n	PAL	*P*-value
Ⅰ (light)	Mostly sedentary position doing reading, studying and talking, or sitting or lying position watching TV and listening to music with 1-h slow walk for walking and shopping	77	1.68 ± 0.21	0.070
Ⅱ (moderate)	Mostly sedentary position doing clerical work and housework with 2-h walk for commuting and shopping, and long hours of standing while meeting people doing housework	63	1.74 ± 0.25	
Ⅲ (light heavy)	In addition to moderate activity, 1 h of brisk walk, bicycle and other vigorous physical activity; mostly standing during farming, fishing with heavy muscular work for 1 h a day	6	1.85 ± 0.31	
Ⅳ (heavy)	Engaged in heavy muscular work for about 1 h a day such as hard training, carrying lumbers, farming in the busy season and so on	4	1.91 ± 0.30	

Abbreviations: PAL, physical activity level. PAL was calculated from total energy expenditure obtained from DLW method divided by basal metabolic rate. *P*-values were calculated by one-way analysis of variance for PAL. Data was modified from Ishikawa-takata et al.'study (2008).

lower literacy levels and limited cognitive abilities as well as difficulties estimating portion sizes for children [52,53]. Recently, Burrows *et al*. [46] systematically reviewed DLW studies to determine which dietary assessment method(s) provides valid and accurate estimates of EI. The critical appraisal process resulted in the inclusion of 15 articles. They classified "misreporting" as follows: adequate reporting, EI/TEE within the 95% confidence limits (0.84-1.16); underreporting, EI/TEE < 0.84; or overreporting, EI/TEE > 1.16. All 15 studies were associated with a degree of misreporting; in particular, significant underreporting of EI was found for estimated food records (19-41% of estimated EI, three out of five studies) and weighed food records (11-27%, one out of two studies), and overreporting for 24-h multiple pass recalls (7-11%, two out of four studies) and FFQs (up to 59%, one out of two studies). The authors note the degree of misreporting may depend on sample size, the dietary assessment method used, and whether parents helped their children complete the survey [46].

It should be noted that "undereating" may be misinterpreted as underreporting. Shahar *et al*. [54] found undereating and not underreporting in elderly subjects aged 70-79 years (n = 296); in that study, TEE and EI were assessed over 2 weeks by the DLW method and a self-reported FFQ, respectively. The authors categorized misreporting as follows: participants with an EI/TEE ratio < 0.77 and > 1.28 were categorized as low and high energy reporters, respectively. The results showed 43% of participants were low energy reporters. Among them, almost 30% were losing weight, probably because they were eating less than their EE level; therefore, these subjects were categorized as undereaters.

There is a vast body of literature related to the underreporting problem in obese individuals. Several studies found a bias of > 35% towards the underreporting of food intake in obese adults, children, and adolescents populations [55-58]. Westerterp [59] demonstrated that TEE linearly increases as body weight increases, although reported EI does not. Studies of the reasons for the underestimation of dietary intake among obese subjects have indicated that body image issues and weight consciousness may play significant roles [60-62].

Needless to say, an underestimation of EI will lead to false conclusions when establishing the nutritional requirements of a certain group; therefore, it is important that populations at risk of underreporting be identified. Because EI derived from any method of food recording can often be imprecise in a given subject group, results from studies using such methods should be interpreted with caution.

CONCLUSIONS

Although the number of applications of the DLW method has significantly increased in many areas of study including nutrition and clinical medicine, the DLW method is not always practical in many developing or Asian countries. To prevent and/or reduce the high prevalence of obesity and various diseases due to excess energy intake, it is essential to determine proper EER equations for general populations or provide an accurate and convenient tool for assessing EI. The DLW method is currently considered the best solution for these problems.

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