Calculation method for daily water intake components using doubly labeled water

Yosuke Yamada (✉ yamaday@nibiohn.go.jp)  
National Institute of Biomedical Innovation, Health and Nutrition  
https://orcid.org/0000-0002-4284-6317

Daiki Watanabe  
National Institute of Biomedical Innovation, Health and Nutrition

Hiroyuki Sagayama  
University of Tsukuba

Aya Itoi  
Kobe Women's University

Tsukasa Yoshida  
National Institute of Biomedical Innovation, Health and Nutrition

Yuya Watanabe  
Meiji Yasuda Life Foundation of Health and Welfare

Hinako Nanri  
National Institute of Biomedical Innovation, Health and Nutrition

Eiichi Yoshimura  
National Institute of Biomedical Innovation, Health and Nutrition

Herman Pontzer  
Duke University

Amy Luke  
Loyola University School of Medicine

Misaka Kimura  
Kyoto University of Advanced Science

Dale Schoeller  
University of Wisconsin - Madison

Motohiko Miyachi  
National Institute of Biomedical Innovation, Health and Nutrition

John Speakman  
University of Aberdeen

Methods Article
Abstract

Daily water intake (DWI) is essential for survival in humans; however, accurate assessment of DWI from drinks and beverages (Wdrinks) or food moisture (Wfoods) is difficult as it depends on self-reported intakes that are prone to inaccuracy. Here, we established an objective method to assess DWI components using doubly labeled water (DLW). Deuterium and H218O were orally administered, and the dilution space and elimination rate of 2H and 18O were measured. DWI was calculated from the deuterium turnover corrected for metabolic water production and insensible water absorption from humidity. Wfoods was estimated using dietary record (Wfoods-DR) or calculated from the total energy expenditure assessed by DLW (Wfoods-DLW). The current results underscore Wfoods-DR underestimation using self-reported dietary assessments, which underestimates food intake. This study proposes novel methods for calculating each DWI component using DLW.

Introduction

Water, a critical but overlooked nutrient, is the principal chemical constituent of the human body, accounting for approximately 65% and 50% of body weight in infants and elderly persons, respectively. Water fills intra- and extracellular compartments, and it serves as a solvent for minerals, vitamins, amino acids, glucose, and many other nutrients. Thermoregulatory, circulatory, and urinary systems are dependent on body water and water balance; water loss and intake are regulated by peripheral and central homeostatic systems. Observational studies suggest that adequate hydration and/or prevention of acute dehydration are associated with reduced risks of developing various morbidities.

Although epidemiological studies have attempted to examine the effects of daily water intake (DWI) on health outcomes, the accumulation of scientific evidence is delayed. A major limitation of the abovementioned studies is that estimated DWI is based on self-reported dietary assessment methods. Results of multi-country comparison studies suggest that the amount of beverage intake depends heavily on a variety of dietary assessment methods, not only on sampling bias or cultural differences. In addition, total water influx does not originate only from Wdrinks but also from Wfoods, metabolic water production (Wmet), respiratory water uptake (Wres), and transcutaneous water uptake (Wtra) (Fig. 1). Restricted food intake enhances drinking behaviors, and metabolic water plays an important role in animals living in environments with limited access to drinking water and food moisture. Thus, to examine the relationship between DWI and health outcomes, it is necessary to assess each source of DWI accurately.

What percentage of DWI originates from Wdrinks or Wfoods? The Third National Health and Nutrition Examination Survey in the United States of America (USA) indicated that approximately 80% of DWI is obtained from drinks and beverages and 20% from food. Moreover, other studies also suggested that 20–30% of DWI originates from food moisture. However, subjective dietary assessment methods underestimate 10–40% of energy and protein intakes compared to assessment with biomarkers.
This fact suggests that subjective dietary assessments underestimate total food intake, resulting in underestimation of Wfoods as well. However, to the best of our knowledge, no previous studies have attempted to calibrate Wfoods using biomarkers.

Several approaches have been proposed for the biomarker calibrations of energy, protein\textsuperscript{20–23}, and intake of various minerals\textsuperscript{24}. The doubly labeled water (DLW) technique is considered a criterion method for energy intake (EI) calibration. Previous studies indicated that the calibrations make associations between nutrient intakes and health outcomes clear\textsuperscript{25,26}. We report here a method for assessing DWI components, particularly differentiating Wfoods from Wdrinks, using DLW. We hypothesized that DWI from food moisture accounts for a much higher percentage of total DWI than presented in current consensus, guidelines, or literature. If this hypothesis is true, the importance of food moisture should be considered in dietary references for water intake and/or water intake advices in public health and clinical settings. Epidemiological studies that examine the health effects of DWI should be conducted to evaluate DWI from both foods and liquids with adequate biomarker calibration using DLW.

Results

The physical characteristics and body composition of the 141 participants are shown in Table 1. Daily water turnover (rH\textsubscript{2}O), energy expenditure, and body composition were measured using the DLW method, as previously described\textsuperscript{21,27}. On day 0, urine samples were collected for the measurement of baseline 2H and 18O enrichment before DLW dose. Each participant drank DLW containing a premixed dose of approximately 0.12 g/kg estimated total body water (TBW) of H\textsubscript{2}O\textsubscript{2} (99.8 at.\%, Taiyo Nippon Sanso, Tokyo, Japan) and 2.5 g/kg estimated TBW of H\textsubscript{2}18O (10.0 at.\%, Taiyo Nippon Sanso)\textsuperscript{28}. Urine samples were collected in the mornings of the next day (day 1), day 2, 8, 9, 15, and 16. The urine samples were stored at -15°C for later analysis by isotope ratio mass spectrometry (IRMS, Hydra 20-20, Sercon, UK). The analysis procedure of IRMS has been described previously\textsuperscript{28}. The dilution spaces of 2H and 18O (Nd and No, respectively) and the elimination rate of 2H and 18O (kd and ko, respectively) were determined\textsuperscript{29}. If 18O enrichment was <8‰, kd and ko were calculated using only data for day 1,2,8 and 9\textsuperscript{30}.

The most recent study proposed a standard calculation methodology for human DLW studies\textsuperscript{31}. The Nd/No of the present study (1.032 ± 0.013) was comparable to that of the abovementioned study\textsuperscript{32}. Thus, TBW was calculated as follows:

\[
TBW = N = \frac{[(No/1.007) + (Nd/1.043)]}{2}\ [1]
\]

The elimination rates of 18O and 2H (ko and kd, respectively) were determined, and carbon dioxide production rate (rCO\textsubscript{2}) (mol/d) was calculated with the assumption that isotope fractionation applies to water in breath and transcutaneous water using Eq. A6 by Schoeller et al.\textsuperscript{33}; the revised dilution space constant was provided by Sagayama et al.. The rCO\textsubscript{2} (L/d) was obtained using the following formula:
The respiratory quotient (RQ) was assumed to be 0.87 based on the food quotient, and total energy expenditure (TEE) was calculated using the modified Weir's equation as follows:

\[
TEE = 1.106 \times r_{CO_2} + 3.94 \times (r_{CO_2}/RQ) \times 4.184 / 10^3 \tag{3}
\]

The quality checklist is described in the International Atomic Energy Agency documents.

**Resources of daily water intake**

The daily total water turnover (TWT, L/d) was calculated using the DLW method according to ref. as follows:

\[
TWT = r_{H_2O} = kd \times Nd \times (1/f) = 1.04 \times kd \times N \tag{4}
\]

where \( f \) is hydrogen isotope fractionation. When body water is maintained, \( r_{H_2O} \) is equal to water efflux and influx (i.e. DWI). Metabolic water production (Wmet; L/d) was calculated from energy expenditure and from assuming a metabolic mixture of carbohydrate, fat, and protein in a caloric ratio based on the ref. as follows:

\[
Wmet = TEE \times [(\%fat \times 0.119) + (\%protein \times 0.103) + (\%carbohydrates \times 0.15) + (\%alcohol \times 0.168)] \tag{5}
\]

Inspiratory water (Wins; L/d) was calculated as:

\[
Wins = \text{respiratory air volume} \times \text{estimated absolute humidity} / 1000 \tag{6}
\]

where respiratory volume is in L/d and absolute humidity is in mg/L, estimated from predicted air temperature. Respiratory air volume was calculated from \( r_{CO_2} \) obtained from DLW, assuming that 3.5% of expired air is \( CO_2 \).

Transcutaneous water influx (Wtra) was then calculated as:

\[
Wtra = 0.18 \times (\text{absolute humidity}/21.7) \times 0.5 \times BSA \times 1.44, \tag{7}
\]

where 0.18 is the rate of transcutaneous absorption in g/m² of body surface area (BSA) in an atmosphere saturated with water vapor (21.7 mg/L). The BSA \( m^2 \) was estimated using the Dubois formula, and a clothing factor of 50% was assumed, as clothing would decrease the rate of evaporation through the skin.
Dietary water intake from preformed water (DWI) was calculated as the difference between rH$_2$O and the sum of all the above-calculated values (Wmet, Wins, and Wtra) as follows$^{27}$:

$$\text{DWI} = \text{TWT} - \text{Wmet} - \text{Wins} - \text{Wtra} \ [8]$$

Wfoods was obtained using the following equation:

$$\text{Wfoods} = \left( \frac{\text{WCF}}{\text{ED}} \right) \times \text{TEE} \ [9]$$

where the water content of food (WCF) and energy density (ED) are obtained in L/kg and MJ/kg, respectively. Wdrinks was calculated as the difference between DWI and Wfoods.

$$\text{Wdrinks} = \text{DWI} - \text{Wfoods} \ [10]$$

**Validity and reproducibility**

Reproducibility of the DLW method was checked by conducting DLW experiments twice among 26 participants. The second measurements were conducted 3 months after the first one, between the end of August and the beginning of September 2012. First, we ensured that the background $^2$H and $^{18}$O isotope abundances were not higher in the second measurement than in the first. To insure that all of the tracers had been eliminated, the oxygen isotope abundance of the second measurement was $-5.1 [0.5]$‰ expressed as $\delta^{18}$O vs. standard mean ocean water (SMOW), and it was not significantly higher than that of the first measurement ($-4.5 [0.6]$‰). The hydrogen isotope abundance of the second measurement was $-37.3 [3.3]$‰ $\delta^2$H SMOW, and it was not significantly higher than that of the first measurement ($-34.0 [4.8]$‰). Instead, the second isotope abundances were significantly lower than the first isotope abundances. Only one out of 26 participants had a higher $^{18}$O isotope abundance ($+0.4$‰). Regarding $^2$H, seven participants (26.9%) had higher isotope abundances ($-0.3$ to $-6.6$‰); however, all of them fell within the range of measurement variations. Therefore, we calculated the second TEE and rH$_2$O using the second baseline isotope abundances as their frameworks. Coefficients of variation (CVs) between two experiments were calculated using the logarithmic method$^{36}$. The CVs were 3.6% (95% confidence interval [CI] 2.5–4.6%), 11.6% (95%CI 8.2–15.2%), and 13.1% (95%CI 9.2–17.1%) for TBW, TDEE, and rH$_2$O, respectively. The CVs, calculated using the within-subject standard deviation (SD) method, were 3.7%, 10.5%, and 13.5% for TBW, TEE, and rH$_2$O, respectively. Within-subject SDs were 1.2 kg, 0.97 MJ/d, and 0.45 L/d for TBW, TEE, and rH$_2$O, respectively. Intraclass correlation coefficients [3,1]) were 0.955, 0.698, and 0.898 for TBW, TEE, and rH$_2$O, respectively.

TWT and its sources in the participants are shown in Table 2. Although Wfood-DR was significantly correlated with total EI estimated using DR ($R^2 = 0.191$, P<0.001), Wfood-DR was not correlated with TEE ($R^2 = 0.000$ P=0.856, Figure 2A). Wfood-DR was significantly lower than Wfood-DLW (P<0.001, Bland-
Altman plot, Table 2 and Figure 2C). These data indicated that DRs could not be used to accurately and precisely assess DWI from food.

EI assessed using DR was significantly lower than TEE (P<0.001, see Bland-Altman plot, Figure 2C). Figure 2D shows the relationship of the percent differences between EI (by DR) and TEE, with the percent differences between Wfood-DR and Wfood-DLW. The percent difference between Wfood-DR and Wfood-DLW was proportionally associated with that between EI by DR and TEE (R² = 0.93, P<0.001). The estimation error of Wfood-DR is dependent on the estimation error of EI by DR.

TEE measured using DLW was correlated with Wfood; therefore, we developed an equation in the model development group (n = 72) as follows (Figure 3A):

\[
W_{\text{food}} (\text{L/day}) = 0.0892 \times \text{TEE} + 0.337 \quad [11]
\]

We applied Eq. 11 in the validated group (n = 69). In Figure 3B, the black line is the y = x line, and the intercept and slope of the regression line are not significantly different from those of the y = x line. Therefore, the equation was validated. We pooled all participants (n = 141) and got the regression line as follows (Figure 3C):

\[
W_{\text{food}} (\text{L/day}) = 0.0914 \times \text{TEE} + 0.315 \quad [12]
\]

After calculation, the proportions of the component of DWI, Wfood and Wfluid were 53% and 47%, respectively.

**Discussion**

First, we discovered that self-report dietary assessment methods systematically underestimate Wfood. The underestimation of Wfood is highly correlated with the underestimation of EI by DR when compared to TEE by DLW. In addition, self-reported Wfood-DR was not significantly correlated with Wfood-DLW. These results suggest that reliable methods are required to accurately and precisely assess Wfood. The newly established equation can reasonably estimate Wfood. This is because Wmet, Wres, Wtra, and Wfood can be assessed using the DLW method, and Wfluid can be estimated as the residual of them from TWT.

It has been generally considered that 70–80% of DWI is obtained from fluids (drinks and beverages) and only 20–30% from foods. However, the present study indicated that approximately 45% and 55% of DWI are from foods and drinks/beverages, respectively. DWI from food accounts for a much higher percentage of total DWI than is revealed by current consensus, guidelines, or literature. This is because dietary assessment methods underestimate DWI from food as well as energy and protein intake, although no previous studies have demonstrated this underestimation. TWT can be measured using the deuterium pool size and the washout, and systematic underestimation of DWI from food induces systematic overestimation of DWI from drinks. Our results suggest that the current guideline for drinking water and
beverages should be revised based on the biomarker method. The current guideline of the Institute of Medicine in the USA\textsuperscript{15} considered only 0.5–0.7 L/d as Wfood. Our participants actually had 1.12 L/d of Wfood, which is almost double the value in the current consensus, although the weight and height of the current participants are lesser than those of the normal US population. DWI in the current consensus led to a recommendation of inflated beverage consumption. In summary, our results emphasized Wfood as an overlooked water source. Moreover, these results underscore the importance of DWI assessment using objective methods and the need for a revision of current guidelines in terms of drinking water and beverages.

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Methods

Study cohorts

This study analyzed subgroups of the Kyoto-Kameoka Study wherein DLW was measured. Details of the abovementioned study have been previously described. We sent mail invitations for face-to-face physical examination to 4831 older adults aged at least 65 years, who lived in Kameoka city, Kyoto, and had responded to a baseline mail survey in February 2012. Of the invited individuals, 1379 participated in a face-to-face physical checkup between March and April, with a participation rate of 30.3%. We advertised the opportunity for individuals to have their energy expenditure measured using DLW, and 147 individuals voluntarily participated in the measurement between May and June 2012. We excluded participants who had missing data in a 7-day dietary record (DR) or DLW (3 people each); therefore, data from 141 individuals (62 women and 77 men) were analyzed. Participants were divided into a model development group (n = 72) and a validated group (n = 69), using the R software function of random number generation.

Dietary records

We used a previously described protocol for dietary assessment. We collected DRs over 7 consecutive days during the DLW method to include both weekdays and weekends. During an informational meeting, the research staff (registered dietitians) were educated on how to administer the DR to the participants, using completed DR sheets as examples. Each participant was provided blank record sheets, wherein to provide their DRs, as well as a digital scale (TANITA, Tokyo, Japan) and paper media for education.
Research dietitians instructed the participants to record every food item and beverage consumed daily during or between meals.

The dietitians checked all completed records at each participant’s home and reviewed them at least twice in a standardized manner. Research dietitians coded and entered completed DRs into an energy and nutrient analysis program called WELLNESS21 software (TopBusinessSystem, Okayama, Japan), which conforms to the Standard Tables of Food Composition in Japan. Participant-recorded foods that were not listed in the Standard Tables of Food Composition in Japan were replaced with similar foods. The DR was used to calculate individual values of EI, WCF (%), ED (MJ/day), and dietary water intake from food (Wfood-DR).

**Ethics**

All participants were informed of the purpose, procedures, and risks of the study, after which they provided written informed consent before participation. The ethics committee of Kyoto Prefectural University of Medicine and the National Institute of Health and Nutrition approved the study protocol (RBMR–E–372 and NIHN187–3). The current analysis used baseline data and did not contain any intervention parts of the study, but the Kyoto-Kameoka study included intervention studies with data on physical activity and nutrition promotion. Therefore, this study has been registered in a clinical trial database (UMIN000008105). The research protocols, outcomes, and measurements including DLW and dietary assessments were fully described at research protocol papers 39,40.

**Declarations**

**Acknowledgments**

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**Author contributions**
YY, DW, HS, HP, MM, JRS, DAS established the concept of this methodology; YY, MK designed research of cohort; YY, HS, AI, TY, YW, HN, EY, MK conducted the study; YY, DW, HS analyzed data; YY wrote the manuscript; AL, HP, JRS, DAS revised the draft substantially; YY had primary responsibility for the final content. All authors read and approved the final manuscript.

Conflicts of interest

None of the authors has conflicts of interest.

Tables

Table 1. Means and standard deviations of participant physical characteristics and variables obtained using the doubly labeled water method.

|                  | n = 141 |
|------------------|---------|
| **Women (%)**    | 45.3%   |
| Height (cm)      | 158.1 ± 8.5 |
| Weight (kg)      | 56.9 ± 9.7 |
| BMI              | 22.7 ± 3 |
| TBW (kg)         | 28.6 ± 5.4 |
| FFM (kg)         | 39.1 ± 7.3 |
| Body fat (%)     | 31.1 ± 6.6 |
| Nd (mol)         | 1654 ± 312 |
| No (mol)         | 1602 ± 299 |
| Nd/No            | 1.032 ± 0.012 |
| Kd               | 0.1025 ± 0.0196 |
| Ko               | 0.1296 ± 0.0217 |
| rCO2 (L/day)     | 375.8 ± 76.3 |

BMI, body mass index; TBW, total body water; FFM, fat free mass; Body fat, percent body fat; Nd, dilution space of $^2$H; No, dilution space of $^{18}$O; kd, elimination rate of $^2$H; ko, elimination rate of $^{18}$O; rCO$_2$, carbon dioxide production rate.
Table 2. Means and standard deviations of total energy expenditure, energy intake, and water turnover values, using the doubly labeled water method and dietary record.

| Variable                        | n = 141 | Mean   | SD     |
|---------------------------------|---------|--------|--------|
| TEE (MJ/day)                    | 8.86    | ±1.8   |
| EI (MJ/day)                     | 8.13    | ±1.24***|
| TWT (L/day)                     | 3.02    | ±0.7   |
| Wmet (L/day)                    | 0.29    | ±0.06  |
| Wres (L/day)                    | 0.13    | ±0.03  |
| Wtra (L/day)                    | 0.09    | ±0.01  |
| DWI (L/day)                     | 2.52    | ±0.65  |
| Wfluid (L/day)                  | 1.39    | ±0.6   |
| Wfood (L/day)                   | 1.12    | ±0.27  |
| Wfood-DLW (L/day)               | 1.12    | ±0.17  |
| Wfood-DR (L/day)                | 0.98    | ±0.22### |
| WCF (kg/kg)                     | 0.69    | ±0.04  |
| ED (MJ/kg)                      | 5.49    | ±0.74  |

TEE, total energy expenditure; EI, energy intake; TWT, total water turnover; Wmet, metabolic water production; Wres, respiratory water uptake; Wtra, transcutaneous water uptake; DWI, daily water intake, Wfluid, preformed water in ingested liquids (drinking water and beverages); Wfood, preformed water in ingested foods; Wfood-DLW, Wfood estimated by DLW only, Wfood-DR, Wfood estimated by DR only; WCF, water content of food; ED, energy density.

*** EI assessed by DR was significantly lower than TEE measured by the DLW method (P<0.001).

### Wfood assessed by DR was significantly lower than Wfood measured by the DLW method. (P<0.001).

**Figures**

![Figure 1](image-url)
Components of total water turnover (TWT). Daily water intake (DWI) can be separated into DWI from ingested liquids (Wdrinks) and from ingested foods (Wfoods).

**Figure 2**

Relationship between Wfoods-DR and TEE (A). Wfood-DR was not correlated with TEE. Bland-Altman plot shows the DR underestimated actual Wfoods (B), which is consistent of the underestimation of energy intake by DR (C). The estimation error of Wfood-DR is dependent with estimation error of EI by DR (D).

**Figure 3**

Relationship between Wfoods and TEE in the model developed group (n = 72) (a). We applied the developed equation in the validated group (n = 69) (b). The intercept and slope of the regression line are not significantly different from those of the y = x line. We pooled all participants (n = 141) and got the regression line (c).