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# Determinant of factors associated with water requirement measured using the doubly labelled water method among older Japanese adults

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## Abstract

*Objective:* Water is an essential nutrient for all organisms and is important for maintaining life and health. We aimed to develop a biomarker-calibrated equation for predicting water turnover (WT) and pre-formed water (PW) using the doubly labelled water (DLW) method.

*Design:* Cross-sectional study.

*Setting:* General older population from the Kyoto–Kameoka Study, Japan.

*Participants:* The 141 participants aged ≥ 65 years were divided into a model developing (*n* 71) and a validation cohort group (*n* 70) using a random number generation. WT and PW was measured using the DLW method in May–June of 2012. In developing the cohort, equations for predicting WT and PW were developed by multivariate stepwise regression using all data from the questionnaires in the Kyoto–Kameoka study (including factors such as dietary intake and personal characteristics). WT and PW measured using the DLW method were compared with the estimates from the regression equations developed using the Wilcoxon signed-rank test and correlation analysis in validation cohort.

*Results:* The median WT and PW for 141 participants were 2·81 and 2·28 l/d, respectively. In the multivariate model, WT ($R^2 = 0{\cdot}652$) and PW ($R^2 = 0{\cdot}623$) were moderately predicted using variables, such as height, weight and fluid intake from beverages based on questionnaire data. WT ($r = 0{\cdot}527$) and PW ($r = 0{\cdot}477$) predicted that using this model was positively correlated with the values measured by the DLW method.

*Conclusions:* Our results showed factors associated with water requirement and indicated a methodological approach of calibrating the self-reported dietary intake data using biomarkers of water consumption.

**Keywords**
Doubly labelled water
FFQ
Water turnover
Pre-formed water
Water requirement

Water is an essential nutrient for all organisms, accounting for 50–70 % of the total body weight in humans, a value that decreases with age[1]. Although humans have homeostatic functions for maintaining body fluid levels, they will die if they do not consume water for a few days[2]. When the body's fluid balance is disturbed by the loss of water,

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humans adjust principally by ingesting water from food and beverages based on the feeling of thirst or hunger[3]. However, older adults exhibit physiological homeostasis dysfunction and reduced body fluid volume, which are independent risk factors for dehydration[1,2,4], and it is, therefore, important to evaluate the amount of water they need to maintain their life and health.

There are two methods for evaluating the required amount of daily water intake: the water balance method (test-weighing technique), which assesses water intake and excretion; and the water turnover (WT) method, which assesses the turnover of fluids in the body. Water requirements calculated by both methods have shown similar results[5,6]. WT can be measured using the doubly labelled water (DLW) method, which uses $^2$H or both, $^2$H and heavy oxygen[6–12], and is considered the gold standard for measuring the daily water requirements of individuals who are not dehydrated[6–12]. The adequate intake of water for maintaining optimal conditions according to the guidelines from WHO[13] and the USA and Canada[14] is 3·2 l/d and 3·7 l/d for adult men and 2·7 l/d and 2·7 l/d for adult women, respectively; however, no targets have been set for older people. The sources of the body's water inputs are preformed water (PW), which includes food and drinks; metabolic water produced by the metabolism of nutrients; respiratory water taken into the body through breathing and transcutaneous water taken into the body via the skin[9,10]. Humans lose body fluids via urine, insensible perspiration, sweat and stool[1,3]. WT differs between regions with different environments[3,12]. Therefore, clarifying the daily water requirements is essential for establishing recommendations on water consumption to prevent dehydration and maintain body fluid levels[15].

Dietary evaluation methods that rely on self-reported data, such as FFQ, dietary records (DR) or 24-h dietary recall (24HR), which are commonly used in nutritional epidemiological studies, are problematic in making accurate assessments of dietary intake owing to systemic errors associated with individual characteristics such as age, gender and BMI[16,17]. For these reasons, the Strengthening the Reporting of Observational Studies in Epidemiology-Nutritional Epidemiology (STROBE-NUT) guidelines recommend using biomarkers for estimating dietary intake[18]. Neuhouser et al.[19] and Watanabe et al.[20,21] reported regression calibration approaches that used objective biomarkers to correct the systematic errors in dietary intake estimated from FFQ. Unlike uncalibrated energy intake, calibrated energy intake estimated using these approaches is strongly associated with the risk of developing diabetes[22], mortality[23] and the prevalence of frailty[24]. Therefore, associations of diseases with self-reported dietary intake without calibration should be observed with caution[22–25]. However, to the best of our knowledge, no regression equations have been developed that calibrate self-reported water intake using water consumption measured with objective biomarkers. The present study aimed to develop a biomarker-calibrated equation for predicting WT using data on dietary intake and individual characteristics obtained from self-reports. We hypothesised that, similar to energy intake and nutrients, it would be possible to develop equations with moderate predictability for WT.

## Methods

### *Study population*

We used data from the Kyoto–Kameoka study on older people (age ≥ 65 years) living in Kameoka City, Kyoto Prefecture, Japan. The details of this study are described elsewhere[20,21,24,26–28]. Briefly, ten of Kameoka's twenty-one districts were selected randomly and postcards were sent to 4831 residents asking them to take part in physical check-up examinations; 1379 took part in physical check-up examinations for the Kyoto–Kameoka study in March and April 2012 (response rate 28·5 %). Of these 1379 participants, 147 individuals participated DLW measurements and 7 d DR in May and June 2012. Participants who did not complete the 7-day DR (*n* 3) or the DLW method (*n* 3) were excluded. In total, 141 people participated in this study. Participants were divided into a model developing (*n* 71) and a validation cohort group (*n* 70), using the random number generation. The development and validation cohort groups were intended to develop the equation for biomarker-calibrated water consumption and confirm the validation of these equations, respectively.

This study's protocol was approved by the ethics committees of the National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN-76–2), Kyoto University of Advanced Science (No. 20–1) and Kyoto Prefectural University of Medicine (RBMR-E-363). Informed consent in writing was obtained from all participants before data collection.

### *Doubly labelled water*

WT and total energy expenditure (TEE) were measured using the DLW method over periods of approximately 2 weeks in May and June 2012. The details of this study are described elsewhere[20,21]. Briefly, urine samples were collected from the participants before drinking DLW on the morning of Day 0 (baseline). After collecting urine samples, the participants drank water mixed with 0·12 g/kg of $^2H_2O$ (99·9 atom %, Taiyo Nippon Sanso, Tokyo, Japan) and 2·5 g/kg of $H_2{}^{18}O$ (10·0 atom %, Taiyo Nippon Sanso, Tokyo, Japan) per total body water estimated from their body weight (measured beforehand). The concentrations of $^{18}$O ($N_o$) and $^2$H ($N_d$) in the urine samples were measured using isotope ratio MS (Hydra 20-20 Stable Isotope Mass Spectrometers; SerCon Ltd, Crewe, UK). The $N_o$ and $N_d$ dilution spaces and the attenuation rates of $^{18}$O ($k_o$) and $^2$H ($k_d$) in the body were assessed with the modified two-point

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method using urine samples collected from days 1 to 16 (mean of the slopes from days 1 to 15 and days 2–16). Total body water was calculated using the $N_o$ and $N_d$ dilution spaces in Equation (1)[29]:

$$TBW = [(N_o/1{\cdot}007) + (N_d/1{\cdot}043)]/2 \quad (1)$$

The carbon dioxide production rate ($r_{CO2}$; mol/d) was calculated using the daily attenuation rates of stable isotopes ($k_o$, $k_d$), total body water and Equation (2)[29]:

$$rCO_2 = 0 \cdot 4554 \times TBW \times (1{\cdot}007k_o - 1{\cdot}043k_d) \times 22{\cdot}26 \quad (2)$$

TEE (kcal/d) was calculated using the Weir's equation (Equation (3)) based on $rCO_2$ and the 24-h estimated respiratory quotient (RQ)[29].

$$TEE = rCO_2 \times [1{\cdot}106 + (3{\cdot}94/RQ)] \quad (3)$$

TEE (kcal/d) estimated using Equation 3 assumes an excellent nutritional status. Assuming that RQ is equal to the food quotient, a value of 0·86 was used for all participants, with reference to previous studies[20,21].

### *Calculation of water consumption using the doubly labelled water method*

WT measured using the DLW method was used to assess daily water requirements. Metabolic, respirometry, transcutaneous and PW and WT were calculated according to Equations 4–8 from a previous study[9–12]:

$$rH_2O = k_d \times N_d \quad (4)$$

where $rH_2O$ is WT (l/d)[9,11,12]. If the equilibrium of fluid in the body is maintained, $rH_2O$, which is the water output, is equal to water input. $K_2$ and $N$ are the attenuation rates and body water content (kg) of $^2$H in the body after stable isotope ingestion, respectively. Equation 4 includes a 4 % correction for isotope fractionation, if 50 % of water output is lost as vapour. Metabolic water ($W_{met}$; l/d) was calculated using Equation 5[9,10,30]:

$$W_{met} = TEE \times (1/100\,000)[0{\cdot}119_{\%fat} + 0{\cdot}103_{\%pro} + 0{\cdot}150_{\%carb} + 0{\cdot}168_{\%alc}] \quad (5)$$

The intake of fat (*%fat*), protein (*%pro*), carbohydrates (*%carb*) and alcohol (*%alc*) per energy intake as estimated from the 7-day DR was multiplied by their coefficients and totalled. Metabolic water was estimated by multiplying this total value by the TEE value obtained using the DLW method. Respirometry water ($W_{res}$; l/d) was calculated by Equation (6)[9,10,30]:

$$W_{res} = [\text{absolute humidity}/1,000] \times 0{\cdot}035rCO_2 \quad (6)$$

This was calculated from the concentration of water in the atmosphere, estimated from the average air temperature and relative humidity during the period when the DLW method was performed. The mean temperature, hours of sunlight, relative humidity and absolute humidity during the study were 20·1°C, 5·5 h/d, 57 % and 9·83 g/m$^3$ in May–June 2012 (spring), respectively. For respiratory air volume, 3·5 % of the inhaled air was assumed to be $CO_2$ and was calculated from the $rCO_2$ obtained using the DLW method. Transcutaneous water ($W_{trans}$; l/d) was calculated using Equation 7[9,10,30]:

$$W_{trans} = [0{\cdot}18_{\text{absolute humidity}}/21{\cdot}7] \times 0{\cdot}5 \times BSA \times 1{\cdot}44 \quad (7)$$

In the current study, the transdermal absorption rate per m$^2$ of body surface area in atmospheric saturated water vapour (21·7 mg/l) was 0·18 g/m$^2$. The body surface area (m$^2$) was estimated using the Dubois equation[31]. Because clothing reduces the rate of evaporation of moisture from the skin, the clothing coefficient was assumed to be 50 %. PW ($W_{pre}$; l/d) was calculated using Equation 8[9,10,30]:

$$W_{pre} = rH_2O - [W_{met} + W_{res} + W_{trans}] \quad (8)$$

This was calculated by subtracting metabolic, respirometry and transcutaneous water from WT. PW includes the fluid consumed from food and drinks.

### *Dietary assessment*

The participants recorded their meals for seven consecutive days, including weekdays and holidays, during May–June 2012; the details of their records are presented elsewhere[28]. Briefly, an investigator (a well-trained, senior dietitian) taught the participants how to record their meals using an example meal record sheet completed at the briefing. The dietitian instructed the participants to record all food and drinks consumed at or between meals. Each participant was provided with blank record sheets for recording meals, a digital scale (TANITA, Tokyo, Japan) and printed educational materials on recording meals. Energy and nutrient intake were calculated using WELLNESS21 software (TopBusinessSystem, Okayama, Japan) based on these DR.

The current study employed a self-administered FFQ, which consisted of forty-seven food and drink items[32]. Assessments of dietary intake using this questionnaire have been validated previously[20,28]. We asked how often they consumed the food and drinks in the FFQ in the past year. For portion sizes, a uniform value for each sex was calculated from the 1-day weighted DR[32]. Energy intake, food weight and fluid intake from drinks were calculated from the intake frequency, and the portion sizes of each food and drink were calculated using a program developed

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based on the Japanese Food Standard Composition List[32]. Calculating the water intake from food using FFQ with this program is impossible. Therefore, as the mean ratio of water in the foods in the DR of this population was 69 %, water intake from food was estimated to be 69 % of the food weight from the FFQ. The estimate of PW by FFQ was calculated from the sum of water intake from food and drinks.

### *Covariates*

In the Kyoto–Kameoka study, a Needs in the Sphere of Daily Life survey (baseline survey) was conducted on July 29, 2011 and constituted questions based on sitting and sleep time. Subsequently, the Health and Nutrition Status Survey (additional survey), which includes the FFQ was conducted on February 14, 2012. The details of these surveys are described elsewhere[26]. Variables with significant associations in a multivariate regression analysis were evaluated as follows: height (response: enter number), weight (response: enter number), sleep time: 'How many hours do you actually sleep for? (This may differ from the time you spend in bed.)' (response: enter number), sitting time: 'How much time do you spend sitting or lying down during the day? (e.g. TV, reading, chatting; not including sleep)' (response: enter number), dentures: 'Do you use dentures?' (response: yes, no), dry mouth: 'Are you bothered by dry mouth?' (response: yes, no), self-reported need care: 'Do you need someone's care and assistance in your daily life?' (response: yes, no), and writing abilities: 'Are you able to fill out the documents you submit to government offices or hospitals by yourself?' (response: yes, no).

We previously reported that self-reported heights and weights were no different from heights and weights measured in a Kyoto–Kameoka study subcohort ($n$ 1169) (mean difference: –0·9 cm in height and 0·4 kg in weight)[27]. The correlation coefficients between the self-reported and actual measurements were 0·970 for height and 0·965 for weight[27]. Further, as a measure of the reproducibility of self-reports, the inter-class correlation coefficients of height and weight were 0·970 and 0·958, respectively[27]. BMI was calculated by dividing the self-reported weight (kg) by the square of the height (m).

### *Statistical analysis*

For descriptive statistics, continuous and categorical variables on participant characteristics in the developing and validation cohorts were expressed as mean and SD and as the number and percentage, respectively. The Shapiro–Wilk test was used to confirm the distribution and normality (skewness, kurtosis) of the WT data measured using the DLW method. This analysis showed that these data had non-normal distributions. Therefore, variables such as WT and PW were shown as the median and interquartile range. To compare the water consumption in participants' characteristics, we used the Mann–Whitney U test and Kruskal–Wallis' test in unpaired samples.

To develop a formula for predicting WT and PW measured using the DLW method, a multivariate linear regression analysis was performed using forward stepwise selection. This model used water consumption measured using the DLW method as the dependent variable. The explanatory variables of this model were all data of the individual characteristics obtained from the Kyoto–Kameoka study questionnaires, including physique, dietary intake estimated from FFQ, oral status, physical activity levels and social and mental health[26]. Following a previous study[19], logarithmic transformation was applied to the regression coefficients of all variables in this analysis (link function = log). The gaussian distribution (family = gaussian) adequately fits the data when compared with the other distributions such as Poisson, gamma and binomial (lowest values of Akaike information criterion). The constructed model was confirmed to meet the conditions of use for linear models (assumption of normality, homoscedasticity and error term independence). A biomarker-calibrated equation was developed to estimate WT and PW using covariates that retained significant associations in this multivariate regression model.

To confirm the validity of the regression equation that was developed, the WT and PW estimates from the regression equation and the DLW method were compared in the validation cohort using the Wilcoxon signed-rank test. The ability to rank individuals in the population of WT and PW estimates from the regression equation was evaluated using Spearman's rank and Pearson's correlation analysis with respect to the values measured using the DLW method. In addition, using the Meng's Z-test[33], we compared the equivalence of validity of the water consumption by the correlation coefficients between the PW estimated from FFQ and the regression equation, against those estimated using DLW method. A two-tailed significance level of 5 % was used in the analysis. STATA MP, version 15.0 (StataCorp LP) was used for all analyses.

## Results

### *Participant characteristics*

Table 1 shows the characteristics of the participants in the developing and validation cohorts. The total participants' mean (SD) age, BMI, total body water and TEE were 72·6 (5·3) years, 22·7 (3·1) kg/m$^2$, 28·6 (5·4) kg and 9037 (1807) kJ/d, respectively. None of the participant characteristics showed significant differences between cohorts.

### *Distribution of the water consumption*

Table 2 indicates the distribution of water consumption measured by the DLW method. The median values of WT



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**Table 1** Comparison of the characteristics of the participants included in the developing and validation cohorts

| | Total (*n* 141) | | Random assignment: Developing cohort (*n* 71) | | Random assignment: Validation cohort (*n* 70) | |
|---|---|---|---|---|---|---|
| | Mean | SD | Mean | SD | Mean | SD |
| Age (years)* | 72·6 | 5·3 | 72·5 | 5·4 | 72·6 | 5·2 |
| Women (*n* (%))† | | | | | | |
| *n* | 64 | | 28 | | 36 | |
| % | 45·4 | | 39·4 | | 51·4 | |
| Population density ≥ 1000 people/km² (*n* (%))† | | | | | | |
| *n* | 56 | | 27 | | 29 | |
| % | 39·7 | | 38·0 | | 41·4 | |
| Height (cm)* | 158·1 | 8·6 | 158·3 | 9·6 | 157·9 | 7·5 |
| Body weight (kg)* | 56·9 | 10·0 | 57·4 | 10·1 | 56·4 | 9·8 |
| BMI (kg/m²)* | 22·7 | 3·1 | 22·9 | 3·1 | 22·6 | 3·2 |
| Total body water (kg)* | 28·6 | 5·4 | 29·2 | 5·7 | 28·0 | 5·1 |
| Total energy expenditure (kcal/d)* | 2160 | 432 | 2256 | 471 | 2064 | 368 |
| (kJ/d)* | 9037 | 1807 | 9439 | 1971 | 8636 | 1540 |
| | *n* | % | *n* | % | *n* | % |
| Current smoker (*n* (%))† | 7 | 5·0 | 3 | 4·2 | 4 | 5·7 |
| Alcohol drinker (*n* (%))† | 112 | 79·4 | 56 | 78·9 | 56 | 80·0 |
| Living alone (*n* (%))† | 14 | 9·9 | 5 | 7·0 | 9 | 12·9 |
| Self-reported need care (*n* (%))† | 2 | 1·4 | 2 | 2·8 | 0 | 0·0 |
| High socio-economic status (*n* (%))† | 44 | 31·2 | 19 | 26·8 | 25 | 35·7 |
| Education ≥ 13 years (*n* (%))† | 45 | 31·9 | 24 | 33·8 | 21 | 30·0 |
| Sleep time (min/d)* | | | | | | |
| Mean | 398 | | 395 | | 401 | |
| SD | 79 | | 89 | | 68 | |
| Sitting time (min/d)* | | | | | | |
| Mean | 311 | | 293 | | 329 | |
| SD | 223 | | 229 | | 217 | |
| Denture use (*n* (%))† | 73 | 51·8 | 40 | 56·3 | 33 | 47·1 |
| No medication (*n* (%))† | 36 | 25·5 | 21 | 29·6 | 15 | 21·4 |
| Dry mouth (*n* (%))† | 44 | 31·2 | 22 | 31·0 | 22 | 31·4 |
| Can write the documents yourself (*n* (%))† | 137 | 97·2 | 69 | 97·2 | 68 | 97·1 |
| | Mean | SD | Mean | SD | Mean | SD |
| Dietary records | | | | | | |
| Energy intake (kcal/d)* | 1943 | 303 | 1949 | 307 | 1937 | 300 |
| (kJ/d)* | 8130 | 1268 | 8155 | 1284 | 8104 | 1255 |
| Pre-formed water (l/d)* | 1·21 | 0·29 | 1·21 | 0·31 | 1·21 | 0·27 |
| Protein intake (% energy/d)* | 15·2 | 1·7 | 15·0 | 1·7 | 15·4 | 1·6 |
| Fat intake (% energy/d)* | 25·3 | 4·8 | 25·0 | 4·4 | 25·6 | 5·1 |
| Carbohydrate intake (% energy/d)* | 56·3 | 5·4 | 56·3 | 5·4 | 56·2 | 5·5 |
| Ratio of water in the foods (%/d)* | 69 | 5 | 68 | 5 | 70 | 4 |
| FFQ | | | | | | |
| Energy intake (kcal/d)* | 1781 | 485 | 1872 | 503 | 1689 | 451 |
| (kJ/d)* | 7452 | 2029 | 7832 | 2105 | 7067 | 1887 |
| Pre-formed water (l/d)* | 1·23 | 0·41 | 1·21 | 0·41 | 1·24 | 0·40 |
| Fluid intake from beverages (l/d)* | 0·579 | 0·332 | 0·556 | 0·347 | 0·602 | 0·317 |

This survey was conducted in spring (May/June 2012). The mean temperature and relative humidity during the survey period are 20·1°C and 57 % in the spring season. BMI was calculated as body weight (kg) divided by height squared (m²). Energy intake conversion factor: 1 kJ = 0·239 kcal.
*Continuous values are shown as mean (SD).
†Categorical values are shown as number (percentage).

and metabolic, respiratory, transcutaneous and PW for all participants were 2·81 l, 0·29 l, 0·13 l, 0·09 l and 2·28 l/d, respectively. When the samples were stratified by age, sex and BMI, the WT was significantly higher in men and individuals of < 75 years with a higher BMI. Similar results were also observed between developing and validation cohorts (see online supplementary material, Supplemental Tables 1–3).

### *Development of a biomarker-calibrated water consumption equation*

Table 3 shows the results of the stepwise multivariate regression model using water consumption measured using the DLW method as the dependent variable. The equations for predicting log-transformed WT and PW consumption measured using the DLW method used variables that exhibited significant relationships (Equations (9) and (10)):



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**Table 2** Distribution of the water consumption calculated by doubly labelled water method according to sex, age and BMI stratified model

| | Water consumption estimated by doubly labelled water method (l/d)* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Water turnover | | Metabolic water | | Respiratory water | | Transcutaneous water | | Pre-formed water | |
| | Median | IQR | Median | IQR | Median | IQR | Median | IQR | Median | IQR |
| Total (*n* 141) | 2·81 | 2·39–3·31 | 0·29 | 0·25–0·33 | 0·13 | 0·11–0·15 | 0·09 | 0·09–0·10 | 2·28 | 1·92–2·77 |
| Sex | | | | | | | | | | |
| Women (*n* 64) | 2·51 | 2·21–2·80 | 0·26 | 0·23–0·28 | 0·11 | 0·11–0·13 | 0·09 | 0·08–0·09 | 2·02 | 1·72–2·31 |
| Men (*n* 77) | 3·11 | 2·79–3·55 | 0·32 | 0·29–0·36 | 0·14 | 0·13–0·16 | 0·10 | 0·09–0·10 | 2·52 | 2·22–2·97 |
| *P*-value | < 0·001 | | < 0·001 | | < 0·001 | | < 0·001 | | < 0·001 | |
| Age (years) | | | | | | | | | | |
| 65–74 (*n* 88) | 2·94 | 2·42–3·36 | 0·30 | 0·25–0·34 | 0·13 | 0·11–0·15 | 0·09 | 0·09–0·10 | 2·40 | 1·94–2·88 |
| ≥ 75 (*n* 53) | 2·73 | 2·35–3·16 | 0·29 | 0·25–0·31 | 0·13 | 0·11–0·14 | 0·09 | 0·08–0·10 | 2·18 | 1·84–2·59 |
| *P*-value | 0·048 | | 0·162 | | 0·135 | | 0·335 | | 0·041 | |
| BMI (kg/m$^2$) | | | | | | | | | | |
| < 18·5 (*n* 13) | 2·34 | 2·00–3·06 | 0·25 | 0·22–0·30 | 0·11 | 0·10–0·13 | 0·08 | 0·07–0·09 | 1·91 | 1·59–2·45 |
| 18·5–24·9 (*n* 91) | 2·81 | 2·42–3·32 | 0·29 | 0·25–0·33 | 0·13 | 0·11–0·15 | 0·09 | 0·08–0·10 | 2·25 | 1·94–2·77 |
| ≥ 25 (*n* 37) | 2·97 | 2·59–3·49 | 0·30 | 0·27–0·36 | 0·14 | 0·12–0·16 | 0·10 | 0·09–0·10 | 2·49 | 2·11–2·97 |
| *P*-value | 0·036 | | 0·008 | | 0·007 | | < 0·001 | | 0·067 | |

This survey was conducted in spring (May/June 2012). The mean temperature and relative humidity during the survey period are 20·1°C and 57 % in the spring season. BMI was calculated as body weight (kg) divided by height squared (m$^2$).
*The values are shown as median (interquartile range). This analysis was used by a Mann–Whitney U test and Kruskal–Wallis' test in unpaired sample.

**Table 3** Regression calibration coefficients for log-transformed water turnover and pre-formed water using a stepwise multiple regression analysis with the water consumption measured by doubly labelled water as a dependent variable

| | Biomarker-calibrated water consumption equation (*n* 71) | | | | | |
|---|---|---|---|---|---|---|
| | RC | SE | 95 % CI | $\beta$ | *P*-value | Collinearity VIF |
| Water turnover | | | $R^2 = 0.652$ (Adjusted $R^2 = 0.601$) | | | |
| Intercept | –0·18 662 | 0·34 544 | –0·86 367, 0·49 042 | | 0·589 | |
| Height (cm) | 0·01002 | 0·00277 | 0·00460, 0·01544 | 0·391 | < 0·001 | 1·870 |
| Weight (kg) | 0·00599 | 0·00241 | 0·00126, 0·01071 | 0·285 | 0·013 | 1·770 |
| Sleep time (min/d) | –0·00064 | 0·00024 | –0·00112, –0·00017 | –0·233 | 0·008 | 1·122 |
| Sitting time (min/d) | –0·00025 | 0·00009 | –0·00043, –0·00007 | –0·231 | 0·006 | 1·065 |
| Fluid intake from beverages (l/d) | 0·12 137 | 0·05569 | 0·01223, 0·23 051 | 0·193 | 0·029 | 1·136 |
| Denture use (Ref, No) | –0·08846 | 0·03803 | –0·16 299, –0·01393 | –0·174 | 0·020 | 1·092 |
| Dry mouth (Ref, No) | 0·10 512 | 0·04102 | 0·02473, 0·18 552 | 0·220 | 0·010 | 1·104 |
| Self-reported need care (Ref, No) | 0·26 623 | 0·08410 | 0·10 140, 0·43 107 | 0·231 | 0·002 | 1·106 |
| Can write the documents yourself (Ref, No) | –0·39 880 | 0·09604 | –0·58 702, –0·21 057 | –0·310 | < 0·001 | 1·087 |
| Pre-formed water | | | $R^2 = 0.623$ (Adjusted $R^2 = 0.568$) | | | |
| Intercept | –0·28 816 | 0·40 740 | –1·08665, 0·51 032 | | 0·479 | |
| Height (cm) | 0·01024 | 0·00328 | 0·00382, 0·01666 | 0·357 | 0·002 | 1·870 |
| Weight (kg) | 0·00588 | 0·00284 | 0·00032, 0·01145 | 0·247 | 0·038 | 1·770 |
| Sleep time (min/d) | –0·00074 | 0·00029 | –0·00131, –0·00017 | –0·238 | 0·011 | 1·122 |
| Sitting time (min/d) | –0·00029 | 0·00011 | –0·00050, –0·00007 | –0·233 | 0·008 | 1·065 |
| Fluid intake from beverages (l/d) | 0·14 856 | 0·06593 | 0·01934, 0·27 777 | 0·204 | 0·024 | 1·136 |
| Denture use (Ref, No) | –0·11 013 | 0·04491 | –0·19 814, –0·02212 | –0·193 | 0·014 | 1·092 |
| Dry mouth (Ref, No) | 0·12 864 | 0·04830 | 0·03397, 0·22 331 | 0·243 | 0·008 | 1·104 |
| Self-reported need care (Ref, No) | 0·29 155 | 0·09518 | 0·10 499, 0·47 811 | 0·229 | 0·002 | 1·106 |
| Can write the documents yourself (Ref, No) | –0·49 041 | 0·10 808 | –0·70 225, –0·27 857 | –0·343 | < 0·001 | 1·087 |

RC, regression coefficient; Ref, reference; SE, standard error; VIF, variance inflation factor.
Information in brackets, reference category or units. Height, body weight, sleep time, sitting time and fluid intake from beverages were modelled as continuous variables.
Positive RC and beta coefficients indicate increased water consumption, while negative coefficients indicate decreased water consumption.

$$\begin{aligned} log\ WT = {} & \beta_0 + \beta_1\ height_1 + \beta_2\ body\ weight_2 \\ & + \beta_3\ sleep\ time_3 + \beta_4\ sitting\ time_4 \\ & + \beta_5\ fluid\ intake\ from\ beverage_5 \\ & + \beta_6\ denture\ use_6(\ 1\ if\ yes, 0\ if\ no) \\ & + \beta_7\ dry\ mouse_7(1\ if\ yes, 0\ if\ no) \\ & + \beta_8\ self\text{-}reported\ need\ care_8(1\ if\ yes, 0\ if\ no) \\ & + \beta_9\ writing\ abilities_9(\ 1\ if\ yes, 0\ if\ no) \end{aligned} \tag{9}$$

where *WT* (l/d) is the WT estimated from the calibration regression equation (Equation 9). The intercept of this equation ($\beta_0$) was –0·18662 l. The coefficients of continuous variables were 0·01002 l (cm), 0·00599 l (kg), –0·00064 l (min/d), –0·00025 l (min/d) and 0·12137 l (l/d) for height ($\beta_1$), weight ($\beta_2$), sleep time ($\beta_3$), sitting time ($\beta_4$) and fluid intake from beverages ($\beta_5$), respectively. The coefficients of the binary variables were –0·08846 l, 0·10512 l, 0·26623 l and –0·39880 l for denture use ($\beta_6$), dry mouth ($\beta_7$), self-reported need care ($\beta_8$) and writing ability ($\beta_9$), respectively. These coefficients were multiplied by the values of the individual's variables (binary variables, 1 or 0; continuous variables, the individual's value). Biomarker-calibrated WT was calculated by exponentially converting the sum of this value and the logarithmic coefficient of the intercept. The coefficient of determination ($R^2$) for this model was 0·652. The biomarker-calibrated PW (l/d) was calculated using Equation (10):

$$\begin{aligned} log\ PW = {} & \beta_0 + \beta_1\ height_1 + \beta_2\ body\ weight_2 \\ & + \beta_3\ sleep\ time_3 + \beta_4\ sitting\ time_4 \\ & + \beta_5\ fluid\ intake\ from\ beverage_5 \\ & + \beta_6\ denture\ use_6(\ 1\ if\ yes, 0\ if\ no) \\ & + \beta_7\ dry\ mouse_7(1\ if\ yes, 0\ if\ no) \\ & + \beta_8\ self\text{-}reported\ need\ care_8(1\ if\ yes, 0\ if\ no) \\ & + \beta_9\ writing\ abilities_9(\ 1\ if\ yes, 0\ if\ no) \end{aligned} \tag{10}$$

The intercept ($\beta_0$) in this equation was –0·28 816 l. The coefficients of continuous variables were 0·01024 l (cm), 0·00588 l (kg), –0·00074 l (min/d), –0·00029 l (min/d) and 0·14 856 l (kcal/d) for height ($\beta_1$), weight ($\beta_2$), sleep time ($\beta_3$), sitting time ($\beta_4$) and fluid intake from beverages ($\beta_5$), respectively. The coefficients of the binary variables were –0·11 013 l, 0·12 864 l, 0·29 155 l and –0·49 041 l for denture use ($\beta_6$), dry mouth ($\beta_7$), self-reported need care ($\beta_8$) and writing ability ($\beta_9$), respectively. These coefficients were multiplied by the values of the individual's variable (binary variables, 1 (yes) or 0 (no); continuous variables: the individual's value). Biomarker-calibrated PW consumption

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was calculated by exponentially converting the sum of this value and the logarithmic coefficient of the intercept. The coefficient of determination ($R^2$) for this model was 0·623.

### *Validation of the developed biomarker-calibrated water consumption equation*

Table 4 compares water consumption estimated using the DLW method and the regression equation that was developed. In the validation cohort, the WT (median difference = 0·20 l; interquartile range: –0·25, 0·55) and PW (median difference = 0·18 l; interquartile range: –0·23, 0·49) estimates from the regression equation were not significantly different when compared with those obtained using the DLW method. WT (Spearman's: $r = 0·527$; Pearson's: $r = 0·530$) and PW (Spearman's: $r = 0·477$; Pearson's: $r = 0·484$) estimated using the regression equation exhibited significant positive correlations with the values measured by the DLW method. In contrast, the PW estimates from FFQ were underestimated by ~50 % compared with the DLW measurements and had a low estimation accuracy (Spearman's: $r = 0·163$; Pearson's: $r = 0·131$) (Tables 5). In addition, the Meng's Z-test comparison revealed a significant difference in the correlation coefficient between PW estimated from FFQ and the regression equation, against those estimated using the DLW method (difference of Spearman's rank correlation coefficient = 0·314; 95 % CI: 0·046, 0·663, *P*-value = 0·024).

## Discussion

This study showed a methodological approach of calibrating the self-reported dietary intake data using biomarkers of water consumption. As far as we know, this was the first study to develop and confirm the validation of an equation for predicting water consumption measured using the DLW method.

The calibrated regression approach has been used in previous studies on the intake of energy[19–21,34], protein[19,34], fats[35], carbohydrates[35], salt[36], potassium[36] and vitamins[37]. These calibrated regression equations have been included with age[19,20,34,36,37], sex[20], physique[19,20,34–37], ethnicity[19,34–37], dietary intake estimated using FFQ[19,20,34,36], income[19,36], smoking[19,36], use of dietary supplements[19,37], physical activity levels[19,37], educational history[19,35–37] and blood and urine biomarkers[35,37]. Our developed regression equation included similar significant variables. Furthermore, in calibration equations created with other nutrients, the median coefficient of determination in equations that only included self-reported items was 0·270 (range: 0·087–0·417)[19,20,34,36], while in equations that included both blood biomarkers and self-reported items, it was 0·497 (range: 0·270–0·689)[35,37]. The coefficient of determination of the regression equation developed in the present study was higher than that in previous studies that only considered self-reported items and was comparable to those of previous studies that used both self-reported items and biomarkers. Logarithmic plots of WT and body mass in humans and other mammals are nearly linear[3], and WT in humans (both men and women) is almost the same as in ungulates that have a similar body mass as humans[3]. Self-reported height and weight estimates have previously been reported to be sufficiently accurate and reproducible as data in this population, compared with objective values[27]. These points may partially explain why the regression equation for WT had a high coefficient of determination despite only using self-reported variables.

In environments with high external temperatures, there is an increased fluid loss due to sweating[1,12], which raises daily water requirements. Because the regression equation that was developed did not consider the influence of seasonal fluctuations in factors, such as temperature and humidity on WT, it cannot be used to evaluate acute water requirements owing to temperature changes. To resolve this problem, further research on the DLW method is required to assess WT in different seasons with different temperatures and humidity values from repeated-measures analysis for the same individuals. If temperature and humidity are higher than when the equation was developed (mean temperature 20·1°C/d), WT estimates from the calibration equation may underestimate the mean value of the population. However, the average annual temperature in Kyoto Prefecture, Japan, where the participants of the present study lived, is ~16°C (~15°C for Japan, overall)[38], and temperatures in spring, which was the season used to create the regression equation, are closer to the average annual value than those in other seasons, suggesting that the spring measurements may better reflect habitual WT.

Previously, prospective cohort studies did not yield consistent results on the association between water intake and total mortality risk in adults[39–42]. A meta-analysis using data from these cohort studies also showed no significant association between total water intake and total mortality risk, indicating a high degree of heterogeneity between the results of the studies included in the analysis[43]. Reasons for this could include differences in the statistical models or the covariates included in the analyses[39], although another reason could be differences in the accuracy of dietary survey results. The dietary assessment methods relying on self-reported data used in these studies are impacted by systematic errors related to individual characteristics[16,17]. Our estimates of PW using FFQ had low accuracy. This may be related to a systematic reporting bias, as questionnaire responses can be modified in the desired direction without any change in actual behaviour[44]. Therefore, accurate evaluations of associations with diseases using water intake estimates from self-reported dietary surveys is difficult. We plan to apply the

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**Table 4** Validation of water consumption estimated using developed calibrated-water consumption equation against water consumption measured using the doubly labelled water method

| | DLW | | Equation | | Median difference* DLW *v.* Equation | | | Correlation coefficient† (*r*) | |
|---|---|---|---|---|---|---|---|---|---|
| | Median | IQR | Median | IQR | Median | IQR | Relative (%) | Spearman's | Pearson's |
| Water turnover (l/d) | | | | | | | | | |
| Total (*n* 70) | 2·72 | 2·35–3·16 | 2·85 | 2·58–3·28 | 0·20 | –0·25–0·55 | 7·3 | 0·527* | 0·530* |
| Sex | | | | | | | | | |
| Women (*n* 36) | 2·46 | 2·17–2·72 | 2·68 | 2·40–2·88 | 0·23 | –0·24–0·60 | 9·5 | 0·019 | 0·228 |
| Men (*n* 34) | 3·05 | 2·73–3·47 | 3·23 | 2·84–3·62 | 0·09 | –0·26–0·55 | 3·3 | 0·504* | 0·432* |
| Age (years) | | | | | | | | | |
| 65–74 (*n* 44) | 2·82 | 2·35–3·26 | 2·96 | 2·59–3·37 | 0·16 | –0·29–0·52 | 5·7 | 0·558* | 0·523* |
| ≥ 75 (*n* 26) | 2·61 | 2·24–2·85 | 2·77 | 2·54–3·02 | 0·22 | –0·11–0·73 | 9·3 | 0·314 | 0·483* |
| BMI (kg/m$^2$) | | | | | | | | | |
| < 18·5 (*n* 8) | 2·23 | 1·98–2·94 | 2·52 | 2·32–3·06 | 0·25 | –0·08–0·32 | 10·0 | 0·429 | 0·413 |
| 18·5–24·9 (*n* 44) | 2·69 | 2·38–3·07 | 2·87 | 2·62–3·18 | 0·18 | –0·26–0·52 | 6·6 | 0·540* | 0·540* |
| ≥ 25 (*n* 18) | 2·92 | 2·58–3·39 | 3·29 | 2·68–3·67 | 0·27 | –0·31–0·91 | 8·3 | 0·414 | 0·454* |
| Pre-formed water (l/d) | | | | | | | | | |
| Total (*n* 70) | 2·19 | 1·93–2·62 | 2·35 | 2·08–2·73 | 0·18 | –0·23–0·49 | 7·6 | 0·477* | 0·484* |
| Sex | | | | | | | | | |
| Women (*n* 36) | 1·99 | 1·72–2·18 | 2·20 | 1·94–2·39 | 0·23 | –0·22–0·49 | 11·1 | 0·002 | 0·217 |
| Men (*n* 34) | 2·50 | 2·21–2·94 | 2·69 | 2·29–2·98 | 0·08 | –0·26–0·50 | 3·6 | 0·462* | 0·394* |
| Age (years) | | | | | | | | | |
| 65–74 (*n* 44) | 2·34 | 1·94–2·67 | 2·44 | 2·08–2·82 | 0·13 | –0·27–0·45 | 5·6 | 0·497* | 0·459* |
| ≥ 75 (*n* 26) | 2·14 | 1·78–2·42 | 2·28 | 2·07–2·48 | 0·22 | –0·18–0·60 | 9·7 | 0·269 | 0·482* |
| BMI (kg/m$^2$) | | | | | | | | | |
| < 18·5 (*n* 8) | 1·82 | 1·58–2·39 | 2·05 | 1·90–2·58 | 0·24 | –0·14–0·30 | 12·6 | 0·286 | 0·299 |
| 18·5–24·9 (*n* 44) | 2·18 | 1·94–2·60 | 2·36 | 2·09–2·64 | 0·15 | –0·24–0·45 | 6·3 | 0·501* | 0·496* |
| ≥ 25 (*n* 18) | 2·40 | 2·06–2·86 | 2·70 | 2·18–3·08 | 0·22 | –0·26–0·77 | 8·7 | 0·360 | 0·427* |

DLW, doubly labelled water; IQR, interquartile range.
*The values are shown as absolute median difference (IQR) and relative difference. Statistical analysis for absolute median difference was used by a Wilcoxon signed-rank test and asterisk marks indicates statistical significance ($P < 0·05$).
†The variables are shown as Spearman's and Pearson's rank correlation coefficient and asterisk marks indicates statistical significance ($P < 0·05$).

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**Table 5** Validation of pre-formed water estimated using FFQ against pre-formed water measured using the doubly labelled water method

| | DLW | | FFQ | | Median difference* | | | Correlation Coefficient† | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | DLW *v.* FFQ | | | | |
| Pre-formed water (l/d) | Median | IQR | Median | IQR | Median | IQR | Relative (%) | Spearman's *r* | Pearson's *r* |
| Total (*n* 70) | 2·19 | 1·93–2·62 | 1·24 | 0·94–1·53 | −1·07 | −1·42 – −0·65* | −46·1 | 0·163 | 0·131 |
| Sex | | | | | | | | | |
| Women (*n* 36) | 1·99 | 1·72–2·18 | 1·16 | 0·75–1·54 | −0·96 | −1·29 – −0·43* | −46·1 | 0·113 | 0·118 |
| Men (*n* 34) | 2·50 | 2·21–2·94 | 1·33 | 1·10–1·54 | −1·14 | −1·63 – −0·71* | −46·5 | −0·007 | −0·115 |
| Age (years) | | | | | | | | | |
| 65–74 (*n* 44) | 2·34 | 1·94, 2·67 | 1·16 | 0·84, 1·49 | −1·18 | −1·65 – −0·79* | −53·5 | 0·246 | 0·202 |
| ≥ 75 (*n* 26) | 2·14 | 1·78, 2·42 | 1·44 | 1·19, 1·59 | −0·75 | −1·09 – −0·27* | −32·7 | 0·279 | 0·229 |
| BMI (kg/m$^2$) | | | | | | | | | |
| < 18·5 (*n* 8) | 1·82 | 1·58, 2·39 | 1·22 | 0·73, 1·41 | −0·84 | −1·23 – −0·43* | −42·2 | 0·286 | 0·411 |
| 18·5–24·9 (*n* 44) | 2·18 | 1·94, 2·60 | 1·21 | 0·90, 1·49 | −1·08 | −1·45 – −0·69* | −49·1 | 0·189 | 0·137 |
| ≥ 25 (*n* 18) | 2·40 | 2·06, 2·86 | 1·49 | 1·04, 1·75 | −0·99 | −1·50 – −0·43* | −39·4 | 0·001 | −0·056 |

DLW, doubly labelled water; IQR, interquartile range.
*The values are shown as absolute median difference (IQR) and relative difference. Statistical analysis for absolute median difference was used by a Wilcoxon signed-rank test and asterisk marks indicates statistical significance ($P < 0·05$).
†The variables are shown as Spearman's and Pearson's rank correlation coefficient and asterisk marks indicates statistical significance ($P < 0·05$).

biomarker-calibrated water consumption estimates from our developed equation to the diet–disease analysis in the Kyoto–Kameoka study. In contrast to costly methods such as DLW method, this approach can calculate water consumption using data from existing cohort studies and may improve statistical power for verifying the associations between diet and disease. It has the potential to provide accurate water consumption targets that can be used while creating guidelines applicable to public health and clinical nutrition aimed at disease prevention.

The main strength of the present study is not merely that an equation was developed to predict water consumption using the DLW method, but that we confirmed the validity of developed equations for WT and PW. These data were essential for confirming the accuracy of the regression equation that was developed; the water consumption estimates from the regression equation had high validity values. However, our research has certain methodological limitations. First, we were unable to evaluate objective indicators of body fluid status in the population, such as serum and urine osmotic pressure and 24-h urine volume[45]. Water consumption measured using the DLW method may contain systematic errors if some of the participants had unstable body fluid status. For TEE measured using the DLW method, all participants were assumed to have an excellent nutritional balance. As there is no guarantee all participants had a perfect nutritional balance, the TEE values may have contained systematic errors. Second, the participants of the present study were only those who agreed to take part in the physical check-up examinations in the Kyoto–Kameoka study. They may have been more health-conscious than those who did not participate. To verify the external validity of this calibration equation, further research on other populations that were not part of this study is needed. Third, the equation that was developed may have contained systematic errors from the use of self-reporting data from mail-in surveys. Our developed equation for predicting water consumption did not include physical activity, which was included in previous studies[12], possibly because we used self-reporting data. In addition, there was approximately 3 (February 14, 2012 (additional survey)) or 10 (July 29, 2011 (baseline survey)) month interval between the measurement of water consumption using the DLW method and the survey with FFQ and other questionnaires. Finally, to develop an equation to predict water consumption measured using the DLW method, all items from the questionnaire obtained from the Kyoto–Kameoka study were included in the analysis. This equation was not evaluated in the Kyoto–Kameoka study and other covariates that may be related to water consumption may have not been considered. This could be the reason for the coefficient of determination ($R^2$) being only moderate. These limitations may hinder the generalisation of the results. Therefore, to determine

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whether the coefficient of determination for the equation to predict WT would increase by including more questionnaire items and objective indicators such physical activity, further validation is needed through a well-designed study that assesses each participant's body fluid status in a larger randomised sample. Because we developed an equation for predicting WT in older people aged 65 years or older, further validation studies are needed to determine whether this equation can be used in people aged under 65 years.

### *Conclusions*

We developed an equation to predict WT and PW measured using the DLW method. Although the water consumption estimates from this equation had high validity compared with measurements from the DLW method, the uncalibrated, PW estimates from FFQ were less accurate. However, using biomarkers to calibrate self-reported estimated dietary intake can partially solve the problems with systematic errors that have hindered nutritional epidemiological studies for decades, which could help bridge the knowledge gap in the relationship between diet and disease.


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## Conflicts of Interest

There are no conflicts of interest.

## Authorship

The authors' contributions are as follows: D. W., T. Y. and Y. Y. formulated the research questions and designed the study; T. Y., A. I., K. I-T., N. E., Y. H., M. K. and Y. Y. obtained the data; D. W., H. N. and C. G. analysed the data; D. W., M. M. and Y. Y. drafted the manuscript; T. Y., H. N., N. E., Y. H., M. M. and Y. Y. provided critical feedback; D. W. had primary responsibility for final contents; and all authors read and approved the final manuscript.


## Ethics of human subject participation

This study was conducted according to the guidelines laid down in the 1964 Declaration of Helsinki and all procedures involving research study participants were approved by the Research Ethics Committee of Kyoto Prefectural University of Medicine (RBMR-E-363), the National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN-76-2) and Kyoto University of Advanced Science (No. 20-1). Informed consent in writing was obtained from all participants before data collection.

## Supplementary material

For supplementary material accompanying this paper visit https://doi.org/10.1017/S1368980024001587

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