

Validity of a selective recall method for assessing water intake and its relationship with hydration status

T. UCHIDA¹, Y. NAKAMURA¹, H. TANAKA², S. NAKAMURA^{2,3},
T. OKAMURA⁴, H. WATANABE¹, N. MURAYAMA¹

¹Suntory Global Innovation Center Limited, Research Institute, Seika-cho, Soraku-gun, Kyoto, Japan

²Graduate School of Science and Technology, Nara Institute of Science and Technology, Ikoma, Japan

³Data Science Center, Nara Institute of Science and Technology, Ikoma, Nara, Japan

⁴Department of Preventive Medicine and Public Health, Keio University School of Medicine, Shinjuku-ku, Tokyo, Japan

Tomoki Uchida and Yumi Nakamura contributed equally to this work

Abstract. – OBJECTIVE: We previously established a descriptive dietary record method that accurately quantifies habitual water intake from food and beverages, to ascertain the relationship between water intake and health. Here, we verified the validity of a selective recall method, which is easy for users to answer and analyze.

PATIENTS AND METHODS: Japanese men and women aged 20-44 years ($n = 16$) and 45-64 years ($n = 16$) participated over three working days and one non-working day. The day following each of the surveyed days, participants collected their first morning urine for urinalysis and completed a selective recall and descriptive dietary record questionnaire.

RESULTS: The two methods of determining water intake were positively correlated ($r = 0.94$, $p < 0.0001$). Water intake volumes from non-alcoholic beverages ($r = 0.94$, $p < 0.0001$), alcoholic beverages ($r = 1.00$, $p < 0.0001$), and food ($r = 0.72$, $p < 0.0001$), calculated using the two methods, exhibited strong correlation. No correlation was observed between urinalysis parameters and total water intake. A significant, negative correlation was observed between urine osmolality and total water intake in men ($r = -0.55$, $p = 0.0011$) and women ($r = -0.51$, $p = 0.0032$) aged 20-44 years.

CONCLUSIONS: Selective recall is a valid method for assessing water intake from food and beverages.

Key Words:

Water intake, Hydration, Water intake assessment, Selective recall method, Urinalysis.

Introduction

Water constitutes 60% of the human body and it is a vital component of life¹. It is essential for maintaining homeostasis; it acts as a medium for the delivery of oxygen, nutrients, hormones, and other substances throughout the body. It is also necessary for the removal of waste products and excess substances². Water is the major component of intracellular and extracellular fluids, such as blood plasma and interstitial fluid. It takes part in biochemical reactions inside the cells and contributes to temperature regulation³. There is an association between water intake and health. Inadequate water intake increases the risk of renal and cardiovascular diseases and contributes to metabolic diseases⁴⁻⁶; in addition, it affects mood and cognitive function⁷⁻⁹. Therefore, maintaining an adequate intake of water is important for reducing the risk of disease and for maintaining daily mental health. In a previous randomized trial, we examined the effect of increasing water uptake to 1.1 L/day for 12 weeks, in healthy Japanese adults. This resulted in decreased systolic blood pressure, increased basal body temperature, reduced blood urea nitrogen concentration, suppressed reduction of the glomerular filtration rate, and changes in the intestinal microbiome¹⁰.

Some countries and organizations in Europe and the USA have defined water as a nutrient and, they have established adequate intake (AI) levels¹¹. The European Food Safety Authority

defines AI as 2.0 L/day for women and 2.5 L/day for men¹². The US Institute of Medicine defines AI as 3.7 L/day for men and 2.7 L/day for women¹¹. In contrast, Japan does not define water as a nutrient, and it has not yet determined AI levels¹³. One of the major reasons for this difference is the insufficient amount of research on water intake in Japanese people, which is required for accurately calculating the AI levels. There is only one report addressing water intake, including food-derived water, in Japanese people¹⁴. The data on water intake from other countries cannot be applied to the Japanese population, because of the unique dietary habits in Japan compared with that in the other countries. Traditional Japanese food consists of cooked rice, soup, and three side dishes, including a main dish of meat or fish and two other dishes of fresh vegetables or simmered food. Compared to the people in Europe and the USA, Japanese people are believed to obtain more water from food, such as cooked rice, soup, and vegetables. Food sources account for 50% of the water intake in Japan¹⁴, while beverages account for the other half. Comparatively, only 20% of water intake in Europe and the USA is derived from food, while 80% is obtained from beverages¹². Additionally, the differences in the physical composition of Japanese people, compared with that of European or American individuals, prevent the direct application of AI data from other countries to the Japanese population.

Dietary surveys can determine nutrient consumption; however, there is no established method for ascertaining water intake, in particular, water intake from food. In our previous study¹⁵, we established the water conversion factor for cooking (WCFC), which accounts for the effect of food preparation on food moisture content. We developed an approach for calculating the total water intake from all foods and beverages; this applies the WCFC to information obtained via the descriptive dietary record method. We used this approach to obtain an accurate understanding of water intake in a clinical study in Japanese people¹⁰. However, the descriptive dietary record method is not well suited to large-scale epidemiological studies, because collecting participant responses and interpreting data are time-consuming processes^{16,17}. Therefore, we have developed the selective recall method as a less time-intensive strategy for calculating water intake. This study analyzed the correlation between the water intake calculated using descriptive diet recording and the selective recall methods in Japanese peo-

ple. We performed general urinalysis to detect the representative indicators of hydration status. The indicators were subsequently used to explore the relationship between water intake and hydration status.

Patients and Methods

Study Design

The schedule for this observational cross-sectional study was as follows: participants were recruited from July 3 to July 10, 2020. An online briefing session was held, and free and voluntary consent was obtained from each participant. The study was conducted over four days, including three working days and one non-working day within the study period, from July 27 to August 2.

Participants

Participant recruitment was limited to employees working at the Suntory World Research Center (Kyoto, Japan). Those who met the selection criteria, based on a self-report, were enrolled. The selection criteria were as follows: (1) men and women aged ≥ 20 to < 65 years and (2) those who read, understood, agreed with, and consented to the explanation of the study in advance. Thirty-two participants, comprising eight men aged ≥ 20 to < 45 years (young men), eight women aged ≥ 20 to < 45 years (young women), eight men aged ≥ 46 to < 65 years (middle-aged men), and eight women aged ≥ 46 to < 65 years (middle-aged women), were included (Figure 1). Exclusion criteria were not applied in this study. The rationales for the sample size are as follows¹⁸: the number of cases required to achieve a correlation coefficient of 0.7 at power = 0.85 and $\alpha = 0.01$ is 21.

Survey Implementation

Participants were assigned any four days within the study period, including three working days and one non-working day, as the survey days. On the day after each survey date, they were asked to (1) collect first morning urine, (2) answer the selective recall method questionnaire, (3) fill in the descriptive dietary record method questionnaire, and (4) answer the other questionnaires on daily life and background information. Answers to the questionnaire were conducted in the order listed above. The selective recall method questionnaire was submitted in Google forms, and once submit-

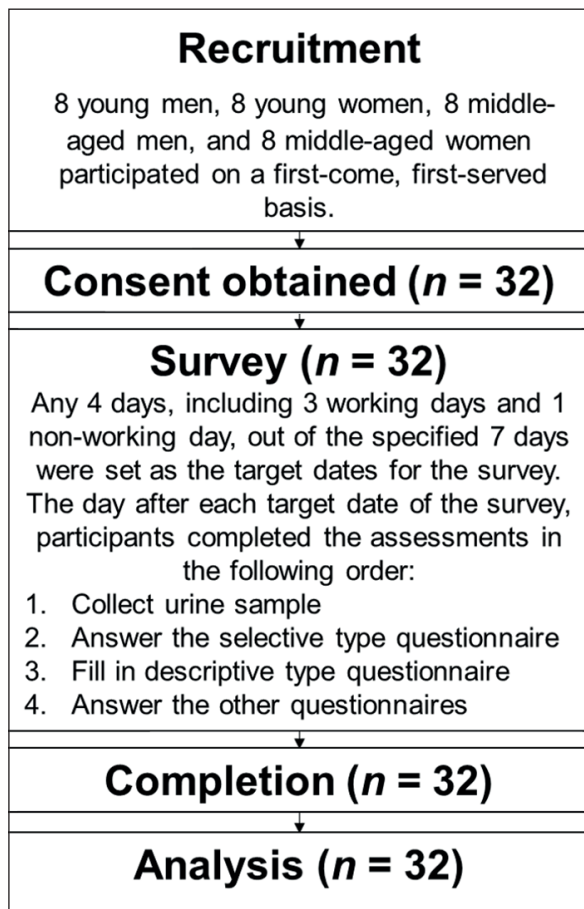


Figure 1. Flowchart of participant recruitment and study.

ted, it could not be corrected by the participants. The two methods were performed on the same day consecutively to minimize recall bias and compare water intake calculated by each method.

Study Outcomes

Water Intake Calculated from a Selective Recall Method

On the day after each survey day, participants were asked to recall their intake of beverages, alcoholic drinks, and food consumption on the previous day. They submitted their responses through Google forms.

The questionnaire for beverages accounted for the following items: water, tea, coffee, milk beverage, and other beverages. The intake from (1) waking up to breakfast, (2) at breakfast, (3) from breakfast to lunch, (4) at lunch, (5) from lunch to dinner, (6) at dinner, (7) from dinner to 30 min before bedtime, (8) 30 min before bedtime, and

(9) from bedtime to waking up, was recorded. The amount consumed was selected from five options: approximately 100 mL, 200 mL, 350 mL, 500 mL, and more than 700 mL. Participants did not provide an answer to this question if they did not drink any beverages.

For alcoholic drinks, the participants chose between the same five volume options for beer, whiskey, sake, shochu, wine, chuhai (a fruit-flavored carbonated alcoholic drink) or sour, and other alcoholic drinks. Participants did not provide an answer to this question if they did not drink any alcoholic beverages. The amounts were set at levels that are easy to understand, considering the volume of commercial beverage products and cups. The classification and approximate volume of each beverage was indicated at the beginning of the questionnaire to make it easier for the participants to track their intake.

For meals, the amount of 40 frequently consumed items from approximately 2.3 million dishes, reported by ~28,000 people in the Japanese application FoodLog (foo.log Inc., Tokyo, Japan), was selected for (1) breakfast, (2) lunch, (3) dinner, and (4) nighttime/snacking. Intake options were: 0.5, 1, 1.5, and more than 2 servings. The 40 items are shown in [Supplementary Table I](#).

The data were multiplied by the WCFC¹⁵ for each beverage, alcoholic beverage, and meal ([Supplementary Table I](#)) to calculate the daily water intake. Water content per 100 g non-alcoholic beverages and alcoholic beverages was taken obtained the Standard Tables of Food Composition in Japan 2010 (Sixth Revision). The water content of each cooked dish was calculated by multiplying the water content of the uncooked ingredients by a cooking factor¹⁹. Water metabolism was included in the water content of food. It was calculated using a standard method, based on the amount of carbohydrates (g), protein (g), and fat (g) in the food²⁰. Based on the water content of 521 dishes, 40 food categories were defined in terms of water content, cooking method, and serving size. The median water content of the dishes in a given food category was used as the water content for that category.

Water Intake Calculated from a Questionnaire Using a Descriptive Dietary Record Method

The day after each survey day, participants filled out a paper-based descriptive dietary record method questionnaire for food and drink

consumed on the previous day (**Supplementary Figure 1**). A dietitian inputted the data from the completed form into the original nutritional calculation software (foo.log.Inc.). The data were multiplied by the WCFC¹⁵ for each food and drink to calculate the daily water intake.

Urinalysis

Osmolarity, and the concentration of aquaporin 2 (AQP2), creatinine (Cre), sodium, and potassium, in the first morning urine collected on the day following each survey day was measured. Osmolarity was measured using the freezing point depression method; AQP2, using the enzyme-linked immunosorbent assay (Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan); Cre, using the enzymatic method; and sodium and potassium, using the electrode method. The measurements were carried out by LSI Medience Co. (Tokyo, Japan). Based on the measured values, AQP2 was evaluated following Cre correction (AQP2/Cre). Sodium excretion was calculated using the following Tanaka method formula²¹.

24 h urinary sodium excretion (g/day) = $21.98 \times [\text{urine sodium concentration (mEq/L)/urine creatinine concentration (mg/dL)}] / 10 \times \text{predicted 24 h urine creatinine excretion}^{*1}]^{0.392}$

*¹Predicted 24 h urinary creatinine excretion (mg/day): $\text{Weight (kg)} \times 14.89 + \text{height (cm)} \times 16.14 - \text{age} \times 2.04 - 2244.45$

Other Questionnaires

Daily activities, such as the number of toilet visits on the day of the study, were assessed based on the answers provided in the daily life questionnaire, completed on the day following each day of the survey. Participants also self-reported their gender, age, height, and weight on a background information form. Body mass index (BMI) was calculated using the formula $\text{BMI} = \text{weight (kg)} / (\text{height (m)})^2$.

Statistical Analysis

The values obtained are presented as mean $\pm$ standard deviation (SD). Correlation analysis was performed using simple linear regression analysis, for the water intake values calculated from the selective recall method and the descriptive dietary record method. Water intake for validation was averaged over 4 days. Daily values of water intake from both methods were used for correlation analysis with the urine test values. The Pearson's correlation test was used to calculate the relationships between the data. Williams's test was applied for

the difference between two dependent correlations that share one variable. Data aggregation and statistical analysis were performed using Microsoft Excel 2013, JMP ver. 14.0.0 (SAS Institute Inc., Cary, NC, USA), and R ver. 4.0.1 (R Foundation for Statistical Computing, Vienna, Austria).

Ethical Standards Disclosure

This study was conducted according to the guidelines laid down in the Helsinki (revised by the Fortaleza General Meeting of the World Medical Association), and all procedures involving human participants were approved by the ethics committee of Suntory Holdings Limited (IRB No.15000139). This study is also in compliance with the Ethical Guidelines for Medical Research Involving Human Subjects (2014 Ministry of Education, Culture, Sports, Science and Technology and the Ministry of Health, Labour and Welfare Ministerial notification No.3). Written informed consent was obtained from all participants.

Results

Study Population

All participants completed the study. The baseline characteristics and survey results are shown in Table I. The mean number of night-time toilet visits was 0.1 times per night (Table I).

Correlation between Water Intake Calculated using the Descriptive Dietary Record Method and the Selective Recall Method

A strong positive correlation was observed between the total water intake calculated using the descriptive dietary record method and that calculated by the selective recall method ($r = 0.94$; $p < 0.0001$; linear regression, $y = 1.20x - 568$; Figure 2a). In addition, the water intake from non-alcoholic beverages ($r = 0.94$; $p < 0.0001$; linear regression, $y = 1.20x - 407$; Figure 2b), alcoholic beverages ($r = 1.00$; $p < 0.0001$; linear regression, $y = 1.05x - 7.66$; Figure 2c), and food ($r = 0.72$; $p < 0.0001$; linear regression, $y = 0.90x + 166$; Figure 2d) recorded using the descriptive dietary record method and the selective recall method exhibited a positive correlation.

Correlation between Urine Osmolarity and Water Intake

It was confirmed whether the correlation between urinary osmolality and water intake could

Table 1. Baseline characteristics for all participants.

Parameter	All aged ≥ 20 to < 65 years	Young men aged ≥ 20 to < 45 years	Young women aged ≥ 20 to < 45 years	Middle-aged men aged ≥ 46 to < 65 years	Middle-aged women aged ≥ 46 to < 65 years
Baseline characteristic					
Age	42.1 (12.6)	33.3 (6.1)	28.1 (2.9)	55.4 (5.5)	51.6 (4.0)
Height (cm)	167.1 (8.1)	172.4 (3.8)	160.5 (6.1)	174.3 (5.4)	161.3 (5.0)
Weight (kg)	59.3 (9.8)	63.1 (8.9)	50.0 (4.9)	69.4 (6.3)	54.8 (4.7)
BMI	21.1 (2.2)	21.2 (2.7)	19.4 (1.3)	22.8 (1.4)	21.1 (1.7)
Water intake (descriptive type)					
Total (mL/day)	2970 (1059)	3534 (1488)	2513 (798)	3312 (968)	2520 (459)
From non-alcoholic beverages (mL/day)	1775 (912)	2501 (1375)	1581 (667)	1617 (557)	1401 (429)
From food (mL/day)	1032 (329)	989 (183)	830 (216)	1251 (420)	1060 (305)
From alcoholic beverages (mL/day)	162 (289)	44 (92)	103 (225)	444 (442)	59 (103)
Water intake (selective type)					
Total (mL/day)	2949 (828)	3474 (1022)	2647 (772)	3102 (824)	2576 (334)
from non-alcoholic beverages (mL/day)	1825 (720)	2413 (936)	1670 (703)	1667 (510)	1549 (360)
from food (mL/day)	963 (256)	1010 (252)	870 (237)	1006 (240)	968 (312)
from alcoholic beverages (mL/day)	161 (274)	51 (93)	107 (198)	428 (403)	60 (106)
Urinalysis					
Urine Osmolarity (mOsm/kg)	604 (247)	610 (236)	649 (270)	663 (226)	493 (227)
Urine Na (mEq/L)	102.4 (54.2)	105.4 (59.7)	110.3 (48.5)	118.3 (56.1)	75.4 (43.6)
Urine K (mEq/L)	28.9 (16.4)	28.8 (16.7)	27.8 (18.2)	30.4 (13.6)	28.4 (17.3)
Urine Creatinine (mg/dL)	133.6 (64.9)	153.2 (67.3)	143.7 (74.6)	141.0 (50.0)	96.7 (52.1)
Urine AQP (ng/mL)	5.01 (5.45)	4.24 (4.29)	6.14 (6.82)	6.74 (6.36)	2.92 (2.65)
Urine AQP2/Cre	0.03 (0.02)	0.02 (0.02)	0.04 (0.02)	0.04 (0.03)	0.03 (0.01)
Urine Na/K	4.1 (2.1)	4.5 (2.4)	4.9 (2.3)	4.1 (1.8)	2.9 (1.1)
Na excretion (mEq/day)	131.1 (26.0)	132.3 (28.1)	125.4 (25.0)	142.7 (25.0)	124.1 (22.5)
Toilet visits					
Day time (times/day)	6.6 (1.6)	7.1 (1.5)	6.5 (1.3)	6.5 (1.3)	6.5 (2.0)
Nighttime (times/day)	0.1 (0.3)	0.1 (0.3)	0.0 (0.0)	0.2 (0.4)	0.2 (0.4)

Data are given as mean (±SD).

be evaluated in the same way using either of the two methods. The correlation coefficient between urine osmolality and water intake calculated using the selective recall method was -0.27 (Figure 3a), while that calculated using the descriptive dietary record method was -0.31 (Figure 3b). There was no significant difference in these correlation coefficients ($r = 0.70$). In addition, there was no significant difference in the correlation coefficients based on sex or age group. Analysis of all participants revealed a weak correlation between the total water intake and urine osmolality (Figure 3a, b). Analysis based on sex and age group showed a significant correlation in the values from young men (Figure 3c, d) and young women (Figure 3e, f); however, there was no significant correlation in the values from middle-aged men (Figure 3g, h) and women (Figure 3i, j).

Relationship between Urine Osmolarity and Urine AQP2/Cre

The correlation between AQP2/Cre, an indicator of reabsorption, and urine osmolality was examined to determine the body's responses to hydration status. Analysis of all participants revealed a positive correlation between urine osmolality and urine AQP2/Cre ($r = 0.67$; $p < 0.0001$; Figure 4a). In addition, a positive correlation was observed in each of the four groups (Figure 4b-e).

Discussion

This study verified the validity of using the selective recall method to calculate total water intake. Specifically, the total water intakes,

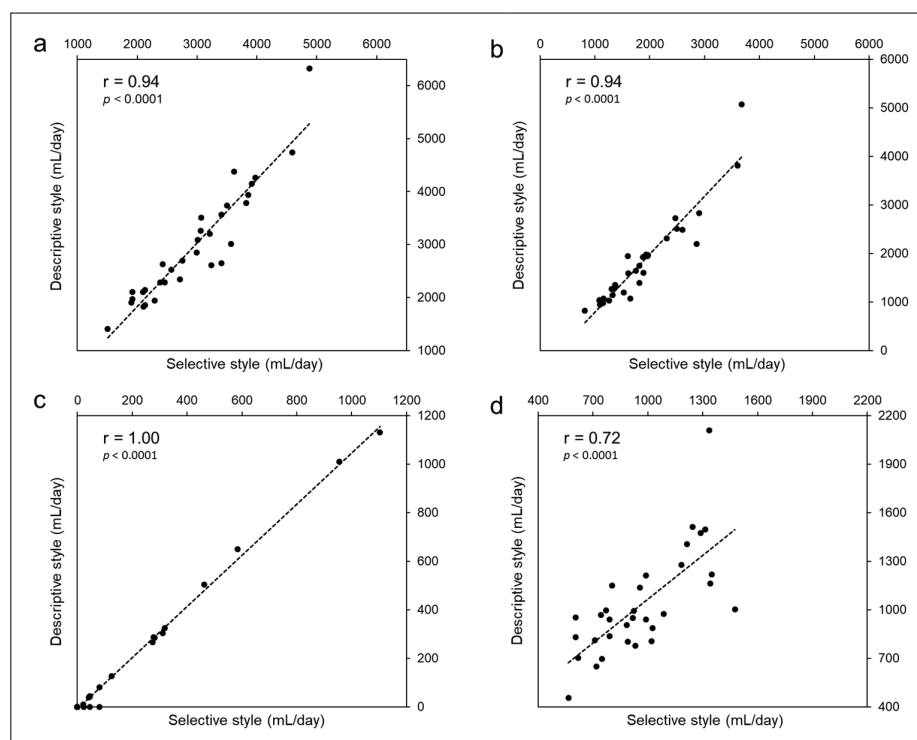


Figure 2. Correlation between total water intake calculated using the descriptive dietary record method and the selective recall method. Correlation for (a) total water intake; (b) water intake from non-alcoholic beverages; (c) water intake from alcoholic beverages; (d) and water intake from food. Data represents mean total water intake over 4 days.

calculated using the descriptive dietary record method and the selective recall method, showed a strong correlation. This demonstrated the validity of the selective recall method. The water intake from non-alcoholic beverages, food, and alcoholic beverages showed a strong correlation between the two survey methods. Therefore, the selective recall method provides a highly accurate understanding of total water intake, for each source of water intake. The descriptive dietary record method documents all food and beverages consumed and it is one of the most accurate diet survey methods^{16,17}. We developed an extremely rigorous technique for verifying water content that involves taking dietary information, collected using the descriptive dietary record method, and multiplying each item by the WCFC¹⁵. However, this method is time and labor-intensive, because the registered dietitian manually enters the paper-based description into the nutritional calculation software. The selective recall method saves time and effort, because the data from the participant is collected online and processed directly. The strong correlation between the values obtained using the descriptive dietary record method and selective recall method indicated that the selective recall method provides a highly accurate understanding of water intake. The correlation between urine

osmolality and water intake was not significantly different between the two methods, and the variability of the scatter plot showed a similar correlation.

Considering that the daily dietary intake is highly variable, validating a method for assessing water intake must involve collecting information on the different foods consumed over several days. Food intake is commonly monitored for 3-7 days²². This study accounted for variation in the diet by surveying food intake over four days consisting of three working days and one non-working day.

The proportion of water intake based on origin was determined using the selective recall method; the ratio of water intake from beverages to that from food was 2.1:1, among all participants. Earlier surveys suggested that the Japanese people derive a greater proportion of their water intake from food than that by people from other countries and that they derive an equivalent proportion of water intake from beverages as from food¹⁴. However, the participants in this study derived a greater proportion of water intake from beverages than from food. The beverage consumption could be higher in this study than expected because the survey was conducted in the summer. This could also be attributed to the characteristic feature of this sample group.

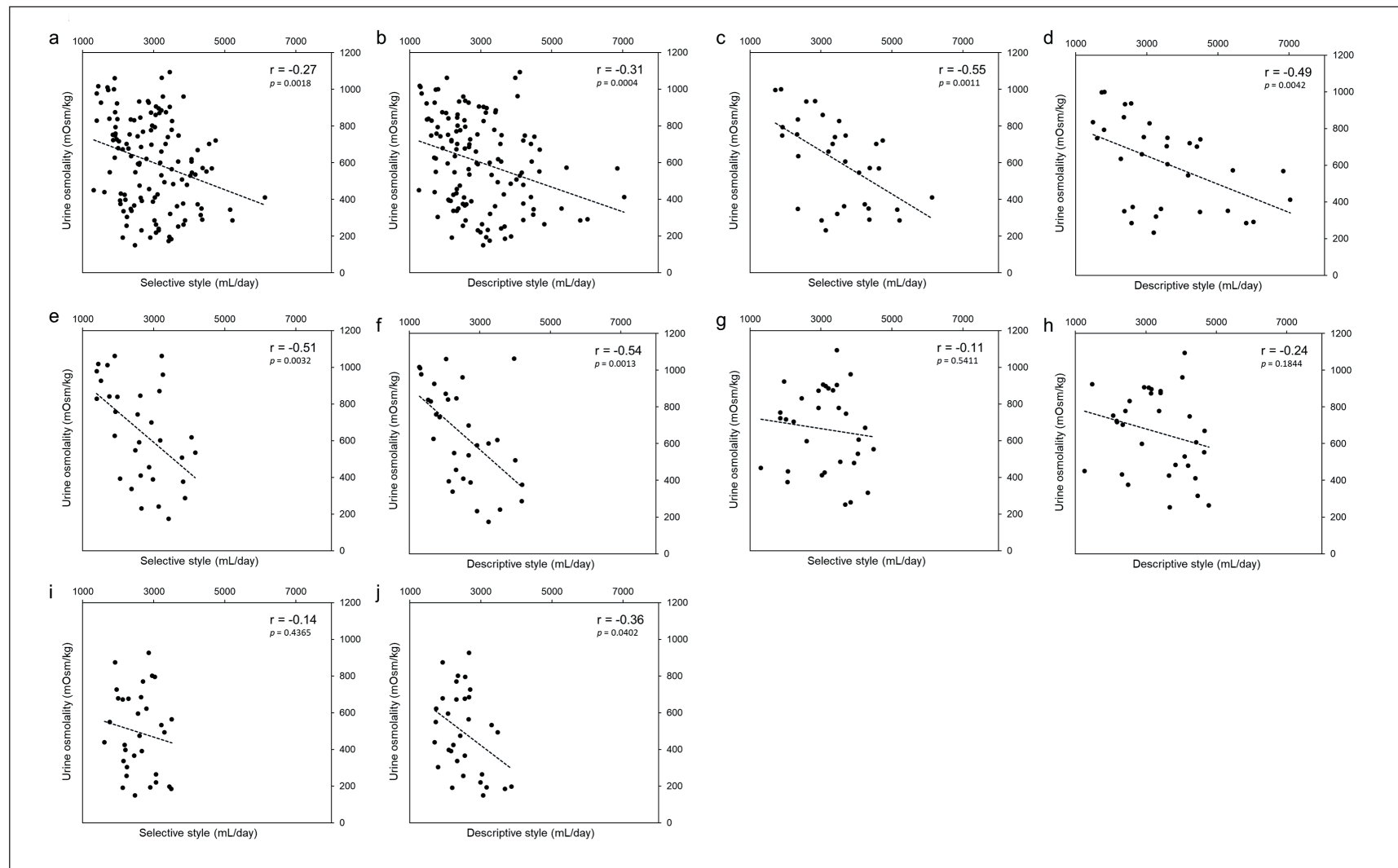


Figure 3. Correlation between urine osmolality and water intake calculated using (a, c, e, g, i) the selective recall method and (b, d, f, h, j) the descriptive dietary record method. Correlation for (a, b) all participants, (c, d) young men, (e, f) young women, (g, h) middle-aged men and, (i, j) middle-aged women. Data represent a single day for each participant group.

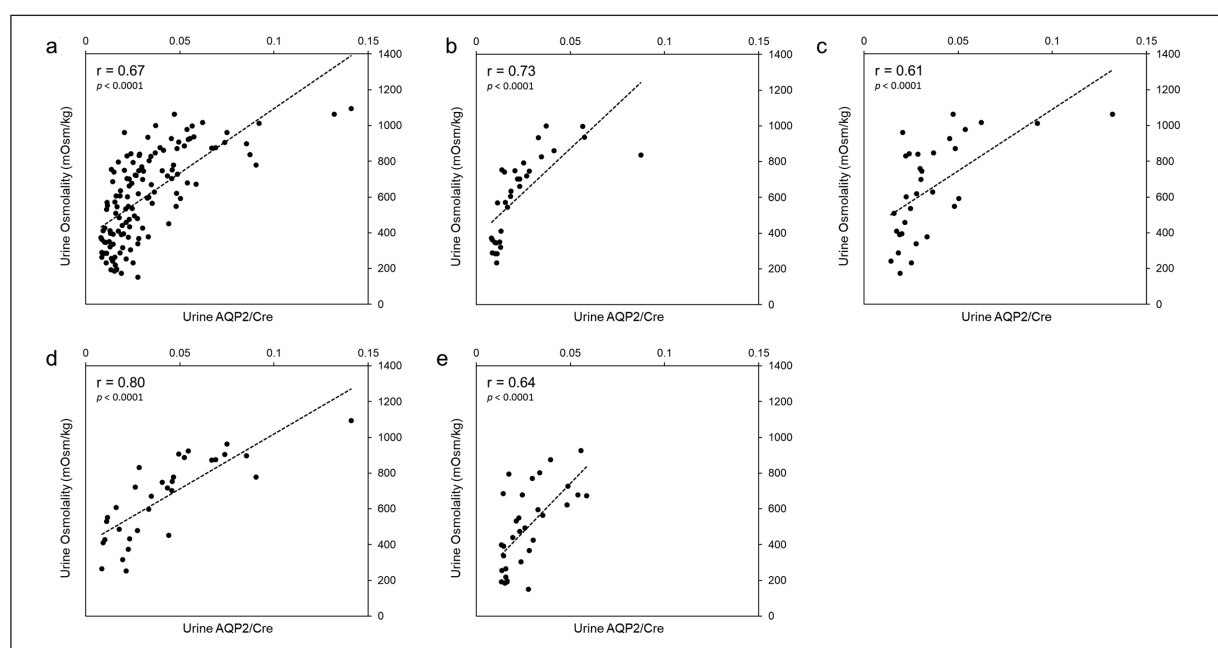


Figure 4. Correlation between urine osmolality and urine aquaporin (AQP2)/creatinine (Cre). Correlation for (a) all participants; (b) young men; (c) young women; (d) middle-aged men; and (e) middle-aged women. Data represent a single day for each participant group.

The 95% confidence limit of osmolality in the first morning urine of young men was 377–1194 mOsm/kg²³; our results were similar. Urine osmolality with euhydration is 818–924 mOsm/kg, and the water intake for that range is 2049–2453 mL/day²³; this is similar to the regression line value of our results. Urine osmolality is higher (924 mOsm/kg) with dehydration²³; however, our results showed that few people were dehydrated, and many were euhydrated to hyperhydrated. Additionally, the correlation between total water intake and urinalysis parameters was analyzed taking into account the sex and age group. Among the young participants, the total water intake correlated with the urinalysis parameters, urine osmolality, and urine AQP2/Cre (Supplementary Figure 2). Urine osmolality is an established indicator of water intake and dehydration status^{24,25}. Among the middle-aged participants, no correlation was observed between the total water intake and urinalysis parameters. Therefore, the body's response to water intake could differ between young and middle-aged adults. This difference could be attributed to the different requirements in terms of water volume and functional differences in renal water regulation between these groups^{26,27}.

Elevated plasma osmolality induces the pituitary gland to secrete vasopressin. This triggers AQP2 translocation to the plasma membrane

on the luminal surface of the kidney collecting duct^{28,29}. Water is reabsorbed via AQP2, thereby regulating the body fluid volume^{29,30}. The quantity of AQP2 in urine is correlated with the quantity of AQP2 translocated to the plasma membrane; it is used as an indicator of resorption^{29,30}. Among middle-aged men, high urine AQP2/Cre levels were observed in some participants with high water intake (Supplementary Figure 2). This is potentially due to the greater water intake from alcoholic beverages. This results in a larger overall water intake, owing to the diuretic properties of alcohol, and increased urine AQP2/Cre levels that promote water resorption. Meanwhile, among middle-aged women, urine AQP2/Cre levels remained low even among those with low water intake (Supplementary Figure 2). This could be because the body meets the required water intake or, alternatively, it does not meet its required water intake, but the resorption response is not properly activated.

Comparing urine osmolality and urine AQP2/Cre revealed a positive correlation among both young and middle-aged participants. Translocation of large amounts of AQP2 to the plasma membrane enhances water resorption volumes, and lower urine output promotes higher urine osmolalities²⁹. This response was evident regardless of the participant sex or age group. Urine was collected immediately upon rising; and therefore,

urinalysis parameters could have been affected by the toilet frequency during bedtime. However, the mean toilet frequency during bedtime was 0.1 ± 0.3 times/night; therefore, the effect of this factor on urinalysis parameters is negligible.

This study has the following strengths. First, it is the first study to evaluate the validity of a selective recall method for assessing water intake, from both beverages and food. This makes assessing water intake easy and accurate for future investigations. Second, we measured not only the fluid intake but also the hydration status through urinalysis. However, general verification only compares the numbers between the questionnaires. We verified the validity of the selective recall method by comparing it with the existing method, and by evaluating the relationship between hydration status and water intake. Third, we conducted the survey on four days, including working and non-working days. Therefore, habitual water intake was calculated taking into account the daily dietary variations in one answer.

This study had some limitations. First, the surveys were conducted only in summer using a relatively small participant group. Second, the participants recruited were employees from Suntory World Research Center; this might have introduced selection bias for attributes pertaining to working for a food company. Individuals with different attributes and from a different region were subjected to the same correlation analysis, considering the water intake from beverages over 3 days. The correlation between the values from the descriptive questionnaire method and selective questionnaire was $r = 0.93$, with a linear regression line $y = 0.96x + 5.88$ (where x represents water intake based on the selective questionnaire, and y represents water intake based on the descriptive questionnaire and general linear model, $p < 0.001$)¹⁰. Therefore, a strong correlation between the descriptive dietary record method and selective recall method was observed in differing populations. Third, the study was conducted during July and August of 2020, when many people refrained from going outdoors, because of the spread of COVID-19 in Japan. This could have resulted in changes to the living circumstances that affected the diet. However, the water intakes based on the two methods were surveyed at the same time; and therefore, they are comparable. Fourth, we collected first morning urine to assess hydration status. 24 h urine collection is the preferred method for assessing hydration status; however, there is a strong correlation between

the osmolality of the first morning urine and that of 24 h urine²³. Therefore, we validated the questionnaire using the first morning urine. Fifth, the study was conducted on Japanese participants, which limits the generalizability of the results. A large-scale study including participants from multiple regions and conducted over all four seasons is needed to examine the potential impact of participant attributes and seasonality.

Conclusion

In this clinical study, there was a strong correlation between the water intake calculated using the descriptive dietary record method and the selective recall method. This is the first study to ascertain the validity of the selective recall method for assessing water intake that includes water intake from food. We are in the process of developing another method of assessing habitual water intake that uses an even simpler questionnaire. This will provide a simple method for epidemiological studies for examining water intake habits and the relationship between these habits and health. These data have the potential to reveal findings that lead to disease prevention and improved disease outcomes, while maintaining and improving overall community health.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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