Energy Requirement Assessment and Water Turnover in Japanese College Wrestlers Using the Doubly Labeled Water Method

Hiroyuki SAGAYAMA1,2,3, Emi KONDO4,5, Keisuke SHIOSE1, Yosuke YAMADA4, Keiko MOTONAGA1, Shiori OUCHI1, Akiko KAMEI3, Takuya OSAWA6, Kohel NAKAJIMA3, Hideyuki TAKAHASHI1, Yasuki HIGAKI7,8 and Hiroaki TANAKA7,8

1 Biotechnology Center, University of Wisconsin-Madison, 425 Henry Mall, Madison, Wisconsin 53706 USA
2 Japan Society for the Promotion of Science, Tokyo 102–0083, Japan
3 Japan Institute of Sports Sciences, Tokyo 115–0056, Japan
4 Department of Nutritional Science, National Institute of Health and Nutrition, National Institutes of Biomedical Innovation, Health and Nutrition, Tokyo 162–8636, Japan
5 Osaka University of Health and Sport Sciences, Osaka 590-0459, Japan
6 COI project center, Juntendo University, Chiba 270–1606, Japan
7 Faculty of Sports and Health Science, Fukuoka University, Fukuoka 814–0180, Japan
8 Fukuoka University Institute for Physical Activity, Fukuoka 814–0180, Japan

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Summary Estimated energy requirements (EERs) are important for sports based on body weight classifications to aid in weight management. The basis for establishing EERs varies and includes self-reported energy intake (EI), predicted energy expenditure, and measured daily energy expenditure. Currently, however, no studies have been performed with male wrestlers using the highly accurate and precise doubly labeled water (DLW) method to estimate energy and fluid requirement. The primary aim of this study was to compare total energy expenditure (TEE), self-reported EI, and the difference in collegiate wrestlers during a normal training period using the DLW method. The secondary aims were to measure the water turnover and the physical activity level (PAL) of the athletes, and to examine the accuracy of two currently used equations to predict EER. Ten healthy males (age, 20.4 ± 0.5 y) belonging to the East-Japan college league participated in this study. TEE was measured using the DLW method, and EI was assessed with self-reported dietary records for ~1 wk. There was a significant difference between TEE (17.9 ± 2.5 MJ·d−1 [4,283 ± 590 kcal·d−1]) and self-reported EI (14.4 ± 3.3 MJ·d−1 [3,446 ± 799 kcal·d−1]), a difference of 19%. The water turnover was 4.61 ± 0.73 L·d−1. The measured PAL (2.6 ± 0.3) was higher than two predicted values during the training season and thus the two EER prediction equations produced underestimated values relative to DLW. We found that previous EERs were underestimating requirements in collegiate wrestlers and that those estimates should be revised.

Key Words doubly labeled water, wrestler, total energy expenditure, energy intake, water turnover

The sport of wrestling is an individual combat Olympic sport that is practiced all over the world. It is a weight-class sport and as elite athletes are involved in daily training and competition, it is essential to determine their energy and water requirements to aid in maintaining optimal weight (1–4). Other sports based on body-weight classification similar to wrestling, such as judo and boxing, also need accurate measures of requirements to establish an energy-and-water-intake plan to accomplish athletes’ target body weight for matches (5–8). The doubly labeled water (DLW) method has been proposed as a criterion method to estimate energy requirement (EER) because it is an accurate and objective method for measuring total energy expenditure (TEE) and water turnover for field athlete studies in free-living conditions with minimum constraints (9, 10). Because of its lower cost, self-reported energy intake (EI) has been commonly used to estimate EER for athletes; however, the self-reported EI measurement has been shown to underestimate EI compared with measured TEE (11). For example, among non-athletes, comparison of self-reported EI with DLW-derived TEE demonstrated that over one-third of individuals underreport EI by more than 25% when using the DLW method in several different population groups (12). The underestimation of EI has also been found in athletes, and the range of error has been reported to be between 0 and −43% (13). Errors in this range plague EI planning for some athletes, because an inadequate EI relative to TEE compromises performance and the benefits of athletic training. To our knowledge, for Olympic sports such as wrestling and other weight-classified sports, no studies have been published using DLW to accurately assess energy requirements and water turnover for the

E-mail: sagayama@wisc.edu
In addition to EER based on self-reported EI, there are methods based on the use of estimated physical activity level (PAL) during training and competition. The DLW method provides a criterion measure for establishing these PAL-based methods when DLW is combined with measurement of resting energy expenditure (REE=basal metabolic rate, BMR) (1). There are EER prediction methods using PAL for various sports; however, no objectively measured PAL values are available for collegiate wrestlers.

The primary aim of this study was to measure TEE using DLW and compare it with self-reported EI for collegiate wrestlers during a typical training period. The secondary aims were to measure the water turnover and the physical activity level (PAL) of the athletes, and to examine the accuracy of two currently used equations to predict EER.

MATERIALS AND METHODS

Subjects. All data were obtained from 10 healthy Japanese male collegiate wrestlers (age 20.4±0.5 y old). The subjects were categorized into either a light weight class (57–66 kg, n=4) or a middle weight class (71–80 kg, n=6) according to a previous study (14). None of them had a history of diabetes or any other metabolic disorder, and all belonged to East-Japan college league and had competed in the world championship (n=1), national (n=4), or/and Kanto region intercollegiate games in Japan (n=10). The number of training days was 6±1 (range 5–7) d and the training time was 172±15 (range 150–204) min·d−1 during the DLW measurement period. The subjects were invited to attend an information meeting, and those interested in participating provided written informed consent. The Ethics Committee of the Japan Institute of Sports Sciences approved the study protocol in accordance with the Declaration of Helsinki (036).

Procedures. The experiment was performed in the winter training season of 2014. The subjects came to the laboratory for a medical check and the DLW dose (day 0) and afterwards (day 7–10). Height was measured on day 0 (isotopic administration day), body weight was measured day 0 and the following days at 7 am and pm on day 8 or 9, whereas REE was measured on the morning of study and afterwards (day 7–10). The subjects were asked to maintain their initial body weight (day 0) during this study period. Percent body fat was calculated by a 2-component model using stable isotope dilution, which is included in the DLW, on day 0. Their characteristics are shown in Table 1.

Total energy expenditure. TEE was measured over about 7 d using the DLW method. Subjects were instructed to maintain normal activity, eating patterns and body weight during this study. They were asked to maintain their body weight during DLW measurement periods. Subjects were asked for urine samples at baseline and after dosing (day 0 baseline, 3 h and 4 h). Subjects were allowed only 280 mL of liquid, which included washout water at the 2.0-h post-dose time point. They were given an oral dose consisting of 18 O-and 2 H-labeled water. The bottle was rinsed twice with 30 mL water. The dose was composed of approximately 1.5 g·kg−1 estimated total body water (TBW) (20 atom% H218O; Taiyo Nippon Sanso, Tokyo, Japan) and approximately 0.12 g·kg−1 estimated TBW (99.9 atom% 2 H2O; Taiyo Nippon Sanso). TBW was estimated as being 60% of the body weight of each subject. After dosing, all subjects collected urine themselves on day 7 in the morning and evening; their samples were analyzed to measure the elimination rates of stable isotope rates at baseline, 3 h, 4 h, day 7 am and day 7 pm. All samples were stored frozen at −30˚C in plastic containers wrapped in tight Parafilm M® (Bemis Co., Inc., Oshkosh, WI).

The urine samples were analyzed using isotope ratio mass spectrometry (Hydra 20-20 Stable Isotope Mass Spectrometers; SerCon Ltd., Crewe, UK). Our previous study provides details regarding the IRMS analysis (8). The 18 O and 2 H dilution spaces (Nd and No) were determined by the plateau method according to Cole and Coward (15). The Nd/No in the present study was 1.031±0.009, which is similar to values reported in previous studies (16, 17). Total body water (TBW) was thus calculated as the mean of Nd and No divided by 1.041 and 1.007 for the dilution space, respectively. Carbon dioxide production was calculated using the 2-point DLW method with equation A6 of Schoeller et al. (9), as modified by Racette et al. (16). The TEE calculation was performed using the Weir formula based on CO2 production rates, and using each assumed food quotient (18, 19). PAL was calculated as TEE divided by REE.

Resting energy expenditure. The REE was measured by indirect-calorimetry after a 10-h fast. Subjects remained awake in a supine position at a comfortable room temperature (24–25˚C). Samples of expired gas were measured for 10 min twice or more, and the average of the two or more values was used after a 30-min resting period in the supine position (20). REE was measured during the study period. This expired gas was collected in a Douglas bag for each time interval. A fast-response mass spectrometer (ARCO-2000 MET and SYSTEM-5L;
Arcoecosystem, Chiba, Japan) was used to analyze the oxygen and carbon dioxide concentrations and expired gas volume per minute (21). The mass spectrometer was calibrated using two different gases and the software was able to calculate the expired minute volume, oxygen consumption, carbon dioxide production, respiratory exchange ratio, and energy expenditure values. The measured REE values were then calculated using a modified Weir equation (18). The REE was defined as BMR in much the same meaning in this study (REE=7BMR).

Energy intake. A survey of food intake was conducted using both self-recorded food intake and visual records obtained using the provided digital camera. All foods were weighed using a portable digital scale. A Japanese registered dietitian checked these dietary records and photographs when they collected them. They then calculated nutrients from the diet record and photographs. Energy intake (EI) was obtained from non-consecutive 3-d dietary records. EI has been defined as self-reported energy intake in this study. All dietary records were used to determine total EI and macronutrient consumption with a computerized nutrient analysis program (Excel Eiyokun ver.6.0, Japan food composition table Version 5, Kenpakusya, Tokyo, Japan). EI and macronutrients for commercially prepared and restaurant food were calculated from manufacturer’s websites or by asking the manufacturer.

Calculation of estimated energy requirement. The energy requirements estimated with EER for the Japanese were calculated using standard methods from the National Institute of Health and Nutrition (NIHN) (Ministry of Health, Labour and Welfare of Japan 2015) (22): EER (kcal·d⁻¹)=BMR (kcal·d⁻¹)×PAL. PAL is divided into three categories: lightly active lifestyle, 1.5 (range 1.40–1.60); moderately active lifestyle, 1.75 (range 1.60–1.90); and vigorously active lifestyle, 2.0 (range 1.90–2.20). A PAL of 2.2, the highest vigorously active lifestyle in the range, was used for all subjects. This equation is described simply as follows:

\[ \text{EER}_{\text{NIHN}} (\text{kcal·d}^{-1}) = \frac{\text{estimated BMR}_{\text{NIHN}}}{2.2} \]

where the estimated BMRNIHN (kcal·d⁻¹)=\left[0.1238 + (0.0481 \times \text{body weight (kg)}) + (0.0234 \times \text{height (cm)}) - (0.0138 \times \text{age (y)}) - 0.5473\right] \times 1.000/4.186 (22, 23).

EERSs for the Japanese athletes were often calculated using the Japan Institute of Sports Sciences (JISS) equation as follows (24):

\[ \text{EER}_{\text{JISS}} (\text{kcal·d}^{-1}) = \frac{\text{estimated BMR}_{\text{JISS}}}{2.0} \]

where the estimated BMRISS (kcal·d⁻¹)=28.5×fat free mass (FFM) (kg). The PAL is divided into three categories in the training season: an endurance sports group, 2.50; a strength, power and sprint sports group, 2.00; a ball game group, 2.00 (23). The PAL of 2.00 on the strength, power and sprint sports group was used for all subjects in this study.

Body water turnover. Body water turnover (\(\text{rH}_2\text{O}\)), comprising metabolic water intake, inspiratory water intake, transtubecaneous water intake and preformed water, was estimated using calculation procedures from Raman et al. (26) and Fjeld and Brown (27).

(a) \(\text{rH}_2\text{O} (\text{L·d}^{-1})=(1/0.98)\times N_d \times K_d\).

(b) Metabolic water (L·d⁻¹)=TEE (kcal·d⁻¹)×(1/10⁵) \([(%\text{fat} \times 0.119) + (%\text{protein} \times 0.103) + (%\text{carbohydrates} \times 0.15) + (%\text{alcohol} \times 0.168)]\).

(c) Inspiratory water intake (L·d⁻¹)=respiratory volume (L·d⁻¹)×absolute humidity (mg·L⁻¹)/1,000. Inspiratory water was calculated assuming a relative humidity of 35% for the winter period at 24˚C, and the respiratory volume was calculated from the \(\text{rCO}_2\) obtained from DLW, assuming 3.5% of inspired air.

(d) Transcutaneous water intake (L·d⁻¹)=(0.18×(absolute humidity (mg·L⁻¹)/217.1)×body surface area (m²))/1.44.

Body surface area was estimated from the DuBois formula (28). A clothing factor of 50% was assumed, as clothing would decrease the rate of evaporation through the skin.

(e) Preformed water intake (L·d⁻¹)=\(a-b-c-d\).

Statistical analysis. The results are presented as mean±standard deviation (SD). An alpha of 0.05 was used as the cut-off point for statistical significance. All analyses were performed using IBM SPSS 23.0 for Mac (IBM, Armonk, NY). The metabolic characteristics were compared using one-way analysis of variance (ANOVA) with repeated measures. When a significant difference was detected, a multiple comparison test was performed using Tukey’s post-hoc test for least significant difference. The other comparisons were evaluated using Student’s paired t-tests. A difference between the light weight and middle weight class couldn’t be assessed because these sample sizes were too small.

RESULTS

Table 1 shows each subject’s characteristics and body composition. There was no significant difference between initial (73.0±7.9 kg) and final body weight (73.2±8.2 kg), and the average body weight was 73.1±8.1 kg. Table 2 shows TEE, self-reported EI, EERNIHN, EERRISS, PAL and the difference between self-reported EI and TEE. The self-reported EI, EERNIHN and EERRISS were all lower than the measured TEE (p<0.05). However, there are no significant differences between self-reported EI, EERNIHN and EERRISS. Nor are there any significant differences among the measured REE, the estimated BMRISS and the estimated BMRISS (Table 3). Table 4 shows reported macronutrient consumption data. The food quotient was 0.88±0.01. Each individual’s food quotient was used to calculate their TEE. Table 5 shows the water turnover for each wrestler in detail.

DISCUSSION

The TEE, self-reported EI and the difference of 10 male wrestlers were measured using the gold standard DLW technique in the first time. Thus, it was possible to determine the energy and water requirement of...
male wrestlers over about 1 wk. In the present study, self-reported EI was 19% (range 3%–45%) lower than TEE and there was a significant difference between TEE (17.9±2.5 MJ·d⁻¹ [4,283±590 kcal·d⁻¹]) and self-reported EI (14.4±3.3 MJ·d⁻¹ [3,446±799 kcal·d⁻¹]). Weight-classified athletes such as wrestlers need to evaluate energy expenditure and physical activity using highly accuracy estimation methods because they need a high degree of weight and diet control for their competitions (29). Previously, we reported weight-classified athletes’ physical activity using a tri- accelerometer and training records (8). There are, however, limitations to the assessment of physical activity using an accelerometer: they are difficult to wear for combat sports players during training, because they might tend to underestimate upper limb movement; there is little movement resistance training; and they do not measure diet-induced thermogenesis or post-exercise oxygen consumption.

Several previous studies have investigated TEE, self-reported EI and the difference using this DLW method for the general population and for other sports (13, 30). However, no similar studies have been performed with male wrestlers or other weight-classified athletes as subjects using the highly accurate DLW methods to estimate energy expenditure in a field study. In the present study, self-reported EI was 19% lower than TEE and there was a significant difference between these two variables, although athletes’ body weight was maintained during TEE measurement periods (Table 2). The difference, i.e.
underreporting, is a well-known general phenomenon, but has not been studied in elite male wrestlers (31–33). The 19% underreporting is not dissimilar to what has been reported in many other studies, but is larger than would be expected based on BMI alone (34) and thus may reflect the effects of worry about weight gain (35). Although many dietary energy studies display underreporting, self reports are still commonly used for estimating EI in athletes. Therefore, we speculate that the potential for underreporting of EI may result in values not be close enough to actual energy requirements and could be insufficient to elevate or maintain body weight. Thus, our observation of 19% underreporting among elite wrestlers should be a caution to those involved in providing dietary advice to wrestlers and possibly to athletes in general.

Comparisons with the TEE of other sports are possible using PAL values, which are relative values estimated from the TEE and REE. We found the mean PAL of male collegiate wrestlers as a second finding. Most of these studies focused on training and/or competition periods, during which PAL is particularly high. The present study reports a PAL for wrestlers of 2.58±0.34, 2.47±0.42 and 2.73±0.06 (mean, light and middle weight class, respectively) during a training period. This is higher than that reported for Japanese adolescents in general (1.97±0.31 in subjects who exercised, and 1.85±0.27 in subjects who did not exercise) (36). This result suggests that the physical activity of collegiate wrestlers during the winter training season is classified as high physical activity when it is compared with previous studies (30). Our TEE and PAL results using the DLW method could serve as a useful reference for light and middle weight class wrestlers.

The EER equation was not fitted for our study, and it appears that the difference in PAL was the primary factor. We used two EER equations including for PAL\textsubscript{NIHN} (2.2) and PAL\textsubscript{JISS} (2.0) as a high activity general population and a strength, power and sprint sports athletes, respectively (22, 25). These PALs would be underestimated for the wrestler. Actually, the EER\textsubscript{NIHN} for PAL is set for active people in general; most athletes would expend even more energy for their training. Otherwise, the two-estimate BMR equation was similar to the measured values. It is possible that the estimated BMR\textsubscript{NIHN} was also appropriate for the collegiate wrestlers because its equation is derived from a large Japanese sample and includes factors such as age, height, weight and sex (23). The estimated BMR\textsubscript{JISS} also was a good predictor equation for athletes in this study. This equation includes FFM as a variable; it is well known that FFM and BMR show good correlation on a regression line, but not excellent. Actually, our measured REE result was significantly and highly positively correlated with FFM (slope=22.8, intercept=234, r=0.74, p<0.05). This slope was close to the coefficient of equation. The BMR\textsubscript{JISS} equation would be easy to use in the field because it uses only FFM as a factor. However, because the relationship between FFM and BMR has a moderate intercept, the use of a simple ratio of BMR to FFM may

| Class                     | Light weight | Middle weight | Total     |
|---------------------------|--------------|--------------|-----------|
| Protein, g·d\textsuperscript{-1} | 115±28       | 136±29       | 123±29    |
| Fat, g·d\textsuperscript{-1}   | 97±19        | 129±14       | 110±23    |
| Carbohydrate, g·d\textsuperscript{-1} | 482±154      | 505±142      | 491±141   |
| P: F: C\textsuperscript{1}   | 14: 28: 58   | 15: 33: 54   | 14: 29: 57|

Values are mean±standard deviations.

\textsuperscript{1} Percentage of the total energy intake.

| Class                     | Light weight | Middle weight | Total     |
|---------------------------|--------------|--------------|-----------|
| rH\textsubscript{2}O (L·d\textsuperscript{-1}) | 4.27±0.60    | 5.11±0.67    | 4.61±0.73 |
| Metabolic water (L·d\textsuperscript{-1})     | 0.53±0.04    | 0.65±0.06    | 0.57±0.08 |
| Inspiratory water intake (L·d\textsuperscript{-1}) | 0.16±0.01    | 0.20±0.02    | 0.18±0.02 |
| Transcutaneous water intake (L·d\textsuperscript{-1}) | 0.07±0.00    | 0.08±0.00    | 0.07±0.00 |
| Preformed water (L·d\textsuperscript{-1})            | 3.51±0.56    | 4.19±0.60    | 3.78±0.64 |

Values are mean±standard deviations.
result in errors for athletes at the extremes of FFM.

Our analysis showed that water turnover was $4.61 \pm 0.73$, $4.27 \pm 0.60$ and $5.11 \pm 0.67 \text{Ld}^{-1}$ (mean, light and middle weight class, respectively) in male collegiate wrestlers during the winter training season as an additional finding. Unfortunately, it is not possible to effectively compare our results with the few studies that investigate water turnover calculation. Westerterp et al. reported that on day 16 of the Tour de France, the cyclists’ water requirements were $10.48 \text{Ld}^{-1}$ (37). Hill and Davies reported that after a 2-wk ultra-endurance run, the runners’ water requirements were $6.08 \text{Ld}^{-1}$ (38). Ruby et al. reported that for wildland firefighters and active college students, the two groups’ water requirements after 5 d were $6.7 \text{Ld}^{-1}$ and $3.8 \text{Ld}^{-1}$ (39), respectively. Our water turnover results of $4.6 \text{Ld}^{-1}$ appear similar to the water needs of active college students, but it might be larger than that of the general student at college (39). These reports are not directly comparable, however, because of the physiological differences and the potential effects of climate and training season between sports. Our data estimated the water turnover in detail, but it is not based on an actual intake. In fact, it is clear from previous data that the amount of water intake is not consistent with the observed water intake in healthy adults (26). Preformed water intake was $3.78 \pm 0.64$, $3.51 \pm 0.56$ and $4.19 \pm 0.60 \text{Ld}^{-1}$ (mean, light and middle weight class, respectively). The preformed water intake constituted about 5% of body weight. Thus, food and fluid restriction in only one day for weight reduction is likely to contribute their body weight reduction and dehydration.

In conclusion, this is the first time that energy expenditure in college wrestlers has been determined using the DLW method. The EI estimated from self-reported dietary records and the two EER equations were under-reported compared with TEE, suggesting that self-reported EI and calculation of EER from these data does not predict energy expenditure in college wrestlers. This suggests that EI based on dietary records is not a good predictor of energy expenditure in college wrestlers. The PAL for college wrestlers during the winter training season was determined to be $2.58 \pm 0.34$. The results will be of value in establishing energy requirements for college wrestlers. The water turnover was $4.61 \pm 0.73 \text{Ld}^{-1}$. Our information may help to provide a reference for the energy and water requirements of wrestlers, nutritionists, and coaches to optimize performance in winter training seasons.

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