Original Article

The relationship between water intake, hydration biomarkers and physical activity of young male athletes in Beijing, China: A cross-sectional study

Xing Wang BM[1], Na Zhang PhD[1,2], Jianfen Zhang PhD[1], Yibin Li MM[1], Yi Yan PhD[3], Guansheng Ma PhD[1,2]

[1]Department of Nutrition and Food Hygiene, School of Public Health, Peking University, Beijing, China
[2]Laboratory of Toxicological Research and Risk Assessment for Food Safety, Peking University, Beijing, China
[3]College of Sports and Human Sciences, Beijing Sport University, Beijing, China

Background and Objectives: To explore the relationship between water intake, hydration biomarkers and physical activity of young male athletes. **Methods and Study Design:** A 7-day cross-sectional study was conducted among 45 male athletes aged 18-25 years in Beijing, China. Total drinking fluids (TDF) was obtained using 7-day 24-h fluid intake questionnaire. Water from food (WFF) was assessed using the methods of food weighing, duplicate portion method and laboratory analysis. Physical activity was evaluated using physical activity energy expenditure (PAEE) and metabolic equivalent of task (MET). **Results:** Totally, 42 participants completed the study. The medians of total water intake (TWI), TDF and WFF of participants were 2771 mL, 1653 mL and 1088 mL respectively. Jonckheere-Terpstra test showed a significant increase trend toward higher TWI and TDF with higher PAEE level (Z=2.414, p=0.016; Z=2.425, p=0.015). Spearman's rank correlation showed that TWI was positively correlated with PAEE (rs=0.397, p=0.009). TDF showed a positive correlation with PAEE and MET (rs=0.392, p=0.010; rs=0.315, p=0.042). The median urine volume was 840 mL, urine specific gravity was 1.020, and 24-h urine osmolality was 809 mOsm/kg. Significant differences were found in plasma cortisol among the four MET groups (χ^2=8.180; p=0.042). **Conclusions:** Young male athletes with higher physical activity level had higher amounts of TWI and TDF than their counterparts but had similar hydration biomarkers. There was a high incidence of dehydration in athletes, and attentions need to be paid on the intake of TDF among them to maintain the optimal hydration status.

Key Words: fluid intake, hydration, dehydration, physical activity, athletes

INTRODUCTION

Accounting for 45-75% of adult body weight, water was an indispensable substance to maintain human life and physiological functions.[1] In general, the intake and discharge of water were in balance to keep the normal hydration status.[2] During exercise, the total body metabolism could increase to 5-15 times the resting rate to support the contraction of skeletal muscles, and approximately 70-90% of this energy was released as heat.[3] Evaporation of sweat was an effective mechanism for the dissipation of heat from the body.[4] Water loss through sweat of trained athletes could reach 1.5-2.0 L/h during intense exercise in a hot environment.[5,6] Excessive perspiration may lead to an increased need for water. A study showed that the total drinking fluids (TDF) of collegiate football players during practices could reach 4.0±1.1 L.[7]

Failure to replenish water in time during exercise might reduce blood volume and increase plasma osmotic pressure, which could lead to hypertonic hypovolemia and affect the function of the body. Existing studies had shown that the athletic performance was best when fluid balance was maintained. When water loss exceeded 2% body weight, aerobic and endurance performance might be reduced, and cognitive ability might be impaired, especially in hot weather.[1,8-11] In addition, dehydration might reduce the sweat, restrict the normal heat dissipation function of the body, and result in heat resistance, which might cause heat stroke and even a threat to life in severe cases.[12,13] Inadequate water intake was also an important factor of muscle spasm and rhabdomyolysis in athletes.[14,15] Besides, exercise-related hyponatremia (EAH) also required vigilance. It usually occurred when large amounts of isotonic or hypotonic fluids were consumed after exercise. And if not corrected in time, it could result in epilepsy, coma, respiratory arrest, and

Corresponding Author: Dr Guansheng Ma, Department of Nutrition and Food Hygiene, School of Public Health, Peking University, 38 Xue Yuan Road, Haidian District, Beijing 100191, China.
Tel: +86-10-8280-5266; Fax: +86-10-8280-5266
Email: mags@bjmu.edu.cn
Manuscript received 20 July 2022. Initial review completed 17 September 2022. Revision accepted 01 December 2022.
doi: 10.6133/apjcn.202303_32(1).0018

even death.[16] Therefore, the water supplement during exercise should be appropriate and needed to be strictly monitored.

Inadequate water intake was common among Chinese people. A survey conducted in 2012 among 1483 adults from four cities in China showed that approximately 32% of the participants drank less fluid than the recommendation of TDF set by the Chinese Nutrition Society in 2007.[17] Another survey conducted in 2016 among 2233 people in 27 cities showed that only 28% of adults met the adequate TDF set by the Chinese Nutrition Society in 2013 (1.7 L/d for males and 1.5 L/d for females).[18] Previous studies had also suggested a large proportion of Chinese residents might be in dehydration status.[19,20] A study conducted in 2016 showed that in male college students the percentage meeting the recommendation of total water intake (TWI) was 25%, with only 35.3% of participants were in optimal hydration status.[19] In 2021, a study about hydration status of male college students aged 18-23 years old showed only 37.1% of the participants in free-living conditions were in optimal hydration status and about 16.7% of them were in dehydration status.[20] Dehydration was common in athletes. A study detected the urine of 263 athletes of the American college league before training and found that 66% of collegiate athletes appeared dehydration.[21] Another study analyzed the urine specific gravity of 14 elite male soccer players and found that the majority were in dehydration status before training.[22] However, there were few researches on water intake and hydration status of athletes in China. More studies were needed in this issue.

Previous studies had shown that the hydration status was closely related to athletic performance.[8] Maintaining proper hydration could not only improve sports performance, but also ensure sports safety. This highlighted the importance of water intake during exercise. In 2004, the Institution of Medicine of the National Academies (IOM) first recognized that physical activity and environmental conditions had a significant impact on the fluid needs of physically active individuals. It was recommended that people who were physically active and exposed to heat needed to consume more water, but there were no definitive recommendations on the amount of adequate water intake for athletes.[1] The relationship between water intake, hydration and physical activity required more in-depth research.

The aims of this study were to investigate the water intake and hydration status of young male athletes, and to explore the relationship between water intake, hydration biomarkers and physical activity, in order to provide a basis for establishing recommendations on adequate water intake for athletes.

METHODS

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by Peking University Institutional Review Board (IRB00001052-19051).

Sample size calculation

Sample size was calculated using PASS 15 Power Analysis and Sample Size Software (2017) (NCSS, LLC; Kaysville, Utah, USA). In a previous study conducted among Chinese young male adults, the standard deviation of TDF was 468 mL.[19] With the δ set at 145 mL, α set at 0.05, and the drop-out rate set at 10%, 45 subjects were needed.

Participants

The participants of the study were recruited from a university in Beijing, China.

Inclusion criteria: Healthy adult male college students aged 18 to 25 with regular exercise training programs (more than 5 times of moderate intensity exercise per week) were included.

Exclusion criteria: Participants were excluded for oral, endocrine, renal, gastrointestinal, metabolic diseases and other chronic diseases or sports injuries, or cognitive impairment. Participants who had taken other health care products such as drugs or vitamins within one month, and who had special conditions during the study period that prevented them from carrying accelerometers were excluded, too.

A total of 45 eligible young male athletes were recruited for this study, and 42 of them completed the study, with a completion rate of 93.3%. The remaining 3 participants failed to completed physical activity monitoring as required. The basic information of them was recorded.

Study design and procedure

A 7-day cross-sectional study was designed and conducted in April-May 2019. On day 1 and day 7, height and weight were measured and recorded in a standard procedure by trained investigators. The averages of the two measurements were used in the final statistics analysis. To assess TDF, participants were asked to complete a self-filled 7-day 24-h fluid intake questionnaire for 7 consecutive days after trained to fill out it correctly. From day 3 to day 5 (including 2 weekdays and 1 weekend), water from food (WFF) was assessed using the methods of weighing, duplicate portion method and laboratory analysis.[19,23] Meanwhile, participants were asked to collect every random urine sample in the consecutive 3 days to measure the urine volume and urine osmolality.[24] On day 4, the fasting blood samples of all participants were collected to test the concentrations of plasma hydration biomarkers. Physical activity monitoring was carried out throughout the whole 7-day follow-up using triaxial accelerometer worn on the right hip. The temperature and humidity in the study site were recorded at 10:00 a.m. each day during the study. The study procedure was shown in Table 1.

Anthropometric measurements

Height and weight were measured by trained investigators following a standard procedure. Height was measured with a height meter (BSM370, BIOSPACE, Korea) with 0.1 cm accuracy. Body weight was measured with a body composition instrument (Inbody 230, Inbody, Korea) with 0.1 kg accuracy. Anthropometric measurements were

Table 1. The study procedure

Project	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Anthropometric measurements	√						√
7-day 24-h fluid intake questionnaire	√	√	√	√	√	√	√
Water from food			√	√	√		
Determination of 24-h urine biomarkers			√	√	√		
Determination of plasma biomarkers				√			
Physical activity monitoring	√	√	√	√	√	√	√
Temperature and humidity record	√	√	√	√	√	√	√

√, The project was taken on that day.

taken on day 1 and day 7. The averages of the two measurements were used in the final statistics analysis.

Body mass index (BMI) = weight (kg) / height (m)^2

Temperature and humidity of the environment

Temperature and humidity were measured to the nearest 0.1℃ and 1% respectively using a temperature hygrometer (WSB-1-H2, Exasace, Zhejiang, China) every morning at 10:00 a.m. at the study site. The average temperature was 24.2±5.8°C, and the average humidity was 29.5±15.8% during the study period.

Assessment of water intake

A validated 7-day 24-h fluid intake questionnaire was used to collect TDF.[25] After trained to fill in the questionnaire correctly, participants were asked to record the time, type, amount, place of drinking and physical activity condition of drinking water each time for 7 consecutive days. The amount of fluid was measured by a cup to the nearest of 5 mL. The physical activity condition of drinking fluids was divided into four conditions: before exercise (within 30 min before exercise), during exercise (between exercises), after exercise (within 30 min after exercise) and non-exercise (not belonging to any of the above three conditions). During the study period, researchers kept in touch with participants every day through telephone and WeChat, an instant messaging application launched by Tencent, for guidance. And the completed fluid intake questionnaires were reviewed by researchers every day to ensure the completeness and accuracy.

WFF was assessed using the methods of weighing, duplicate portion method and laboratory analysis by trained investigators. The samples of food were weighed before and after participants ate and the backup food samples collected for 3 consecutive days. All foods were weighed accurately by trained investigators using an electronic balance (YP20001, SPC, Shanghai, China).[20] The food samples were sealed in an airtight bag and stored in a refrigerator at 4 °C before measurement. The determination of water in food was measured according to National Food Safety Standard GB 5009.3-2016 Determination of Water in Food by professional laboratory analysts.[26] Parallel samples were made for each food, and the error between the two results was less than 5%. Water intake from fruits was assessed using China Food Composition Tables Standard Edition (2018).[27]

Exercise-related fluid intake (EFI) (mL) = drinking fluids before exercise (mL) + drinking fluids during exercise (mL) + drinking fluids after exercise (mL).

Non-exercise-related fluid intake (NEFI) (mL) = TDF (mL) - EFI (mL).

Personal food consumption (g) = weight before eating (g) - weight after eating (g).

Food water content (%) = [weight of containers and samples before drying (g) - weight of containers and samples after drying (g) / weight of laboratory samples (g)] ×100%.

WFF (mL) = personal food consumption (g) × food water content (%) / 1.0 (g /mL).

TWI (mL) = TDF (mL) + WFF (mL).

Determination of urinary biomarkers

The 24-h urine was defined from the second urine of the first day to the first urine of the second day. Participants were asked to collect each random urine sample using a customized urine collection bag (maximum volume 600 mL, made of polyethylene terephthalate) and sent to the designated laboratory by themselves as soon as possible for 3 consecutive days. Before measurement, the urine was stored in cold storage at 4°C. Urine volume was measured using electronic balance (YP20001, SPC, Shanghai, China) to the nearest 0.1 g.

Urine osmolality was tested with the freezing point descent method using an osmotic pressure molar concentration meter (SMC30C, Tian he, Tianjin, China) with 1 mOsm/kg accuracy. Hydration status was defined according to the osmolality of urine. The optimal hydration was defined as urine osmolality ≤500 mOsm/kg, middle hydration was defined as 500 mOsm/kg <urine osmolality ≤800 mOsm/kg, dehydration was defined as urine osmolality >800 mOsm/kg.[19,28] Urine specific gravity (USG) was tested with uric dry-chemistry method using an automatic urinary sediment analyzer (H-800, Dirui, Changchun, China). The concentration of chlorine (Cl) in urine was tested with an automatic biochemical analyzer (AU 5800, Beckman, Brea, CA, USA) with the ion-selective electrode potentiometer method. The urine volume, number of urinations, USG and 24-h urine osmolality were the average of the consecutive 3 days.

Determination of Plasma Biomarkers

A 5 mL fasting blood sample was collected on day 4 to test the plasma biomarkers. The concentrations of plasma potassium (K), sodium (Na) and Cl were tested using an automatic biochemical analyzer (AU 5800, Beckman, Brea, CA, USA) with the ion-selective electrode potentiometer method. The concentrations of plasma cortisol, testosterone and copeptin were measured using an IMARK enzyme reader (Bio-RAD 680, Bole, USA).

Table 2. The characteristics of participants

Group	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)
Total	20.8±1.0	178.6±4.8	71.2±7.3	22.3±1.8
PAEE				
Gp1	20.5±0.8	176.0±3.5	68.9±5.58	22.2±1.1
Gp2	20.9±1.2	177.6±4.4	68.3±5.2	21.6±1.4
Gp3	21.3±1.1	180.7±6.6	72.5±7.9	22.2±1.4
Gp4	20.5±1.0	180.3±3.0	75.1±8.8	23.1±2.8
F	1.309	2.435	2.149	1.193
p	0.285	0.080	0.110	0.325
MET				
Gm1	20.5±0.9	177.2±4.1	68.4±5.7	21.7±1.3
Gm2	20.8±0.9	180.5±7.6	74.6±8.1	22.9±1.9
Gm3	21.0±1.3	177.0±2.7	68.1±3.1	21.7±0.8
Gm4	20.9±1.1	180.1±3.3	74.1±9.3	22.8±2.7
F	0.366	1.552	2.749	1.365
p	0.778	0.217	0.056	0.268

PAEE: physical activity energy expenditure; MET: metabolic equivalent of task.
Values were shown as ± standard deviations.

Physical activity monitoring

Generally, physical activity energy expenditure (PAEE) referred to the total amount of energy expended in physical activity each day, comprehensively considering the intensity, duration and individual weight of the physical activity. Metabolic equivalent of task (MET) was used to represent the energy cost of physical activity as a multiple of resting metabolic rate. One MET was defined as the physical activity intensity in a position of sitting at rest. It was equal to 3.5 mL oxygen per kilogram body weight per minute or 1 kcal of energy per kilogram of body weight per hour.[29] It was usually used to reflect the intensity and duration of daily activities. Both PAEE and MET were used to indicate the intensity of human physical activity in this study. With reference to previous studies, considering the convenience in population studies and the accuracy of the results, we used an ActiGtraph WGTX3-BT triaxial accelerometer to monitor the physical activity of participants.[30,31]

Firstly, the height, weight and other basic health information of participants were input into the triaxial accelerometers (WGT3X-BT, Actigraph, USA). Then, the accelerometers were initialized with a sampling period of 60 seconds, a sampling rate of 30 Hz, and a sampling interval of 10 seconds. The accelerometers were attached on the right hip of the participants with the charging ports upward. All participants were required to wear the accelerometer continuously for 7 days, which could not be removed except for swimming, bath and sleeping. The monitors began to record data from the 0:00 of day 1 after issuance, and finished recording data at 24:00 on day 7, and were recovered on the eighth day. After data collection, the analysis software Actigraph Actilife Version 6.11.4 was used for processing. Valid data days referred to a day with 10 hours or more wear time, and a set of valid data included at least 2 weekdays and 1 weekend.[32] The built-in equations in the software were used to calculate the energy consumption. Freedson VM3 Combination (2011) equation was used to predict PAEE. Freedson Adult (1998) equation was used to predict MET. And Troiano Adult (2008) equation was used to classify physical activity.

In this study, PAEE and MET were the average of all-day activity, reflecting the overall intensity of physical activity throughout the day. In this study, the PAEE of all participants was 851.9±250.6 kcal and the MET was 1.45±0.19. Participants were divided into quartiles of 11, 10, 11, 10 according to PAEE and MET, respectively. The corresponding PAEE of Gp1, Gp2, Gp3 and Gp4 were 294.9-696.3 kcal, 697.8-839.6 kcal, 839.8-979.3 kcal and 1007.1-1563.2 kcal, respectively. And the corresponding MET of Gm1, Gm2, Gm3, Gm4 were 1.08-1.30, 1.31-1.40, 1.44-1.60, 1.62-1.84, respectively.

Statistics

The EpiData3.1 software was used to establish the database, and data entry was carried out by double-entry method to check and clean up the wrong items in time. Statistical analysis was performed using SPSS 19.0 statistical software package. Means ± standard deviations (SD) were used to describe the centralized and discrete trend of the data with normal distribution. Medians and quartile ranges (QR) were used to describe the data with skewed distribution. The differences of basic characteristics among groups were analyzed with one-way ANOVA. Kruskal-Wallis H test was used to compare the differences in water intake, plasma hydration biomarkers, and urinary hydration biomarkers of participants with different physical activity levels. Jonckheere-Terpstra test was used to evaluate the trend of water intake and hydration as physical activity level increased. Spearman's rank correlation coefficient was used to measure the association between water intake and physical activity. Two-sided tests were performed, and the significance level was set at 0.05 ($p<0.05$).

RESULTS

The characteristics of participants

A total of 45 eligible male college student athletes were recruited, and 42 of them completed the study, with a completion rate of 93.3%. No statistically significant differences were found in age, height, weight and BMI among the four PAEE groups and the four MET groups. The characteristics of participants were shown in Table 2.

Water intake of participants with different physical activity levels

The median of TWI of young male athletes was 2771 mL, with a percentage of 45.2% meeting the recommendation of TWI for male adults (3.0 L/d) set by the Chinese Nutrition Society in the Dietary Reference Intakes for Chinese: 2013 edition.[33] The medians of TDF and WFF were 1653 mL and 1088 mL respectively. The proportion of meeting the recommendation of TDF (1.7 L/d) was 50.0%. The medians of EFI and NEFI were 329 mL and 1314 mL respectively. Water intake of different PAEE and MET groups were shown in Table 3.

Statistically significant differences were found in TWI and TDF among the four MET groups (χ^2=11.787, p=0.008; χ^2=11.658, p=0.009). The TWI of Gm1 (2532 mL) was significantly lower than Gm2 (3305 mL) and Gm4 (3282 mL) (p<0.05). The TDF of Gm1 (1398 mL) was significantly lower than Gm2 (2172 mL) and Gm4 (2007 mL) (p<0.05). Jonckheere-Terpstra test showed that there were statistically significant trends of higher TWI and TDF with higher PAEE levels, with Gp1 consuming 2413 mL, 1422 mL, Gp2 consuming 2599 mL, 1681 mL, Gp3 consuming 3019 mL, 1602 mL and Gp4 consuming 3421 mL, 2109 mL (Z=2.414, p=0.016; Z=2.425, p=0.015).

There were significant differences in EFI among the four PAEE groups (χ^2=13.902, p=0.003) and four MET groups (χ^2=14.757, p=0.002). The EFI of Gp4 (574 mL) was significantly higher than Gp1 (172 mL) and Gp2 (211 mL) (p<0.05). And the EFI of Gm4 (721 mL) was significantly higher than Gm1 (179 mL) and Gm3 (304 mL) (p<0.05). Jonckheer-Terpstra test showed significant increase trends toward higher EFI with increasing PAEE and MET levels (Z=3.582, p<0.001; Z=3.020, p=0.003). However, there was neither significant differences nor significant trends in WFF and NEFI among PAEE and MET groups (p>0.05).

Spearman's rank correlation showed that PAEE was positively correlated with TDF and TWI (r_s=0.392, p=0.010; r_s=0.397, p=0.009). And MET showed a significantly positive correlation with TDF (r_s=0.315, p=0.042).

Hydration biomarkers of participants with different levels of physical activity

The median urine volume was 840 mL and the median number of urinations per day was 4.0. The median of 24-h urine osmolality was 809 mOsm/kg and the median of USG was 1.020. There were no statistically significant differences in urine volume, the number of urinations, USG, 24-h urine osmolality among the four PAEE and four MET groups (p>0.05). Based on 24-h urine osmolality, the proportion of optimal hydration, middle hydration and dehydration in the 42 participants were 19.0%, 28.6% and 52.4%, respectively. Based on the random urine osmolality, the ratio of optimal hydration, middle hydration, dehydration of random urine was 0.02, 0.18, 0.66, respectively. The urinary hydration biomarkers of participants with different PAEE and MET levels were shown in Table 4. There were no statistically significant differences in plasma copeptin, testosterone, K, Na and Cl among different PAEE and MET groups (p>0.05). But significant differences were found in plasma cortisol among the four MET groups (χ^2=8.180, p=0.042). The plasma cortisol concentration of Gm3 (73.50 ng/mL) was significantly lower than Gm2 (94.21 ng/mL) and Gm4 (97.08 ng/mL) (p<0.05). See in Table 5.

DISCUSSION

In this study, the TWI and TDF of young male athletes

Table 3. Water intake of participants with different PAEE and MET levels

Group	TWI (mL)	TDF (mL)	WFF (mL)	EFI (mL)	NEFI (mL)
Total	2771 (1066)	1653 (857)	1088 (570)	329(389)	1314 (620)
PAEE					
Gp1	2413 (1586)	1422 (989)	1052 (465)	172 (419)	1227 (673)
Gp2	2599 (814)	1681 (596)	972 (516)	211 (143)	1404 (651)
Gp3	3019 (787)	1602 (487)	1222 (508)	403 (544)	1241 (435)
Gp4	3421 (1042)	2109 (848)	1179 (683)	574 (427) †,‡	1439 (939)
χ^2 (K-W test)	6.083	6.129	2.641	13.902	0.307
p (K-W test)	0.108	0.106	0.450	0.003*	0.959
Z (J-T test)	2.414	2.425	0.977	3.582	0.123
p (J-T test)	0.016**	0.015**	0.329	<0.001**	0.902
MET					
Gm1	2532 (847)	1398 (371)	1052 (456)	179 (210)	1227 (552)
Gm2	3305 (1015) §	2172 (665) §	1130 (427)	375 (601) §	1583 (794)
Gm3	2356 (980) ¶	1534 (483) ¶	890 (452)	304 (340)	1156 (373)
Gm4	3282(711) §,††	2007(1069) §	1136(561)	721(507) §,††	1187(827)
χ^2 (K-W test)	11.787	11.658	3.888	14.757	6.270
p (K-W test)	0.008*	0.009*	0.273	0.002*	0.099
Z (J-T test)	1.583	1.549	0.775	3.020	-0.932
p (J-T test)	0.113	0.121	0.439	0.003**	0.351

TWI: total water intake; TDF: total drinking fluids; WFF: water from food; EFI: exercise-related fluid intake; NEFI: non-exercise-related fluid intake; PAEE: physical activity energy expenditure; MET: metabolic equivalent of task.
Values were shown as medians (quartile ranges).
*p<0.05 there were statistically significant differences between different PAEE or MET groups; **p<0.05 there was statistically significant trend with the PAEE or MET level increase.
†p<0.05 compared with Gp1; ‡p<0.05 compared with Gp2; §p<0.05 compared with Gm1; ¶p<0.05 compared with Gm2; ††p<0.05 compared with Gm3.

Table 4. Urinary hydration biomarkers of participants with different PAEE and MET levels

Group	Urine volume (mL)	USG	24-h urine osmolality (mOsm/kg)	The number of urinations	Average urine volume per time (mL)	Optimal hydration ratio	Middle hydration ratio	Dehydration ratio
Total	840 (418)	1.020 (0.008)	809 (314)	4.0 (1.4)	217 (117)	0.02 (0.30)	0.18 (0.24)	0.66 (0.57)
PAEE								
Gp1	719 (605)	1.018 (0.007)	706 (377)	4.0 (1.7)	188 (123)	0.08 (0.44)	0.22 (0.22)	0.46 (0.55)
Gp2	808 (575)	1.023 (0.006)	865 (310)	3.8 (1.3)	220 (113)	0.08 (0.35)	0.17 (0.32)	0.71 (0.55)
Gp3	938 (395)	1.023 (0.007)	839 (249)	3.7 (2.0)	217 (253)	0.00 (0.15)	0.14 (0.31)	0.86 (0.70)
Gp4	855 (237)	1.018 (0.008)	784 (386)	4.2 (1.8)	213 (82)	0.05 (0.31)	0.22 (0.27)	0.58 (0.54)
χ^2 (K-W test)	1.895	4.132	2.539	0.399	1.004	1.367	2.269	2.900
p (K-W test)	0.594	0.248	0.468	0.940	0.800	0.713	0.518	0.407
Z (J-T test)	1.156	0.259	0.123	0.271	0.707	-0.613	-0.270	0.506
p (J-T test)	0.248	0.795	0.902	0.786	0.479	0.540	0.787	0.613
MET								
Gm1	1095 (1091)	1.022 (0.010)	828 (423)	4.0 (1.3)	240 (134)	0.00 (0.50)	0.18 (0.21)	0.67 (0.50)
Gm2	844 (309)	1.023 (0.009)	748 (248)	4.5 (3.0)	195 (99)	0.09 (0.33)	0.25 (0.30)	0.45 (0.57)
Gm3	829 (430)	1.020 (0.008)	845 (340)	3.7 (1.3)	224 (145)	0.00 (0.30)	0.09 (0.24)	0.89 (0.56)
Gm4	837 (271)	1.018 (0.006)	777 (362)	3.7 (1.8)	219 (81)	0.05 (0.31)	0.28 (0.31)	0.52 (0.42)
χ^2 (K-W test)	0.583	3.114	1.774	1.818	2.245	0.310	5.238	3.955
p (K-W test)	0.900	0.374	0.621	0.611	0.523	0.958	0.155	0.266
Z (J-T test)	-0.146	-0.981	-0.685	-0.745	0.236	-0.156	0.315	-0.079
p (J-T test)	0.884	0.327	0.493	0.456	0.814	0.876	0.752	0.937

USG: urine specific gravity; PAEE: physical activity energy expenditure; MET: metabolic equivalent of task.
Values were shown as medians (quartile ranges).

Table 5. Plasma hydration biomarkers of participants with different PAEE and MET levels

Group	Copeptin (pmol/L)	Testosterone (nmol/L)	Cortisol (ng/mL)	Plasma K (mmol/L)	Plasma Na (mmol/L)	Plasma Cl (mmol/L)
Total	1.66 (0.19)	16.95 (2.00)	86.88 (21.59)	4.17 (0.88)	139.8 (5.3)	102.89 (12.06)
PAEE						
Gp1	1.81 (0.29)	16.91 (3.36)	89.11 (27.72)	4.05 (0.55)	139.0 (6.6)	106.39 (11.13)
Gp2	1.67 (0.14)	16.52 (1.88)	83.85 (26.21)	4.32 (1.09)	139.9 (5.5)	107.63 (14.74)
Gp3	1.56 (0.22)	17.34 (1.44)	85.61 (14.66)	4.19 (0.84)	140.2 (4.4)	101.64 (11.13)
Gp4	1.65 (0.17)	17.06 (1.50)	95.96 (23.58)	4.53 (1.32)	139.8 (5.8)	103.09 (12.68)
χ^2 (K-W test)	5.952	2.603	2.024	1.400	1.327	2.690
p (K-W test)	0.114	0.457	0.568	0.705	0.723	0.442
Z (J-T test)	-1.057	0.831	0.752	0.393	0.494	-1.101
p (J-T test)	0.290	0.406	0.452	0.694	0.621	0.271
MET						
Gm1	1.69 (0.32)	17.30 (3.09)	85.93 (18.16)	3.91 (0.64)	139.8 (6.4)	102.89 (12.58)
Gm2	1.68 (0.16)	16.28 (2.27)	94.21 (16.33)	4.36 (1.64)	140.4 (4.6)	103.09 (10.15)
Gm3	1.60 (0.16)	17.30 (0.78)	73.50 (25.49) †	4.14 (1.06)	140.2 (5.2)	107.42 (10.72)
Gm4	1.65 (0.24)	17.24 (2.06)	97.08 (23.58) ‡	4.38 (1.43)	139.1 (5.9)	102.06 (12.68)
χ^2 (K-W test)	1.219	4.346	8.180	2.137	0.528	1.413
p (K-W test)	0.749	0.226	0.042*	0.544	0.913	0.702
Z (J-T test)	-0.832	1.123	0.056	0.494	-0.067	0.314
p (J-T test)	0.405	0.261	0.955	0.621	0.946	0.753

K: potassium; Na: sodium; Cl: chlorine; PAEE: physical activity energy expenditure; MET: metabolic equivalent of task.
Values were shown as medians (quartile ranges).
* $p<0.05$, there were statistically significant differences between different MET groups.
† $p<0.05$ compared with Gm2.
‡ $p<0.05$ compared with Gm3.

were 2771 mL and 1653 mL respectively. The percentage of the participants meeting the recommendation of TWI set by the Chinese Nutrition Society was only 45.2%, which was higher than that in previous studies conducted among male college students and young adults in China.[19,34] However, the recommendation was set for the general population, the proportion of dehydration in young male athletes was still high in this study. As shown in the results of this study, only 19% of the participants were in optimal hydration status based on 24-h urine osmolality, 66% of the participants were in dehydration status throughout the day based on random urine osmolality. Besides, the median 24-h urine osmolality and USG of young male athletes in this study were obviously higher than those of Chinese male college students in previous studies.[19] Previous studies had also shown poor hydration status in athletes.[35,36] In a study of elite Brazilian young athletes, the urine specific gravity pre-game was 1.021±0.004, which suggested that the players were at the lower limit for significant dehydration.[35] Another study of 29 National Basketball Association players showed that 52% players were in dehydration status at the start of a game (USG >1.020).[36] Overall, the TWI and TDF of young male athletes were higher than the general population, and dehydration was more common in athletes.

Moreover, in this study, the TWI, TDF and EFI showed significant increase trends with increasing physical activity levels. But there were no significant differences or trends in NEFI and WFF. It could be inferred that physical activity mainly affected water intake by affecting exercise-related fluid intake, and had little effect on water intake during non-exercise periods in this study. Besides, Spearman's rank correlation analyses showed that the PAEE had a significant positive correlation with TWI and TDF. And MET had a significantly positive correlation with TWI. It suggested that TWI and TDF might increase with physical activity level. Previous studies had also indicated the association of water intake and physical activity.[37,38] A study based on data from the US National Health and Nutrition Examination Surveys 1999-2006 found that the average physical activity during a typical day was an independent influence on water intake from beverages and TWI.[37] Another study based on data from Australian 2011-2012 National Nutrition and Physical Activity Survey found that the total time of physical activity in the past week was associated with TWI in adults.[38] Therefore, physical activity should be included when considering water intake recommendations.

As early as 1989, the National Research Council of the United States issued an announcement that 1.0-1.5 mL/kcal could be used as the recommended value for the additional water intake of adults due to physical activity in practice.[39] The American College of Sports Medicine in its latest statement recommended that weight changes could be used to determine the amount of sweat loss during exercise, and to develop specific fluid replenishment programs.[8] However, it was worth noting that the sweating rate during exercise would vary with factors such as body size, environmental conditions, exercise intensity, and adaptation status, and it was difficult to accurately estimate the amount of sweating.[40] What's more, to formulate accurate recommendations of water intake and provide scientific guidance for athletes, there was a long way to explore these various factors, including environmental temperature, humidity, psychological stimulation of athletes, drinks type, and so on.[41,42]

In this study, there were significant differences in the plasma cortisol among the four MET groups. Some previous studies had found a clear correlation between elevated cortisol concentrations and decreased hydration status.[43-45] A systematic review and meta-analysis in 2020 showed that cortisol was significantly elevated with dehydration.[43] And a study of male tennis players found that mild dehydration (<1.5% of body weight) during singles matches was associated with increased salivary concentrations of cortisol.[44] An observational study of wrestlers also found a significant positive correlation between plasma osmotic pressure and blood cortisol concentrations.[45] Other studies have also found that physical activity was associated with increased cortisol concentrations in the blood.[46,47] A study found that professional soccer players had elevated blood cortisol concentrations after the pre-season period.[46] Another study of male adolescent athletes found significant increases in blood cortisol concentrations post-exercise and 30 minutes post-exercise.[47] These suggested that the changes in plasma cortisol in this study might be related to changes in hydration status. However, definitive conclusions about the relationship between physical activity level and cortisol could not be drawn in this study. With the exception of cortisol, most of the urinary and plasma hydration biomarkers in this study did not show the differences between groups with different physical activity levels. This might be due to the fact that there were many related factors between physical activity and hydration status. In this study, we conducted a preliminary analysis and further researches were needed to supplement the results.

In terms of strengths, this study was the first time to investigate the water intake and hydration status of young male athletes in China and to explore the relationship between water intake, hydration biomarkers and physical activity level. This study also had weaknesses. Participants in this study were all male, and the overall age was relatively young, which might not be representative of all athletes. More studies needed to be conducted among female athletes and athletes of other ages in the future. Besides, our study was conducted among 42 participants, further researches needed to be carried out in a large population.

Conclusion

Young male athletes with higher physical activity level had higher amounts of TWI and TDF than their counterparts but had similar hydration biomarkers. There was a high incidence of dehydration in athletes, and attentions needed to be paid on the intake of TDF to maintain the optimal hydration status.

ACKNOWLEDGEMENTS

We thank the participation of the subjects in Beijing, China.

AUTHOR DISCLOSURES

The authors declare no conflict of interest.

This research was funded by the National Natural Science Foundation of China (No.81673146) and CNS Research Fund for DRIs (Grant No. 2021-Water).

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