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ORIGINAL INVESTIGATION

Reliability of Urinary Dehydration Markers in Elite Youth Boxers

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Purpose: To determine the reliability and diagnostic accuracy of noninvasive urinary dehydration markers in field-based settings on a day-to-day basis in elite adolescent amateur boxers. ***Methods:*** Sixty-nine urine samples were collected daily from 23 athletes (17.3 ± 1.9 y) during their weight-stable phase and analyzed by field and laboratory measures of hydration status. Urine osmolality (U_{OSM}), urine specific gravity (U_{SG}), total protein content (T_{PC}), and body-mass stability were evaluated to determine fluid balance and hydration status. Overall macronutrient and water intake were determined using dietary records. According to their anthropometric characteristics, athletes were assigned into 2 groups: lightweight (L_{WB}) and heavyweight (H_{WB}) boxers. ***Results:*** Data presented on U_{OSM} demonstrated a uniform increment by 11.2% ± 12.8% (L_{WB}) and 19.9% ± 22.7% (H_{WB}) ($P < .001$) over the course of the study, even during the weight-stable phase (body mass, ICC = .99) and ad libitum fluid intake (42 ± 4 mL · kg^{-1} · d^{-1}). The intraclass correlation coefficients (ICCs) ranged from .52 to .55 for U_{SG} and .38 to .52 for U_{OSM}, further indicating inconsistency of the urinary dehydration markers. Poor correlations were found between U_{SG} and T_{PC} metabolites ($r = .27$, $P = .211$). ***Conclusions:*** Urinary dehydration markers (both U_{SG} and U_{OSM}) exhibit high variability and seem to be unreliable diagnostic tools to track actual body-weight loss in real life. The ad libitum fluid intake was apparently inadequate to match acute fluid loss during and after intense preparation. The applicability of a single-time-point hydration-status assessment concept may preclude accurate assessment of actual body-weight deficits in youth boxers.

Keywords: combat sports, urine, validity

Urine specific gravity (U_{SG}) is a fast, noninvasive measure of urine concentration commonly used to characterize hydration status of athletes. Therefore, the National College Athletic Association (NCAA) imposed U_{SG} assessment in 1998 as a mandatory regulation to prevent the occurrence of tragic deaths associated with vigorous weight-reduction efforts in college wrestling.[1] Such an approach was found suitable to attenuate health-related consequences of weight-loss protocols in adolescent wrestlers.[2] Conversely, the International Olympic Committee does not have established policies (besides official weigh-in) to discourage aggressive weight-reduction despite the tragic events in combat sports, including death.[3,4] Recently, Reljic et al[5] proposed that adverse health-related issues in adolescent boxers originate from body-fluid manipulations, primarily achieved by acute dehydration, to meet competitive body weight. For example, whole-body fluid manipulations (by 4%) during adolescence may affect body development through changes in endocrine mechanisms, causing a reduced number of growth-hormone receptors in wrestlers.[6] Pettersson and Berg[7] recently criticized the efficiency of official weigh-in to prevent the occurrence of excessive hypohydration prevalence, as ~90% of athletes were characterized as hypohydrated on competition day via U_{SG} measurement. The aforementioned authors suggested that Olympic combat athletes who weigh in during the morning of their bout (judokas and boxers in particular) are at greater risk of serious hypohydration than other combat athletes, mainly due to shorter recovery time between official weigh-in and the beginning of competition.[7]

Although U_{SG} and urine osmolality (U_{OSM}) were suggested as the most commonly used dehydration markers among Olympic combat athletes,[8] an ongoing debate persists in the literature regarding noninvasive whole-body fluid-deficit characterization in this athletic community. For example, a cross-sectional study of Fernandez-Elias et al[9] recommended U_{SG} as a valid alternative to track fluid deficit in Spanish combat athletes on the basis of the 79% common variance with U_{OSM}. Likewise, another study found U_{SG} to be a superior dehydration index to track for low-level whole-body fluid deficit (eg, 1–3%) compared with plasma osmolality in well-trained athletes.[10] Alternatively, a recent study established that urinary dehydration markers (both field and laboratory) exhibit large variability and inconsistency (U_{OSM} increased by ~16% over a 7-d period) in elite youth boxers, regardless of stable body mass or adequate macronutrient intake.[11] Sing and Peters[12] reported poor correlation between percentage change of body mass and U_{OSM} ($r = .09$, $P > .05$) during a 3-day running competition, implying discrepancy in measurement resolution during an actual competition. Finally, Cheuvront et al reported U_{OSM} as highly variable and inconsistent,[13] recommending that spot urine specimens should not be used as the primary method for measuring hydration status in athletes.[14] Thus, it seems that the equivocal findings regarding assessment of an athlete's hypohydration level may preclude any accurate determination of actual body-fluid balance.

Aside from the obvious subsequent fluid deficits, several other important factors may interfere with the diagnostic accuracy of U_{SG}. Factors such as increased muscle mass,[15] urine metabolite clearance,[16] and high-protein diets[17] have been documented to artificially increase urine concentration, independently of actual hydration status. Indeed, several studies on combat athletes observed urine concentration increase, resulting in a subsequent violation of the U_{SG} cutoff (>1.020 g/mL), even during the weight-stable period.[11,18–20] For example, Reljic et al[19] demonstrated that regardless of body-water and plasma-volume maintenance, U_{SG} readings increased from 1.018 ± 0.008 to 1.025 ± 0.008 g/mL over

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a 1-month investigation in a control group of elite German combat athletes. Considering these issues, reports on the relative reliability of noninvasive urinary dehydration markers on a day-to-day basis in combat athletes remain scarce. Applicability of noninvasive screening tools to distinguish between hydrated and dehydrated athletes is debatable, which is supported by the conflicting evidence in the current literature and generates a need for further investigation. The equivocal findings in literature may partially originate from factors such as diversity in methodology, including differences in research design (eg, real-life vs controlled laboratory settings, cross-sectional vs randomized investigations) that preclude a conclusive position stand in recent research. Moreover, previous field-based research in combat sports was dominantly single-sampling time-point assessment, focused solely on the widely accepted theoretical postulate of urinary-dehydration marker cutoff-point violation (1.020 g/mL), established in artificial laboratory settings. Alternatively, in real-life settings combat athletes typically fail to meet guidelines for fluid replacement during intense preparation[5,7,11,20] irrespective of ad libitum access to fluids, thereby causing relevant fluctuations in their hydration status.

Therefore, the aim of this study was to evaluate the interday or relative reliability of urinary dehydration markers in real-life settings. Additionally, this study examined the influence of urine protein metabolites on the diagnostic accuracy of U_{SG} in youth boxers during their preparation period.

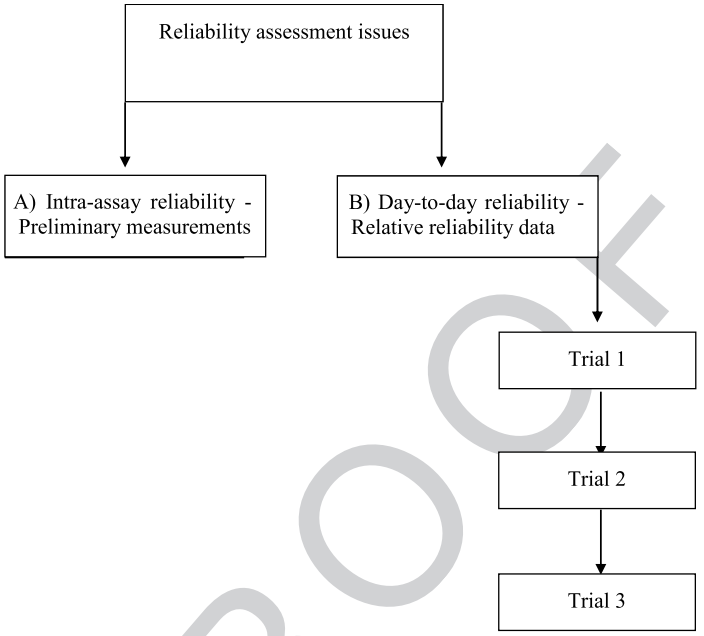

Figure 1 — Reliability of urine-concentration analysis. (A) Reproducibility of the measurement resolution itself. (B) Relative reliability—stability of data acquired throughout the investigation.

Methods

Participants

Twenty-three youth boxers (mean ± SD age 17.3 ± 1.9 y, body height 1.74 ± 0.08 m, body-mass 66.8 ± 11.8 kg), all national champions from 3 different European countries (including 14 medal winners from previous European Youth Champions), volunteered to participate in the present study. A medical-history questionnaire was administered prior to any data collection. All boxers reported a negative doping result from a previous national championship, with no previous history of any renal or urinary tract disease or any known cardiovascular disease. The present study was approved by the University of Split institutional research ethics board prior to data collection, in accordance with the Declaration of Helsinki. Written informed consent was obtained from each athlete prior to participation in the study. For boxers under the age of 18 (n = 20, age range 17–18 y), written informed consent was provided by their parent or legal guardian.

Study Design

This observational research was performed during 1 month of a mutual training camp in June 2015 in Subotica, Serbia, prior to a continental championship. Athletes were instructed to withdraw from caffeine, diuretics, and dietary supplements and sauna exposure over the course of the study. National-team managers agreed that during the baseline week of preparation, all boxers would follow the same training, diet, and rest patterns suggested by a previous investigation for youth boxers.[11] To mimic real-life settings, researchers did not interfere with training load. The present study was separated into 2 protocols: preliminary measurements and urine-sampling trials (details outlined in Figure 1). First, measurements included preliminary determination of body-mass stability immediately on waking (at 7:15 AM, days 1–3), measurement of anthropometric characteristics, answering the validated questionnaire on weight-loss history, and explanation by a trained dietitian on how to document dietary intake. To remove possible confounding factors of weight-loss habits, a validated questionnaire on weight management and supplement consumption was given to all athletes.[21] Based on their previous weight-management history (time span from previous weight-loss experience, supplement consumption), 23 boxers were selected for inclusion in the study, while 3 were excluded for not meeting pretesting guidelines. Next, in the second part of the study, urinary dehydration markers were measured on a daily basis for 3 consecutive days, in parallel with body-mass determination, in accordance with the American College of Sports Medicine's recommendations.[22] Athletes did not train between 8:00 PM and 8:00 AM prior to the urine-concentration assessment; they had free access to their water bottles throughout the entire investigation, including their boxing practice; and investigators did not encourage boxers to hydrate. Finally, in agreement with the previous studies,[23] athletes were assigned into 2 groups: lightweight (L_{WB}, from flyweight to welterweight, n = 11) and heavyweight (H_{WB}, from middleweight to superheavyweight, n = 12) boxers.

Anthropometric Measurements

Body mass was determined by using Tanita BC-418 (Tanita Corp, Japan) while athletes were barefoot and wearing only dry underwear. Percentage body fat and muscle-mass proportion were estimated according to the equations established by Carter[24] and recently applied in an athletic population by Hamouti et al[23] via 6-site skinfold-thickness measures (eg, subscapular, suprailiac, abdominal, triceps, front thigh, medial calf) taken to the nearest 0.2 mm (in triplicate, whereas the mean values were computed for further analysis) and 2-site skeletal breadths (eg, bicondylar and

radioulnar) on the right side of the body, using a Harpenden skinfold caliper and anthropometer (Holtain Ltd, UK).

Urinary Analysis

All boxers were provided with 3 sterile containers to collect a first morning urine specimen, and a total of 69 urine specimens were analyzed for field and laboratory measures of hydration status. Athletes turned in their first morning urine specimens before mandatory weigh-in scheduled at 7:15 AM by providing a small urine sample collected midflow from the first void. The U_{SG} readings were immediately analyzed using an AtagoPal-10s refractometer (Tokyo, Japan), which provides readings accurate to 0.001 unit. The refractometer was calibrated with distilled water before use, and a glass pipette was used to apply the urine sample to the instrument. Urine sampling was performed by the same qualified staff. Additional laboratory processing of the urine samples was handled by an experienced biochemist. Within 30 minutes after collection, the urine specimens were delivered to the Medical Biochemistry and Hematology Laboratory Humanlab, Subotica, for further analysis and were assessed at laboratory temperature (20–22°C). The samples were transported in a Styrofoam box containing ice packets with an absorbent material to avoid unnecessary mechanical disturbance. U_{OSM} was determined via freezing-point depression (−80) osmometry (Advanced Instruments, Automated Osmometer, Norwood, MA). Total urine protein content (T_{PC}) was analyzed via turbidimetry using benzenthonium-chloride determined at 404 nm (Cobas U-411 automated analyzer, Roche Diagnostics, Indianapolis, IN), following established protocols.[25]

The U_{SG} and U_{OSM} cutoff criteria for hypohydration were based on published guidelines[22] and similar investigations[9] and defined as $U_{OSM} > 701$ mOsmol/kg and $U_{SG} > 1.020$ g/mL, whereas T_{PC} cutoff (>0.10 g/dL) followed a recommendation previously reported in the literature.[26] The temperature and relative humidity in the testing facility ranged from 15°C to 18°C and from 45% to 50%, respectively, for all trials.

Dietary Intake

Dietary intake was constantly recorded over the course of the study. A trained dietitian monitored portion sizes, overall dietary intake, and volume of liquids ingested throughout the study, following previously established guidelines.[5,7,27] All boxers ingested the same meals provided by hotel staff and received instructions from the nutritionist on how to document dietary intakes, with particular attention to measuring accurate fluid volumes. Water intake was calculated indirectly from athletes' reported fluid and solid-food intakes; overall macronutrient composition was analyzed using an Open Platform for Clinical Nutrition from a Slovenian food-composition database, in agreement with Pakkala et al.[28]

Statistical Analyses

All data are presented as mean ± SD. Normality was confirmed using the Shapiro-Wilk test for all variables except T_{PC}. Differences in anthropometric characteristics between groups were tested using an unpaired *t* test. Reliability (interassay and intra-assay) of all dependent variables was estimated using intraclass correlation coefficient (ICC). To assess the interday (relative) reliability of U_{SG}, U_{OSM}, T_{PC}, and body mass in both groups, ICC and standard error of estimate (SEM) were calculated followed by the coefficient of variation (CV), which indicates within-subject variation. The U_{SG} and U_{OSM} values were entered into a 2-way repeated-measures analysis of variance (ANOVA) with group (L_{WB}, H_{WB}) as between-subjects variable and time as within-subject variable to detect any systematic biases between items. For post hoc analysis, the Bonferroni test was adopted to determine multiple comparisons. For nonnormally distributed T_{PC}, Friedman ANOVA was applied. Pearson correlation coefficient was calculated by using the mean data of each subject during the testing between U_{SG} and U_{OSM} readings and between T_{PC} and U_{SG} readings. The degree of effect was determined for dependent variables using partial eta-squared (η_p^2). The level of significance ($\alpha = .05$) was divided by 3 ($\alpha/3$) to yield a type I error rate of 0.0167 for post hoc 1-way ANOVA with 80% power to detect a difference.

Results

Intra-assay reliability results for all dependent variables demonstrated an ICC of .99 (95% CI .998 for U_{SG} to .999 for body mass). The intra-assay reliability of the skinfold-thickness measures (ICCs and 95% CI) were very reliable for the subscapular (.980; .961–.991), suprailiac (.985; .971–.993), abdominal (.99; .977–.994), triceps (.981; .980–.995), front thigh (.940; .910–.978), and medial calf (.978; .957–.990). Preliminary body-mass readings (days 1–3) were stable with ICC = .99 and F test = 0.2, $P = .621$, confirming that all boxers were in a weight-stable period. Differences between L_{WB} and H_{WB} in anthropometric characteristics are presented in Table 1.

Reliability analysis showed low ICCs for U_{SG} (ICC = .52–.54) and U_{OSM} (ICC = .38–.58) in both groups (Table 2). For U_{SG}, a significant time effect was observed ($F = 12.3$, $P = .001$, $\eta_p^2 = .4$), whereas no significant interaction and group effects were found (interaction, $F = 2.7$, $P = .331$; group, $F = 1.0$, $P = .272$). For L_{WB}, a post hoc test demonstrated no differences between time trials ($F = 3.0$; $P = .091$), whereas for H_{WB} there was a significant increase in U_{SG} ($F = 8.9$, $P = .001$, $\eta_p^2 = .5$) from trial 1 to trial 3, with no significant differences between other trials ($P = .110$, $P = .181$).

For U_{OSM}, a time effect was observed ($F = 13.3$, $P = .001$, $\eta_p^2 = .4$), whereas no significant interaction or group effects were found (interaction, $F = 1.2$, $P = .901$; group, $F = 0.1$, $P = .322$). For both L_{WB} and H_{WB}, post hoc showed increases of 11.2% ± 12.8% (L_{WB} $F = 5.2$, $P = .001$, $\eta_p^2 = .4$) and 19.9% ± 22.7% (H_{WB} $F = 10.2$, $P = .001$, $\eta_p^2 = .5$), respectively, between trials 1 and 3, with no significant differences between other trials (L_{WB} $P = .20$, $P = .27$; H_{WB} $P = .32$, $P = .410$). Reliability analysis showed excellent body-mass stability during the day-to-day urine-sampling protocol (ICC = .99). T_{PC} readings increased from trial 1 to trial 3 by 29.5% ± 51.8% in H_{WB} ($P = .001$).

Table 1 Anthropometric Characteristics of the Boxers

	Lightweight		Heavyweight	
Variable	Mean ± SD	Range	Mean ± SD	Range
Age (y)	17.2 ± 0.4	16–18	17.5 ± 0.7	16–19
Body mass (kg)	57.4 ± 4.6*	51–64	76.9 ± 7.7	65–91
Body height (m)	1.69 ± 0.06*	1.6–1.8	1.79 ± 0.08	1.7–1.9
Body fat (%)	8.2 ± 2.3*	6–8	10.6 ± 3.3	8–12
Muscle mass (kg)	37.7 ± 3.0*	34–41	49.5 ± 4.5	42–55
Sum of 6 skinfolds (mm)	38.3 ± 4.3*	33–44	56.1 ± 12.8	43–79
Experience (y)	7.1 ± 1.2	6–8	7.0 ± 1.1	6–8

*Different from heavyweight ($P < .05$).

High positive correlation was observed between mean U_{SG} and U_{OSM} values ($r = .87$, $P < .001$, 95% CI = .72–.94), whereas the association between U_{SG} and T_{PC} was not ($r = .27$, $P = .210$, 95% CI = .16–.62). As evident from mean values, the overall dietary intake was stable throughout the study (Table 3). The average values for urinary indices were $U_{SG} = 1.028 \pm 0.003$ g/mL and $U_{OSM} = 1036 \pm 158$ mOsmol/kg. The average weight loss during training was 1.9% ± 3.2% of body mass. Finally, data concerning the boxing-training protocols during the investigation period are presented in Table 4.

Discussion

This was the first study involving Olympic combat athletes that evaluated relative reliability of urinary dehydration markers. Data presented on U_{SG} and U_{OSM} demonstrated high variability and inconsistency (ICC = .38–.55) even during the weight-stable period and ad libitum fluid intake. Apparently, the ad libitum fluid intake was inadequate to match acute fluid loss after intense preparation. Finally, the urine protein metabolites did not interfere consistently with the diagnostic accuracy of U_{SG} readings, as poor correlations were observed.

Reliability of the Urinary Dehydration Markers

The present study aimed to examine the measurement resolution of urinary dehydration markers by following previously established guidelines for field-based investigations.[7,11,12] First, even with the weight-stable period, U_{OSM} readings increased by ~11% (L_{WB}) and ~20% (H_{WB}), suggesting inconsistency and high day-to-day variability for both groups of boxers during the preparation period. Despite the replication of daily training routines and ad libitum dietary and water intake, we observed low ICCs for both urinary dehydration markers in these athletes. Generally, an increase in urine concentration originates from whole-body fluid deficit and subsequent body-mass reduction. Alternatively, research summarized by Akerman et al[29] suggested that the concentrating ability of the kidneys is enhanced by 40% to 50% after short-term hypohydration. Indeed, short-term hypohydration may occur in combat athletes who perform repeated bouts of intense training the same day or on consecutive days,[30] whereas the desire to drink does not normally occur until water loss reaches a mild hypohydration threshold (2% of body mass).[31] Accordingly, several underlying mechanisms could explain the whole-body fluid fluctuations, since the consecutive boxing workload was imposed throughout our investigation.

In a study looking at the increase of U_{SG} readings, Hamouti et al[10] suggested that it was due to the acute response of the renal system itself to 3-hour intense exercise in heat, as opposed to reflecting a hypohydrated state exclusively. This was based on findings that the concentration of urea in the urine continued to increase (from 327 to 520 mmol/L) 11 hours postexercise, reflecting the renal system's ability to concentrate urine. Thus, the inconsistency of the urinary dehydration markers (ICC = .38–.55) does not exclusively originate from whole-body-weight deficits in these athletes. Indeed, the onset of excessive sweat secretion (1.2–1.5 L/h) after consecutive boxing sessions may affect osmotic equilibrium further, leading to excessive

Table 2 Reliability Analysis of Urinary Dehydration Markers, Mean ± SD

Variable	Trial 1	Trial 2	Trial 3	ANOVA	CV	SEM	ICC	95% CI
Lightweight boxers								
urine specific gravity	1.027 ± 0.004	1.029 ± 0.003	1.029 ± 0.002	3.02	0.008	0.001	.52	.20–.78
urine osmolality	987 ± 127	1038 ± 68	1085 ± 80[b]	5.16[a]	0.03	57.5	.38	.05–.72
total protein content	0.11 ± 0.07	0.12 ± 0.06	0.12 ± 0.05	0.31[c]	0.09	—	—	—
body mass	57.4 ± 4.6	57.5 ± 4.7	57.5 ± 4.8	0.22	0.002	1.12	.99	.998–.999
Heavyweight boxers								
urine specific gravity	1.025 ± 0.005	1.027 ± 0.004	1.030 ± 0.002[b]	8.90[a]	0.001	0.001	.54	.23–.83
urine osmolality	952 ± 179	1031 ± 164	1033 ± 127[b]	10.20[a]	0.04	82.3	.58	.21–.81
total protein content	0.09 ± 0.04	0.09 ± 0.03	0.11 ± 0.03	3.31[c]	0.08	—	—	—
body mass	76.9 ± 7.7	76.9 ± 7.6	76.9 ± 7.7	0.30	0.001	1.03	.99	.998–.999

Abbreviations: ANOVA, analysis of variance; CV, coefficient of within-subject variation; ICC, intraclass correlation coefficient; SEM, standard error of the estimate. [a] Significant time main effect ($P < .05$). [b] Significantly different from trial 1 ($P < .05$). [c] Significant time effect for nonparametrically distributed data ($P < .05$).

Table 3 Mean Macronutrient- and Water-Intake Values, Mean ± SD

	Lightweight Boxers			Heavyweight Boxers				
Variable	Trial 1	Trial 2	Trial 3	Trial 1	Trial 2	Trial 3	All athletes	95 % CI
Energy intake ($kcal \cdot kg^{-1} \cdot d^{-1}$)	34 ± 4	34 ± 4	35 ± 3	35 ± 2	35 ± 4	35 ± 3	35 ± 3	28–38
Water intake ($mL \cdot kg^{-1} \cdot d^{-1}$)	42 ± 4	42 ± 6	43 ± 5	43 ± 3	42 ± 5	43 ± 4	42 ± 4	34–49
CHO intake ($g \cdot kg^{-1} \cdot d^{-1}$)	2.9 ± 0.4	2.8 ± 0.3	2.8 ± 0.4	3.0 ± 0.2	2.9 ± 0.3	3.0 ± 0.3	2.9 ± 0.3	2.6–3.2
Protein intake ($g \cdot kg^{-1} \cdot d^{-1}$)	2.1 ± 0.3	2.0 ± 0.2	2.0 ± 0.3	2.0 ± 0.4	2.1 ± 0.3	2.1 ± 0.1	2.1 ± 0.3	1.8–2.4
Fat intake ($g \cdot kg^{-1} \cdot d^{-1}$)	1.7 ± 0.1	1.8 ± 0.2	1.7 ± 0.2	1.9 ± 0.2	1.8 ± 0.2	1.8 ± 0.3	1.8 ± 0.2	1.6–2.0

Note: All calculations based on preliminary body-mass measurement. Macronutrient- and water-intake data were obtained over the course of the study.
Abbreviations: 95% CI, confidence interval; CHO, carbohydrate.

Table 4 General Training Distribution During the Investigation Period

Training schedule	Monday [a,b]	Tuesday [b]	Wednesday [b]	Thursday [c]	Friday [c]	Saturday [c]	Sunday
Morning (8:00–9:30 AM)	5-km run; 3×3 SB	8-km run; 3×3 SB	5-km run; 5×3 SB	8-km run; 3×3 SB	5-km run; 3×3 SB	Rest	8-km jogging
Evening (6:00–7:30 PM)	9R×3:30 min SSD; 3R×5 min HBW	10R×3:30 min SSD; 3R×5 min HBW	6R×3 min RS; 5×3 SB3; R×5 min HBW	10R×3.30 min; SS	9R×3:30 min SSD; 3R×5 min HBW		3R×3.30 min SS; 3R×3:30 min SSD; 3R×3 min HBW

Abbreviations: HBW, heavy-bag workout; R, round; RS, rope skipping; SB, shadow boxing; SS, sparring session; SSD, sport-specific drills.
[a] Day 1 of the investigation. [b] Preliminary data collection. [c] Urine sampling.

fluctuations in plasma osmolality of 5 to 10 mOsmol/kg.[32] Such small changes in plasma osmolality are buffered by the large oscillations in U_{OSM} via the exquisite renal sensitivity to hormonal disturbance (elevated ADH), whereby an ~1-mOsmol/kg increase in plasma osmolality can dictate a rise in U_{OSM} by ~90 mOsmol/kg.[33] It is important to note that renal blood flow declines by 30% to 40% in trained athletes during intense exercise and causes acute suppression in the urine-concentrating ability of the renal system.[34] However, the urine samples in this investigation were collected after 12 hours had passed from previous training; therefore, it is likely that a surrogate mechanism of kidney function (eg, the autoregulation of glomerular filtration rate that enables renal water retention and subsequent concentrated urine output) is possible here.

Maughan et al[35] proposed that excessive rates of anaerobic glycolysis induced by high-intensity exercise (>70% VO_2max.) result in an increase in osmolality of active muscles due to an accumulation of glycolytic intermediates, leading to a whole-body osmolality increase. This is particularly noteworthy for boxers, since the metabolic demands of high-level boxing rely on anaerobic glucose breakdown to fuel energy requirements. Data concerning the overall macronutrient and water intake are consistent with observations of Reljic et al,[5] who also reported an exceptionally low dietary intake (total energy intake 31 ± 8 kcal/kg and 1.8 L of water consumed) in a control group of youth German boxers during their weight-stable period. Pettersson and Berg[7] reported water intake of 38 ± 11 mL/kg body mass in judo and boxing athletes who attempted to maximize rehydration over a 4- to 6-hour recovery period between official weigh-in and beginning of the competition; they observed higher values of $42 \pm 4\ mL \cdot kg^{-1} \cdot d^{-1}$. Apparently, the combination of inadequate carbohydrate and water intake and intense boxing preparation caused inconsistent first morning urine readings in these highly trained athletes. This explains the discrepancy between data presented and the reliability analysis shown by Hamouti et al,[23] who reported satisfactory daily U_{SG} values (ICC = .76) in parallel with a basic training regimen for their athletes, whereas our athletes were engaged in intensive sport-specific preparations. Likewise, Shirreffs and Maughan[18] have documented that boxers do produce higher first morning U_{OSM} values (775 ± 263 vs 627 ± 186 mOsmol/kg, $P < .05$) than non-weight-class athletes.

Methodological Issues and Measurement Resolution

Typically, previous combat sports investigations[9,36] have used cross-sectional research designs to determine diagnostic accuracy of multiple dehydration markers, usually sampled on the competition day, when body mass is at its most unstable. For example, Oppinger et al[36] criticized hydration-status assessment via U_{SG} due to a 37% U_{SG} false-positive rate among 51 male wrestlers. Meanwhile, in the aforementioned investigation, the authors did not observe supplement use and history of fluid consumption prior to testing, which were both recently confirmed to be important methodological prerequisites of such studies.[9] Notably, regardless of a high day-to-day variability of both U_{SG} and U_{OSM}, correlation analysis ($r = .87$, $P < .001$) corroborates the report on the strong association between urinary dehydration markers by Fernandez-Elias et al,[9] who imposed U_{OSM} as a valid, noninvasive hypohydration marker for combat athletes. However, the aforementioned study may have overemphasized the role of correlation analysis in the context of actual measurement validity of whole-body fluid deficits, especially based on single urine samples taken at 1 time point before a competition. Indeed, their conclusions on U_{OSM} validity originated from a cross-sectional study with no data supporting the reliability assessment.[9] Reliability is generally accepted as a stepping stone in validity analysis, so the question remains as to what extent U_{OSM} could represent a valid measure of hypohydration for combat athletes. In this context, a review article criticized the use of a single-time-point hydration-assessment concept and suggested that spot urine specimens should not be used as the primary method for assessing hydration status,[14] as the urinary index can be confounded by fluid intake, diet, and exercise, leading to false positive findings.

Factors Influencing Diagnostic Accuracy of USG

This was the first study to report T_{PC} readings on a day-to-day basis in combat sports research, which is important to determine the diagnostic accuracy of U_{SG}. It has been previously documented that strenuous exercise reduces the glomerular filtration rate and thereby increases concentration of urine protein metabolites and influences the U_{SG} accuracy independent of hydration status.[16] In H_{WB} group T_{PC} values increased by ~30% in parallel with U_{SG} and U_{OSM} increments. Conversely, Hamouti[23] reported a high positive correlation between U_{SG} and urine protein metabolites ($r = .92$, $P < .01$), and that study found poor correlations between U_{SG} and T_{PC} ($r = .27$, $P = .210$), implying that the interassay reliability of single urine sampling of T_{PC} may remain skewed. This is rather noteworthy, as the presence of T_{PC} could potentially mask actual fluid-deficit characterization via U_{SG}. Indeed, Fernandez-Elias et al[9] showed that regardless of an overall high-positive correlation between U_{SG} and U_{OSM} ($r = .89$, $P < .001$), U_{SG} measures were not as tightly correlated in their most severely dehydrated group ($r = .61$) as in the moderately or less dehydrated athletes; certainly, this discrepancy between groups was likely influenced by the presence and/or clearance of metabolite concentration in the single urine sample, although T_{PC} was not measured in that study.

This study followed established protocols[5,7,12] to generalize the renal function of highly trained athletes in a field-based setting and was restricted to quantifying hydration markers in a real-life scenario during which athletes were not accessible to undergo a 24-hour urine-sampling protocol. Although dietary intake was standardized in terms of range of foods on offer and meal times, the exact quantities of food and fluids ingested were not independently weighed in this study. The overall macronutrient and water intake was summarized in grams as suggested by Pettersson and Berg.[27] Nevertheless, we can advocate for internal validity maintenance during this research, as boxers maintained the same training patterns during the study without any endorsement of weight-reduction practices or use of high-protein diets.

Practical Applications

Urinary dehydration markers continued concentrating over the course of the study, despite body-weight stability and ad libitum fluid intake. Thus, coaches and athletes should reconsider their current fluid-replacement strategies during intense preparation periods, since ad libitum fluid intake was apparently insufficient to attenuate excessive fluctuations in U_{SG} and U_{OSM} readings. Therefore, stand-alone hydration-status assessment, derived from single urine-specimen readings, appears to be an inappropriate diagnostic tool to screen for whole-body-weight reductions, especially in real-life scenarios and during an intense preparation

period. Note that habitually low dietary intake appears to be overlooked in terms of the possible detrimental health consequences in adolescent boxers and therefore may require further intervention of the governing authorities, parents, and coaches to increase awareness of health-related issues originating from excessive hypohydration. Correspondingly, an intervention from a trained dietitian to improve athletes' macronutrient and water-replacement habits is essential.

Conclusions

Although all procedures were standardized followed by a weight-stability period, ad libitum fluid consumption, and macronutrient intake, daily urinary dehydration markers were highly variable, even at the early stages of the preparation camp. Apparently, ad libitum fluid intake did not attenuate the increment of urinary dehydration markers in these boxers during an authentic preparation period. Total urine proteins were not correlated to U_{SG}, indicating that the interassay reliability of urine sampling may remain skewed, especially for samples that have increased protein content. Thus, despite being a popular concept in the combat-sports community, field determination of hydration status via urinary indices could be misleading. Collectively, one should avoid assessing hydration status via urinary dehydration markers based on single sampling time point, which is typically done to determine hydration status of combat athletes on competition day.

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Queries

Q1. Please ensure author information is listed correctly here and within the byline.

Q2. “the association between USG and TPC was not ” . . . what? Please clarify.

Q3. Please provide volume editor(s) name for ref 24.