


# Sex differences in hydration status among adolescent elite soccer players

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## ABSTRACT

Hydration of athletes may affect performance and health status likely with differences among sexes. We studied the impact of sex on hydration behaviour in adolescent athletes. Hydration status and urine markers were investigated in 7 female and 7 male elite 16 years-old soccer players in temperate climate (21-24°C). Participants consumed water *ad libitum* during the first training session (LIB), whereas in the second session (HYD) they drank a water amount matching 70% of sweat loss from the LIB session. Post-training performances were evaluated by Yo-Yo intermittent recovery level 1 (IR1) test and countermovement jump (CMJ). Body mass values were recorded and urine samples were collected before and after each experimental session. Males drank a double amount of water in HYD (1.19±0.21 kg) compared to LIB (0.62±0.19 kg;  $p=0.001$ ; ES=2.88), resulting in a lower percentage body mass loss (HYD -0.95±0.63% versus LIB -1.59±0.33%;  $p=0.044$ ; ES=-1.35); total distance of Yo-Yo IR1 was higher, albeit not significantly, in HYD (2953±779 m) than in LIB (2103±939 m); CMJ performance was unchanged. In females, water drunk, body mass, Yo-Yo IR1 and CMJ did not vary in HYD versus LIB sessions. In adolescent males a 70% sweat replacement personalized hydration regimen reduced body mass loss and tended to improve performance, whereas in females *ad libitum* water drinking allowed to maintain hydration status (<1% body mass loss). Our results suggest that coaches and athletes themselves should consider a personalized hydration regimen for adolescent male soccer players, whereas *ad libitum* drinking seems suitable for females. **Keywords:** Dehydration; Drinking to thirst; Female athletes; Male athletes; Gender; Urine biomarkers; Weight loss; Sweat.

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## INTRODUCTION

Optimisation of athletes' hydration status is an issue of continuing research. The euhydrated state was shown to attenuate thermal, physiological and perceptual strain (Ersoy et al., 2016; Maughan & Shirreffs, 2008). Conversely, improper hydration strategies before, during, and after sport activity not only can impair athletic performance (Cheuvront et al., 2005; Maughan & Shirreffs, 2010), but may also adversely impact athletes' health (Benton et al., 2015; Siegel, 2015). Specifically, an improper hydration status was associated with development of hyperthermia, increased heart rate, increased glycogen use, and increased rate of perceived exertion (Logan-Sprenger et al., 2015; Périard et al., 2014). Interestingly, a study on 107 suboptimally hydrated young male soccer players (age 11-16 yr) showed that *ad libitum* fluid intake did not prevent dehydration under warm weather conditions (Arnautis et al., 2015). Furthermore, a recent study (Buoite Stella et al., 2017) performed on athletes (age: 8-63 years, median: 21±13 years) across all types of sport showed that only 6.5% out of 352 athletes reported fluid intake during their regular training at or above currently NATA and ACSM suggested hydration guidelines, namely drinking between 0.2-0.3 L for every 10-20 min of exercise performed (Goulet, 2012; Sawka et al., 2007). Thus, individual hydration should be monitored to ensure that each athlete is euhydrated before and after sport activity (Ersoy et al., 2016; Maughan & Shirreffs, 2008; Périard et al., 2014).

General recommendations about athlete hydration include avoiding an excessive body mass loss (Goulet, 2012; Sawka et al., 2007). Although the threshold is still debated, body mass losses exceeding 2% of pre-exercise body mass are commonly associated to adverse effects (Cotter et al., 2014; Goulet, 2012). A smaller exercise-induced body mass loss (below 1%) is considered a condition not affecting performance (Périard et al., 2014; Phillips et al., 2014), even if it can increase heart rate, finally resulting in cardiovascular stress (Casa et al., 2010). On the other hand, a post-exercise increase of body mass due to overdrinking beyond thirst should also be avoided (Almond et al., 2005; Cotter et al., 2014; Sawka et al., 2007). Of concern, overdrinking has been associated to a condition defined "exercise induced hyponatremia", which can have severe health consequences, up to life-threatening hypotonic encephalopathy (Almond et al., 2005; Hew-Butler et al., 2017). This hydration strategy was put under debate after the tragic death of two female charity marathon runners in 2003 that practiced it (Almond et al., 2005).

The *ad libitum* strategy of fluid intake, where athletes are free to drink on their will, started to be suggested likely because of the fear that planned drinking might cause overdrinking and thus exercise-induced hyponatremia (Almond et al., 2005; Judge et al., 2016; Siegel, 2015). However, despite the *ad libitum* strategy is generally expected to avoid detrimental levels of dehydration even in young athletes (Rowland, 2011), it is not always sufficient to result in euhydration (Purcell, 2013; Arnautis et al., 2015; Ersoy et al., 2016). Consequently, recent research points to optimise hydration through personalized strategies (Maughan & Shirreffs 2008; Périard et al., 2014).

Following this line of thinking, a study performed on ten 23 years-old male tennis players (Périard et al., 2014) evaluated the effects of a hydration strategy during the tennis match that provided an amount of fluids corresponding to 70% of athletes' sweat loss, as compared to a previous similar match where the same athletes could drink *ad libitum*. Both hydration regimens resulted in minimal body mass loss (<1%), suggesting that the athletes were able to spontaneously prevent dehydration (Périard et al., 2014). Similarly, a group of adolescent male soccer athletes spontaneously replaced on average about 71% of fluid losses across the training sessions by *ad libitum* drinking, finally resulting in minimal hypohydration (<1%) (Phillips et al., 2014).

Hydration status among athletes can differ according to the sport played and the elite and non-elite athletic level. Soccer is an outdoor team sport, where athletes usually show moderate-to-intense metabolic activity (Shirreffs et al., 2005). Outdoor physical exercise implies exposure to variable conditions of temperature, humidity, and wind; as a consequence, athletes might experience a variable grade of dehydration (Williams & Blackwell, 2012), especially when exercising in hot conditions (Racinais et al., 2015). As a matter of fact, an about twice higher sweat rate was reported for a group of elite professional soccer players training in the heat (Shirreffs et al., 2005) compared to a group of young male soccer players exercising at a lower ambient temperature (about 21 °C).

Some evidence in adults indicates that males compared to females have greater sweat losses, resulting in different exercise induced changes in body composition and hydration status, although fluid intakes (expressed per unit body mass and unit time) were similar (Weitkumat et al., 2012). Similarly, a group of adolescent male swimmers lost more fluid relative to initial body mass but drank no more than females (Higham et al., 2009). Paucity of information is available on adolescent female soccer players (Gibson et al., 2012), as the majority of studies have been carried out only on males (Arnaoutis et al., 2015; Da Silva et al., 2012; Ersoy et al., 2016; Gordon et al., 2015; Phillips et al., 2014; Williams and Blackwell, 2012). Notably, females can differ from males for many hormonal and physiological characteristics (Cauci et al., 2017; Stachenfeld, 2008), including antidiuretic hormone (ADH) responses through ADH receptors affecting hydration status (Liu et al., 2011). No study, so far, explored sex differences in adolescent soccer players undergoing a similar training protocol under the same environmental conditions.

The aim of this study was to explore sex differences in hydration behaviour, pre- and post- exercise differences in hydration status, urine parameters, and performances in adolescent elite soccer players undergoing an intensity-matched training session. In addition, we wanted to assess the effects of a specific personalized hydration strategy (HYD) based on a fixed 70% replacement of sweat loss in respect to a previous *ad libitum* session (LIB), a protocol adopted previously by other authors in different sports (Périard et al., 2014). The effects on performance of the two fluid consumption strategies were evaluated both in male and in female athletes by specific sport tests, namely the Yo-Yo intermittent recovery level 1 (IR1) (Bangsbo et al., 2008), and the countermovement jump (CMJ) test (Rodriguez-Rosell et al., 2017).

## MATERIAL AND METHODS

### **Participants**

Fourteen Caucasian adolescent under-17 National league soccer players including 7 females and 7 males were enrolled (they were components of the adolescent female and male teams of the same soccer society in Tavagnacco and Udine, respectively). Inclusion criteria were age (all athletes had to be 16 years-old) and the regular participation to the usual training sessions. The weekly exercise volume (including training and games) was about 5 hours for females and 8 hours for males; the average number of matches played per year was 44 for both sexes.

The data collected on the 14 adolescents concerning the main outcome of the study, that is the difference between sexes in the amount of water drunk in the two sessions (LIB and HYD), were used as preliminary data to calculate the number of volunteers to be recruited. Post hoc analysis revealed a power of 0.91, for an effect size of 2.45 and assuming a two tailed  $\alpha$  error <0.01. On the basis of this result, recruitment was stopped.

The study was approved by the Local Ethics Committee, accordingly with the Declaration of Helsinki. Each participant and his/her parents were informed of the study aims, requirements and risks before entering the study. Each participant and parents gave written informed consent.

### **Study design**

The entire study was conducted in temperate climate. Participants were invited for assessment of baseline total body mass and lean/fat mass the week before the first experimental sessions. Subsequently, two experimental training sessions were carried out during which athletes performed a soccer training as close as possible to the usual ones; the training consisted first in 5 minutes of moderate running, 20 minutes of conventional soccer warm up followed by 40 minutes of simulated match; just thereafter, the athletes were asked to complete the performance tests to assess the possible effects of dehydration. The Yo-Yo IR1 test (Bangsbo et al., 2008) provides a simple and valid way to obtain information about an individual's capacity to perform repeated intense exercise (i.e. to maximally activate the aerobic system) and is widely used to examine changes in performance (Bangsbo et al., 2008; Krstrup et al., 2003). In turn, the countermovement jump (CMJ) test is used to assess explosive power (Rodriguez-Rosell et al., 2017), i.e. the maximal power output developed by the lower leg extensor muscles during a voluntary abrupt vertical jump. Because of its high reproducibility and simplicity, CMJ is appropriate also for field conditions (Castagna & Castellini, 2013; Rodriguez-Rosell et al., 2017).

The two experimental sessions differed only for the adopted hydration strategy: in the first session (LIB) athletes were allowed to drink water ad libitum, i.e. at their will; in the second session (HYD), accordingly to a protocol adopted previously by other authors on adults (Périard et al., 2014), the individual hydration strategy was based on a fixed 70% replacement of each athlete's sweat loss in the LIB training session (calculated as the difference in post- minus pre- session body mass plus the weight of water drunk). None of the females were menstruating in either session. The phase of menstrual cycle was not acknowledged or recorded, since it has been reported previously that the menstrual cycle phase may have a minimal effect on fluid balance (Maughan et al., 1996). All the training sessions were performed in the afternoon between 4.00 PM and 5.30 PM, and lasted approximately 100 min; they were performed on April-June in Udine, a city located in the Northeast Italy, in a temperate climate (temperature ranging from 21 °C to 24 °C, wind from 7 to 14 km/h and relative humidity from 45 to 56%).

### **Procedures**

The week before the first experimental sessions, for the baseline assessment, each participant was weighed nude by a digital balance assessing 0.1 kg (EP1270, Laica, Italy) and lean and fat mass were assessed by measurement of 7 skin folds according to sex (Jackson and Pollock 1978; Jackson et al., 1980). Body mass index (BMI) was calculated by body mass (kg) divided for square of stature (m<sup>2</sup>). Average body mass was 59.8±6.9 kg and 67.2±6.0 kg in females and males, respectively, and stature was 1.65±0.04 m and 1.79±0.07 m, respectively. None of the athletes were obese (i.e., BMI over 30 kg/m<sup>2</sup>); BMI was not statistically different between sexes (21.9±2.9 kg/m<sup>2</sup> and 20.9±1.8 kg/m<sup>2</sup>, in females and males, respectively). Females had 2.6-fold higher fat mass (9.9±2.6 kg versus 3.8±0.7 kg; p=0.001), and 2.9-fold higher fat mass percentage than males (16.3±2.5% versus 5.7±0.6%; p=0.001).

In the preliminary visit, all the participants performed a baseline assessment of the Yo-Yo IR1 (Bangsbo et al., 2008) and CMJ test (Rodriguez-Rosell et al., 2017) to familiarize themselves with the performance tests (data not shown).

For both experimental sessions (LIB and HYD), upon arrival at the training ground, participants provided a urine (pre-exercise) sample, and then were weighed nude by a digital balance assessing 0.1 kg (EP1270, Laica, Italy), as for the baseline evaluations. Thereafter, athletes performed a standard soccer training; at the end of the training, athletes performed the two performance tests, i.e. the Yo-Yo IR1 test and the CMJ test, as described below; a urine (post-exercise) sample was then collected and finally body mass of each participant was determined with the same methods used in the pre-session. Thus, body mass of athletes was consistently determined with void bladder. Participants were asked to collect all urine in-between the start and the end of the training; no participant, however, had the necessity to empty his/her bladder throughout the experimental sessions.

In the first session athletes were provided with personalized labelled bottles filled with water at ambient temperature and were allowed to drink ad libitum (LIB). Each personalized bottle was weighed before and after the experimental session and the weight difference (in grams) was recorded. Players were instructed to drink only from their own bottles and not to spit out any drink; no restrictions were imposed about how quickly the fluid had to be ingested throughout the experimental session. All measurements of water amounts were performed by use of a balance assessing 0.1 grams (Gibertini 2002, Italy). The difference in athlete's body mass between post- and pre-session plus water drunk was assumed to represent total sweat loss. In the second experimental session (HYD), where hydration strategy was individualized for each athlete, a personalized bottle, containing the grams of water balancing 70% of expected sweat losses, was given to each athlete to be fully consumed during the session. Participants were allowed to request more water; two participants (a female and a male) requested an additional bolus of water (100 g) that was recorded. During both experimental sessions participants were allowed to drink only one type of commercial no gassed water (Levissima naturale oligominerale, San Pellegrino S.p.A., San Pellegrino Terme, Bergamo, Italy). Composition of water was the following: Na<sup>+</sup> 1.9 mg/L, K<sup>+</sup> 1.6 mg/L, Ca<sup>2+</sup> 20.4 mg/L, Mg<sup>2+</sup> 1.8 mg/L, HCO<sub>3</sub><sup>-</sup> 57.4 mg/L, residual dry weight at 180 °C, 80.0 mg/L, pH at spring 7.8.

No food and/or carbohydrate supplementation was admitted during the experimental sessions. Alimentary habits before sessions were those habitually used by athletes before training sessions. Each participant was asked to replicate the same meal before the two experimental sessions (LIB and HYD) (Périard et al., 2014).

### **Performance tests**

The Yo-Yo IR1 test consists of 2x20m shuttle runs at increasing speed, interspersed with 10-second of active recovery (controlled by a pre-recorded instruction voice). An individual is running until he/she is not able to maintain the speed; the distance covered at that point and the speed reached are the test result. The Yo-Yo intermittent recovery level 2 (IR2) test would likely have been a better test to assess performance in male adolescent soccer players; nevertheless, it was discarded because it was expected that females would have great difficulties in performing the Yo-Yo IR2 test (Bangsbo et al., 2008).

The CMJ tests were performed with the Ergotester, which has an optical acquisition system (Globus, Italy). Flight times (s) are measured, from which the test results, i.e. the jump height (cm) and the vertical speed, are calculated (Rodriguez-Rosell et al., 2017). Each athlete was required to perform three jumps and the best values were retained. To minimize the contribution of the upper limbs, adolescents were asked to keep their hands on the hips during the jumps.

### **Laboratory analyses**

Urine samples were analysed soon after collection in a clinical laboratory. Urine specific gravity (USG) in g/mL was determined by refractometry (iChem@VELOCITY™ system; Beckman Coulter, Inc) using Iris

Diagnostics Automated Chemistry Consumables. Urine electrolyte composition was determined by Cobas 8000 ISE module instrumentation (Roche Diagnostics, USA), by ion-selective electrode system. Analytical imprecision (CV) of clinical biochemical methods were: 0.04% for USG, 0.36% for sodium concentrations, 0.63% for potassium concentrations, and 0.71% for chloride concentrations.

**Statistical analyses**

The Shapiro-Wilk test showed that all the parameters were normally distributed. Means, standard deviations (SD), and percent change ( $\Delta\%$ ) were calculated for all continuous variables of hydration status, urine markers and performance parameters. Significant differences were identified using two sided t-tests, unpaired (for gender effect) or paired (for experimental session effect), as appropriate. All the statistically significant differences were also confirmed by use of the appropriate non-parametric tests. Statistical significance level was set to  $p < 0.05$ . Effect sizes (ES) for both paired and unpaired data were calculated by Cohen’s d, i.e. as the ratio between the difference among the two average values and the pooled standard deviation; ES thresholds of 0.2, 0.5, and 0.8 representing small, moderate, and large differences, respectively, were used (Sullivan & Feinn, 2012). Statistical calculations were performed using the Statistical Package for Social Sciences (SPSS for Windows, SPSS Inc., Chicago, IL, USA).

**RESULTS**

**Performance tests**

Table 1 shows performance characteristics of 7 female and 7 male elite adolescent soccer players at the end of the LIB and HYD sessions. As expected, males had better performances than females for all Yo-Yo IR1 test parameters ( $p < 0.001$ ) and CMJ values ( $p < 0.001$ ). In females no significant differences were found between the LIB and HYD sessions for both Yo-Yo IR1 and CMJ tests. Among males, Yo-Yo IR1 test performances in LIB session, as evaluated by either total distance (m) or final running speed (km/h), were somewhat worse compared to the HYD session, albeit the difference was not statistically significant. The males CMJ performance parameters did not differ between the LIB and HYD sessions.

Table 1. Performance of the adolescent soccer players after the two training sessions

	Yo-Yo IR1		CMJ		
	Total distance (m)	Final speed (km/h)	Flight time (s)	Height (cm)	Vertical speed (m/s)
<b>Females (n=7)</b>					
LIB	1297±434**	15.7±0.6**	0.47±0.03**	26.8±3.0**	2.28±0.13**
HYD	1233±411**	15.6±0.6**	0.47±0.03**	27.3±3.2**	2.31±0.14**
$\Delta\%$	-4.9%	-0.9%	1.0%	2.0%	1.0%
ES	-0.15	-0.23	0.16	0.17	0.17
<b>Males (n=7)</b>					
LIB	2103±939	16.7±1.5	0.55±0.01	37.5±1.4	2.71±0.05
HYD	2953±779	18.0±1.1	0.55±0.02	37.5±2.4	2.71±0.09
$\Delta\%$	40.4%	7.7%	0.0%	0.0%	-0.1%
ES	0.99	0.98	-0.01	-0.01	-0.05

\*\* $p < 0.001$ ; significantly different by comparison of females and males

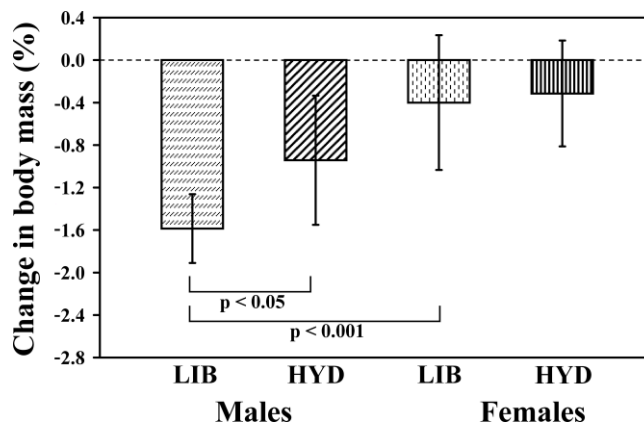
**Water and weight balances**

Table 2 summarizes body mass and body mass changes, as well as amounts of water drunk and sweat loss, of all players in LIB and HYD sessions. The pre- sessions body masses were not significantly different in both sexes. Among females, pre- and post- session body masses did not differ significantly in both LIB and HYD sessions; among males, post- session body masses were significantly lower than pre- session body masses in both LIB and HYD sessions ( $p=0.001$  and  $p=0.009$ , respectively). Specifically, in LIB session, body mass loss in males ( $-1.07\pm0.25$  kg) was significantly larger than in females ( $-0.23\pm0.33$  kg,  $p=0.001$ ,  $ES=2.91$ ), but not so in the HYD session. Interestingly, among males body mass loss change was higher in LIB session ( $-1.07\pm0.25$  kg) compared to the HYD session ( $-0.64\pm0.44$  kg;  $p=0.049$ ;  $ES=-1.24$ ).

Table 2. Body mass, body mass changes, amounts of water drunk and of sweat loss of the adolescent soccer players before and after the experimental sessions

	<b>Body mass Pre (kg)</b>	<b>Body mass Post (kg)</b>	<b>Body mass Change (kg)</b>	<b>Water Drunk (kg)</b>	<b>Sweat Loss (kg)</b>
<b>Females</b>					
LIB	59.8±6.9	59.6±6.9	-0.23±0.33	0.81±0.36	1.04±0.25
HYD	59.9±7.1	59.7±7.1	-0.17±0.26	0.74±0.17	0.91±0.30
Δ%	-	-	-25.0%	-8.6%	-12.2%
ES	-	-	-0.19	-0.26	-0.46
<b>Males</b>					
LIB	67.0±5.8	65.9±5.7*	-1.07±0.25§#	0.62±0.19	1.69±0.32#
HYD	66.8±5.8	66.1±5.7*	-0.64±0.44	1.19±0.21§#	1.83±0.48#
Δ%	-	-	-40.0%	93.2%	8.6%
ES	-	-	-1.24	2.88	0.36

\*  $p<0.05$ ; significantly different by comparison of pre vs. post session values in the same sex;  
 §  $p<0.05$ ; significantly different by comparison of LIB and HYD session values in the same sex;  
 #  $p<0.01$ ; significantly different by comparison of females versus males.

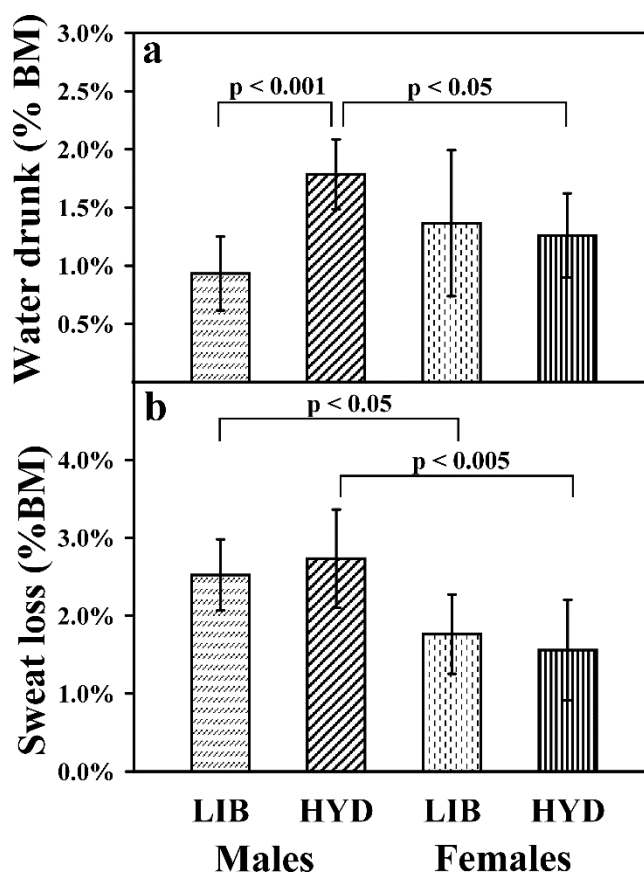


Histogram illustrating the mean percentage changes in body mass between pre- and post- experimental sessions (LIB and HYD) in the adolescent soccer players according to sex. Bars are standard deviations.

Figure 1. Changes in body mass across the experimental sessions

Percentage body mass changes of athletes is illustrated in Figure 1 as mean ( $\pm$ SD). Percentage body mass loss in males was significantly larger as compared to females in the LIB session ( $p=0.001$ ,  $ES=2.53$ ); a body mass loss was observed also for the HYD sessions, but the difference was smaller and not statistically significant ( $p>0.05$ ). Among females, no significant findings were observed by comparing the percentage of body mass changes in LIB ( $-0.40\pm0.62\%$ ) and HYD sessions ( $-0.30\pm0.48\%$ ). Conversely, among males a significantly lower percentage of body mass loss was observed in the HYD session ( $-0.95\pm0.63\%$ ) compared to the LIB session ( $-1.59\pm0.33\%$ ;  $p=0.044$ ;  $ES=1.35$ ). All the participants, except one (a male athlete who reached 2.1% loss of body mass in the LIB session) had a percentage of body mass loss  $<2.0\%$ .

Regarding the amounts of water drunk, as summarized in Table 2, in the LIB session females drank ( $0.81\pm0.36$  kg) a slightly greater amount of water compared to males ( $0.62\pm0.19$  kg,  $p>0.05$ ). However, sweat loss in females ( $1.04\pm0.25$  kg) was significantly lower than in males ( $1.69\pm0.32$  kg,  $p=0.001$ ,  $ES=2.26$ ). In the HYD session females drank ( $0.74\pm0.17$  kg) and sweat ( $0.91\pm0.30$  kg) less than males ( $1.19\pm0.21$  kg and  $1.83\pm0.48$  kg, respectively;  $p=0.001$  and  $ES > 2.33$  for both). Of note, the controlled administration of water in the HYD session (corresponding to 70% of sweat loss in the LIB session) had no significant effects among females, both as amount of water drunk and sweat loss ( $p>0.05$ ,  $ES < 0.5$  for both). Conversely, among males the amount of water drunk was about doubled in HYD versus LIB sessions ( $p=0.001$ ,  $ES=2.88$ ), despite sweat loss was not significantly different ( $p>0.05$ ).



Histograms illustrating the mean amounts of water drunk (panel A) and of sweat loss (panel B), expressed as percentage body mass (BM) in adolescent soccer players in the two experimental sessions (LIB and HYD) according to sex. Bars are standard deviations.

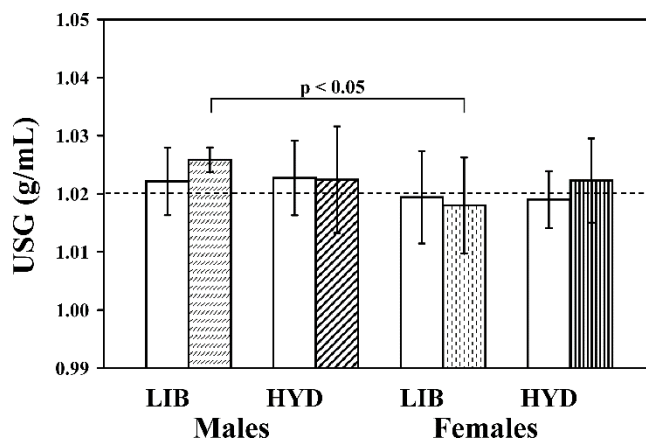
Figure 2. Amounts of water drunk and of sweat loss across the experimental sessions



The amounts of water drunk and sweat loss, expressed as percentage of body mass, are shown in Figure 2 for LIB and HYD sessions. Consistently with the absolute values, among males the amount of water drunk normalized for body mass in the HYD session was almost double than in the LIB session, despite a similar sweat loss. Among females no significant differences were observed.

**Urine analyses**

Analysis of urine specific gravity (USG) showed that this parameter did not differ between pre- and post-session in either hydration regimen (LIB and HYD) and in both sexes ( $p > 0.05$ ,  $ES < 0.67$ ) as shown in Figure 3. However, post-LIB USG in females was significantly lower than post-LIB USG in males ( $p = 0.046$ ,  $ES = 1.52$ ).



Histogram illustrating the mean urine specific gravity (USG) between pre- and post- experimental sessions (LIB and HYD) in the adolescent soccer players according to sex. Bars are standard deviations. The horizontal dotted line is the usual considered threshold for hypohydration (1.020 g/mL).

Figure 3. Urine specific gravity before and at the end of the experimental sessions

Table 3. Urine electrolytes concentrations of the adolescent soccer players before and after the experimental sessions

	<b>Na<sup>+</sup></b>			<b>K<sup>+</sup></b>			<b>Cl<sup>-</sup></b>		
	Pre	Post	ES	Pre	Post	ES	Pre	Post	ES
<b>Females</b>									
LIB	154±109	107±71	-0.43	52.8±26.8	67.1±19.1	0.54	167±123	115±82	-0.43
HYD	138±41	121±75	-0.42	69.2±33.0	80.1±32.5	0.33	153±52	146±100	-0.14
LIB+HYD	145±75	114±70*	-0.41	61.8±30.1	74.2±26.8	0.42	159±86	131±89	-0.32
<b>Males</b>									
LIB	163±52	113±45	-0.96	57.4±19.6	82.6±22.5	1.28	160±51	129±53	-0.60
HYD	163±52	99±57**	-1.22	56.9±19.2	71.2±32.3	0.74	159±52	98±55*	-1.18
LIB+HYD	163±50	106±49**	-1.14	57.2±18.7	77.0±27.4*	1.06	160±50	113±54**	-0.93

\* $p < 0.05$ ; \*\* $p < 0.01$ ; significantly different by comparison of pre vs. post session values in the same sex

Table 3 summarizes urine electrolytes concentrations before and after the LIB and HYD sessions according to sex. Overall, combining all study subjects and the two hydration regimens, urine sodium concentrations showed a profile of decreasing values in the post-sessions compared to the respective pre-sessions ( $p=0.001$ ). However, only one significant difference was noted, specifically for males post-HYD versus pre-HYD ( $p=0.001$ ;  $ES=-1.22$ ).

Over all study subjects and combining the two hydration regimens, urine potassium concentrations profile showed increasing values in the post-sessions compared to the respective pre-sessions ( $p=0.002$ ). No significant differences were observed in  $K^+$  concentrations between post- and pre- values in each specific session.

Over all study subjects and combining the two hydration regimens, urine chloride concentrations profile showed decreasing values in the post-sessions compared to the respective pre-sessions ( $p=0.005$ ). However, the only significant difference was the lower chloride concentration in males by comparing post-HYD versus pre-HYD session ( $p=0.003$ ;  $ES=-1.18$ ).

## DISCUSSION

To our knowledge, this is the first study on hydration profiles in adolescent elite soccer players which makes a direct sex comparison on subjects with the same ethnic background undergoing the same experimental protocol. Behind the expected differences in male vs. female sport performances (evaluated by Yo-Yo IR1 test and CMJ), and body fat composition, we observed some significant sex differences.

Main result of the present investigation was the striking difference between sexes in relation to water drunk in the ad libitum (LIB) versus the controlled hydration (HYD) sessions. Males drunk a significantly lower amounts of water in the LIB compared to the HYD session, whereas females drunk similar amounts of water in the two sessions. This hydration behaviour likely is responsible for the different profile of body mass changes (post- minus pre-exercise), taken as index of hydration status, observed in the two sexes. In fact, body mass changes in females did not vary comparing the LIB session versus the HYD session. Conversely, hydration status in males, as evaluated by absolute body mass loss, was improved in the HYD ( $-0.64\pm 0.44$  kg) session as compared to the LIB session ( $-1.07\pm 0.25$  kg;  $p<0.05$ ;  $ES=-1.24$ ).

By considering the percentage of body mass loss, in our study the average value (combining LIB and HYD sessions) was  $-0.4\%$  among females and  $-1.6\%$  among males; only one male participant had a body mass loss value below  $-2.0\%$  in the LIB session, whereas no participant reached this threshold in the HYD sessions. Our results are in line with the data reported previously in male adolescent tennis players (Périard et al., 2014) who underwent an identical experimental LIB and HYD hydration regimen protocol, where minimal body mass losses ( $\sim 0.3\%$ ) were found. Moreover, a study on adolescent 17 years-old soccer players (Phillips et al., 2014), allowed to drink water ad libitum during two training sessions, also showed a minimal body mass loss ( $<1\%$ ) in a cool temperature of  $13-17^\circ\text{C}$ .

Interestingly, in the LIB session our male players drunk an amount of water ( $0.62\pm 0.19$  kg) close to that observed in other young elite soccer players of similar age ( $0.43\pm 0.19$  kg) (Phillips et al., 2014) whereas in the HYD session our athletes drunk an almost double amount of water (i.e.  $1.19\pm 0.21$  kg). In a hotter environment (about  $37^\circ\text{C}$ ), the young tennis players studied by Périard et al. (2014) drunk ( $\sim 2$  L/h) and sweat ( $\sim 1.6$  L/h) more than our male soccer players.

The female soccer players of the present investigation showed in both experimental sessions a percentage body mass loss ( $-0.40 \pm 0.62\%$  in LIB and  $-0.30 \pm 0.48\%$  in HYD sessions) that was about half the value reported for Canadian junior women's soccer players ( $-0.84 \pm 0.07\%$ ) studied in a cool climate (Gibson et al., 2012). This result might be at least partially explained by the greater amount of water drunk by our female athletes ( $0.81 \pm 0.36$  kg in LIB and  $0.74 \pm 0.17$  kg in HYD sessions) as compared to the low fluid intakes ( $<0.25$  L) observed by Gibson et al. (Gibson et al., 2012).

It should be noticed that different methods and markers may be used to define hydration status. Beyond body mass loss, which defines acute fluid losses, hydration status in athletes is usually monitored by urine specific gravity (USG) (Fernández-Elías et al., 2014; Oppliger et al., 2005), the threshold of  $>1.020$  g/mL being considered an index of hypohydration (Armstrong & Maresh, 1998; Ersoy et al., 2016; Hamouti et al., 2013; Phillips et al., 2014; Sawka et al., 2007). We observed significantly higher USG values in males vs. females in the post-LIB measurements ( $ES=1.52$ ); this finding further supports the notion that in an ad libitum hydration regimen, at the end of training male soccer players are more dehydrated compared to females. No other significant findings were observed using as marker of hydration status the USG values. Likely in our study the amount of hydration changes were not large enough to overcome physiological balancing by the endocrine system controlling water balance (Ersoy et al., 2016; Maughan & Shirreffs, 2008). Of note, variable findings were observed by other authors on the association of USG with hydration status in athletes (Chlíbková et al., 2014).

Electrolytes concentrations in urine can also provide information on hydration and electrolyte balance in athletes. Physical effort typically increases both antidiuretic hormone (ADH) and aldosterone levels, with higher increased levels observed in dehydrated athletes (Freund et al., 1991; Maresh et al., 2004). Especially the rise of aldosterone induces lower urine concentrations of  $Na^+$  and increased concentrations of  $K^+$  (Chlíbková et al., 2014). The hormonal effects of exercise in our athletes are attested by the observed profile of decreasing values of urine sodium and the increase in urine potassium likely reflecting the expected increase of aldosterone levels (Maresh et al., 2004). The phenomenon was less pronounced in the female players, where the changes in electrolytes concentrations reached statistical significance only pooling together the two experimental sessions (LIB + HYD). Conversely, in males a significant decrease in sodium was observed in the HYD session. Moreover, in males pooling together LIB and HYD session also the increase in potassium became significant. These results support the hypothesis that in male players the hydration status at the end of the training session was more adversely affected than in females.

Regarding the Yo-Yo IR1 performance tests, major sex differences were noted with males performing better than females. Moreover, in females no significant differences were observed in the Yo-Yo IR1 test between LIB and HYD sessions. Conversely, in males a lower performance was observed in the LIB session compared to the HYD session. This finding supports the importance of the hydration status in determining the athletes' ability to repeatedly perform intense exercise, further suggesting that the ad libitum strategy appears appropriate only for females. Conversely, for male soccer adolescent athletes, personalized strategies should be recommended to optimise hydration status (such as water integrating 70% of sweat loss), properly monitored by athletes themselves as well as coaches (Rowland, 2011).

In our study, we did not observe significant differences in CMJ comparing LIB and HYD sessions in males and females. In this respect, our results differ from those reported by Mohr & Kustrup (2013) who showed a deteriorated CMJ performance by 6% after a soccer game; nevertheless, those male athletes lost on average 3.1% of body mass exercising in a hot temperature ( $30^\circ C$ ), whereas our male players showed in the LIB

session a body mass loss of  $-1.59 \pm 0.33\%$ . The effects of dehydration on muscle strength and power accounting also for the ambient temperature, however, need to be further explored (Pallarés et al., 2016).

Limitations of our study are the lack of serum and sweat biomarkers, and the lack of evaluation of biological age, heart rate, and internal temperature during the training sessions. It should be also acknowledged that some body mass changes during exercise might be due to water formation during substrate oxidation and release of water linked with glycogen.

Future studies are warranted to better assess the best hydration strategy to be adopted in adolescent soccer players, with special attention to males. In our study, the ad libitum strategy in males was the worst choice. Conversely, 70% replacement of sweat loss, as was proposed by Périard and colleagues (2014) in tennis players, in our study resulted in an improved hydration status in male athletes, but it was not totally sufficient to completely avoid a body mass loss. It remains to be determined whether a replacement over 70% of sweat loss would result in further improvement in athletic performance, particularly in adolescent males.

## CONCLUSION

The detrimental effects of hypohydration on the athletic performance are well documented by numerous studies which involve mainly adult athletes, rather than young athletes. Moreover, some evidence suggests that different effects might occur according to sex. We adopted a controlled hydration regimen previously used in adult athletes to assess whether it was beneficial in comparison to an ad libitum drinking regimen also among adolescent athletes and accounting for sex differences.

Our study evidenced for the first time sex difference in the hydration habits of elite adolescent soccer players, with females showing better spontaneous ability to compensate exercise induced body water changes. It remains to be determined whether the different distribution of ADH receptors between sexes (Liu et al., 2011) might at least in part be the major determinants of different responses to modifiers of the hydration status. Males benefit more than females of a controlled strategy (consisting in 70% replacement of body mass loss during a similar training session with ad libitum drinking) to maintain a good hydration status in temperate climate. It is noteworthy that in our study male performance was affected by the hydration strategy, although body mass loss did not exceed on average 2%. Our findings suggest that special attention should be given to develop optimal personalized hydration strategies during soccer games specifically focused on sex differences, so that health risks and performance decrements can be limited. Indeed, the ad libitum hydration strategy appears inadequate for male adolescent soccer players, whereas coaches may be confident on female soccer players ability to spontaneously maintain an adequate hydration status. Conversely, practitioners should pay attention to the individual habitual spontaneous hydration habits of male athletes in order to determine whether intervention strategies above ad libitum drinking are warranted. Future studies are warranted to depict in greater detail the personalized sex tailored hydration strategy able to maximize the individual athletic performance.

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## CONFLICTS OF INTEREST

None. Maria Pia Francescato, Ilaria Venuto, Alex Buoite Stella, Giuliana Stel, Franco Mallardi, and Sabina Cauci had no conflict of interest.

## AUTHOR'S CONTRIBUTION

SC, IV and ABS conceived and designed the experiments. IV, ABS, and GS performed the experiments. MPF, IV and SC analyzed the data. SC and GS contributed for reagents/materials/analysis. MPF, SC, FM, IV and ABS wrote the paper. All authors read and approved the final manuscript.

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