Title: Assessment of Physical demands and Fluid balance in elite female handball players during a 6-day competitive tournament

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Abstract

Little data exists on drinking behaviour, sweat loss and exercise intensity across a competitive handball tournament in elite female athletes. Heart rate (HR), fluid balance and sweat electrolyte content were assessed on 17 international players across a 6-day tournament involving 5 games and 2 training sessions played indoors (23 ± 2°C, 30 ± 2% relative humidity). Active play (effective) mean HR was 155 ± 14 bpm (80 ± 7.5% HR_{max}) with the majority of time (64%) spent exercising at intensities >80% HR_{max}. Mean (*SD*) sweat rates during games was 1.02 ± 0.07 $L \cdot h^{-1}$ and on 56% of occasions fluid intake matched or exceeded sweat loss. A significant relationship was observed between estimated sweat loss and fluid intake during exercise (r^2 = 0.121, P = 370.001). Mean sweat sodium concentration was 38 ± 10 $mmol \cdot L^{-1}$, with significant associations observed between player sweat rates and time spent exercising at intensities >90% HR_{max} (r^2 = 390.181, P = 0.001). Fluid and electrolyte loss appear to be work rate dependent in elite female handball players, whom appear well capable of replacing fluids lost within a tournament environment. Due to large between-athlete variations, a targeted approach may be warranted for certain players only.

Introduction

Handball is a fast paced body-contact Olympic sport played by two competing teams of 7 players (including a goalkeeper) on an indoor court (40m x 20m) over two 30 minute periods. It is generally recognised that games are played at high-intensity due to the nature of rolling substitutions and recent rule changes on starting the game from the centre after a goal is scored. Despite its popularity, a paucity of data exits to describe the games physical demands. Recent

research on elite male handballers has shown that players cover a mean distance of 4370 ± 702m during games, most of which is spent performing low-intensity aerobic exercise actions interspersed by a short duration of very high-intensity anaerobic actions (Póvoas et al., 2012). In the latter study, players were observed to exercise at a mean intensity of 82 ± 9.3% of HR_{max}. To our knowledge, only one study describes the physical demands within the elite women's game with players observed to cover distances of 4002 ± 551m and exercise at high mean relative workloads (79.4±6.4% VO_2-max) during match-play (Michalsik et al., 2013).

Handball is played indoors, and although most playing halls have temperature controlled environments, high-intensity exercise may result in thermal stress and subsequent disruption of body water and electrolyte balance. Team sport players have been shown to lose variable amounts of electrolytes in sweat (Kurdak et al., 2010; Maughan et al., 2007b) and the magnitude of solute loss appears dependant on both sweat rate and composition. In soccer, large individual variations in player sweat response are known to occur as a result of differences in environmental temperature and humidity, work-rates, state of acclimation and individual fitness (Maughan, Watson, Evans, Broad & Shirreffs, 2007). When exercising in the heat, a 1-3% loss in BM due to sweat losses has been reported to result in elevated HR, core temperature (Tc), rating of perceived exertion, and plasma osmolality (Montain & Coyle, 1992; Buono & Wall, 2000). More recently, it has been observed that muscle glycogenolysis significantly increases early in exercise with a BM loss of < 2% and this appears related to a rise in core and muscle temperature (Logan-Sprenger et al., 2013). However, available data has shown that values in excess of 2% BM are needed before aspects of team sport physical and cognitive performance begin to be negatively affected (Baker et al., 2007; McGregor et al., 1999). While many studies have investigated fluid balance in team sport athletes during practice or competition in the field (Maughan et al., 2005; Kurdak et al., 2010; Maughan et al., 2007) few, if any, have explored changes in these variables across a

competitive tournament. In particular, there are no data available on women's handball. Olympic handball teams may play up to 5 games in 9 days during group stages of a major competition. Since prior hypohydration will amplify the effects of any fluid deficit incurred during exercise, added emphasis on fluid-electrolyte replacement may be required during such time periods to ensure adequate recovery between games. For this reason, understanding the pattern of fluid and electrolytes losses during a tournament may assist with the provision of targeted interventions in players with high sweat rates or where failure to replace lost fluids might raise concerns of an ergolytic effect.

The main aim of this investigation was to observe normal fluid balance behaviour and quantify electrolyte losses across a competitive tournament in a team preparing for the Olympic Games. Secondary aims were to describe the physical demands of elite women handball players and investigate whether or not fluid-electrolyte losses could be explained from exercise intensity and effort distribution.

Methods

Participants. Data were collected on an international team (n = 17) of female handball players (26 ± 9 yr, 1.72 ± 0.06 m, 70.7 ± 8.5 kg: mean ± SD) during a tournament scenario over six days.

Experimental design.

The team participated in two training sessions and five competitive friendly games against top club opposition whom were normally playing at the highest standard in European handball competition (Figure 1). Data collection took place at two training venues seven months before the 2012 Olympic Games which the team under investigation subsequently participated. Games were played according to international handball federation rules and with the exception of game 4 (lasted 90 minutes due to a prior agreement between coaching teams), consisted of two periods of 30 minutes. The team

under investigation recorded three wins and two losses during the investigation. Training sessions lasted 104 ± 2.1 minutes, consisting of warm-up, calisthenics, position-specific drills, small-sided games and technical drills. Environmental conditions during the games/training sessions are shown in Table 1 and all measurements were recorded with a portable thermohygrometer (Higbo, Oregon Scientific, Berkshire, UK).

Study approval was obtained from the national governing body involved and collection of data arose as part of the service provision supplied to the team in preparation for competition (Winter & Maughan, 2009). All athletes agreed to take part and signed consent was obtained. To ensure player confidentiality, data were anonymized before analysis.

Testing procedures Players wore shorts and short sleeve T-shirts throughout testing. On the morning of training or games, players provided a urine sample (first void) into a pre-labelled urine collection pot (60 ml, Greiner Bio-One Ltd, Stroud, UK). Urine refractive index (Pocket Osmocheck, Vitech Scientific Ltd, Sussex, UK) were subsequently determined to provide an index of pre-exercise hydration status (Shirreffs, 2003; Sparks and Close, 2012). Players were provided with dietary plans by the affiliated nutritionist in the lead up to training/games. As per norm, food was self-prepared in large player groups within nearby shared accommodation to the team home venue and this was not altered during the investigation. Neither dinner nor breakfast was recorded or standardized so as to avoid interference with player's normal dietary/fluid habits. Player menstrual state was not recorded during the 6-day tournament.

Over a 15-min period (-45 to -30 min) before the start of each game/training, players were weighed (nude) to the nearest 20 g on a calibrated electronic scale (Marsden W/M, Oxford, UK). After entry to the playing court, players were fitted with a heart rate (HR) strap and individual HR data was subsequently collected wirelessly at one-second intervals (Polar Team System, Polar Electro,

Kempele, Finland). Following team warm-down, players were re-weighed (towelled dry nude) on the same calibrated scale. Body mass changes were used as a proxy measure for sweat losses. While this represents a limitation of the study, previous work has indicated that this represents a realistic field measurement to estimate hypohydration (Maughan et al., 2007a). Players were asked to refrain from eating between weighing points but were free to drink fluids *ad libitum* and whenever breaks in play were permitted (1 minute time-out per-half is allowed by handball rules). Players were asked to consume drink products which they would normally consume during everyday practice and games. This included either tap water or tap water mixed with sugar free cordial for the current player group. Observed checks confirmed that no player consumed commercial drink products or electrolyte sachets during exercise. Therefore electrolyte intake during sessions/games was considered negligible.

All bottles were individually numbered and players drank from bottles assigned to them by team staff. All players were left to behave as they wished with regard to fluid consumption. During the time period from weighing players before and after exercise, each player's fluid intake was measured to the nearest 0.001kg on a calibrated scale (Adam Equipment Ltd., Milton Keynes, UK). A volume of 1 mL ingested fluid was assumed to weigh 1 g. In the event of players wishing to urinate during the analysis period, they were accompanied by research staff to the toilet and provided with a pre-weighed urine collection bag with funnel (P-Bag, Medipost Ltd, Dorset, UK). Filled urine collection bags were subsequently weighed to aid in determination of fluid balance. All bottles/collection devices were weighed out of view of the players at all times.

Sweat collection and analysis

Before three of the five games, players were prepared for sweat sample collection using methods outlined previously (Maughan et al., 2007b; Maughan & Shirreffs, 2008). Briefly, players had

absorbent patches (Tegaderm + pads, 3M, Loughborough, UK) placed on four body locations (chest, upper forearm, back and thigh). Before attaching patches to the skin, the locations were washed thoroughly with deionized water and dried with electrolyte-free gauze. At the end of each game, patches were removed with sterile forceps and each patch was subsequently placed into pre-weighed screw-top containers (20 ml, Sterlin, Cambridge, UK) until analysis. No patch showed signs of undue saturation (e.g. dripping) across the period of investigation. Following collection, samples were stored at 4°C before analysis (within one week of collection).

To determine electrolyte concentrations, each sweat patch and container were weighed to the nearest 4 decimal places before 1 mL of deionized water was added. After mixing, the sample was analysed for sodium and potassium concentration using flame photometry (Sherwood 410, Sherwood Scientific, Cambridge, UK) and for chloride concentration using the mercuric thiocynate method (Randox, Crumlin, UK). All samples were analysed in duplicate and intra-run coefficients of variations were <5%.

Heart rate data

Player heart rate max data (HR_{max}) was obtained in the weeks leading up to experimentation using an incremental running test to exhaustion (Yo-Yo IE2 test) conducted by strength and conditioning staff. Confirmatory HR_{max} analysis was achieved by comparing these results to those achieved previously (-4 months) using a laboratory incremental test to exhaustion. Previous work has shown that no systematic differences occur between HR_{max} achieved using the Yo-Yo IE2 test and incremental treadmill test in intermittent sport (Bradley et al., 2011).

Heart-rate readings were classified into 5 intensity zones, ranging from 50-59%, 60-69%, 70-79%, 80-89% and 90-100% HR_{max} for assessment of physiological load in team handball (Póvoas et al.,

2012). The percentage of time spent exercising in each zone was determined to provide an indication of exercise intensity for each player and all HR values were analysed during the first and second halves. Given that team handball rules allow for unlimited substitutions, it is unusual that one athlete plays an entire game. Furthermore, a one-minute time-out period is allowed for each team per-half. Therefore, HR during the games were analysed (a) as total HR (i.e. HR during the total game time) to provide an index of global cardiovascular load and (b) HR during active play time (effective HR) in order to classify game demands during time when a player was actually on the playing court (Póvoas et al., 2012). The time period for the half-time break was excluded from HR analysis.

Calculations

Sweat rate (SwtR: $L \cdot h^{-1}$) was estimated as net body mass loss (kg) during the training session/game plus the total fluid intake divided by the exercise time (min). Corrections for any individual player urine loss were also made.

SwtR = {[pre-exercise mass (kg) – post-exercise mass (kg)] – urine volume (L) + fluids consumed (L)} / time (h)].

Fluid intake ($L \cdot h^{-1}$) was calculated as total fluid intake during the recorded exercise session divided by the exercise time (per hour). Changes in BM before and after exercise were to assess acute changes in hydration status (% fluid deficit). Net balance of respiratory and metabolic contributions to BM loss was considered negligible as observed previously under similar environmental conditions for indoor team sport (Hamouti et al. 2010). Sweat electrolyte losses (in grams) were calculated from the sweat electrolyte concentration, the molecular weight of the electrolyte, and the total sweat loss of the individual (Maughan et al., 2007a). It was assumed that the mean sodium concentration (from 4 collected sites) represented mean whole-body sodium concentration although it is acknowledged that this may be an overestimate (Shirreffs & Maughan, 1997).

Statistical analysis

Data sets were initially tested for normality of distribution using the Shapiro–Wilk test. Comparisons between data were then made using paired t-tests and a one-way repeated measures analysis of variance (ANOVA). Differences in regional sweat electrolyte concentration were determined by 1-way ANOVA and with Tukey's test of honestly significant difference. Relationships between parameters were performed using a least-squares regression model, and the coefficient of determination (r^2) is reported (Maughan et al, 2005). All statistical analyses were performed using SPSS v. 20. Data are presented as mean ± standard deviation (SD) with range of data given in parentheses. Statistical significance was set at $P < 0.05$.

Results

Heart rate

Player HR_{max} achieved during incremental running test was 191 ± 7 bpm^{-1}. Peak HR over the 5 games (182 ± 9 bpm^{-1}; 93 ± 5% of HR_{max}) was significantly higher than values recorded in training (169 ± 13 bpm^{-1}; 86 ± 5% of HR_{max}); $P < 0.05$. Player effective mean HR (155 ± 14 bpm^{-1}; 80 ± 8% of HR_{max}) was also significantly higher than values observed in training (129 ± 10 bpm^{-1}; 65 ± 5 % of HR_{max}) and total mean game HR (131 ± 14 bpm^{-1}) ($P < 0.05$). Average time spent by players in "active" play during games was 33 ± 15 minutes.

No between game-half differences were observed for either total or effective mean player HR (Figure 2). Furthermore, no significant differences were observed between game halves at different interval percentages of players' HR_{max} (Figure 3). The majority of effective game time (64%) was spent exercising at intensity >80% HR_{max}, with 23% of game time spent exercising >90% HR_{max}. No between game differences were observed for percentage of effective or total game time spent any of the designated HR zones.

222
223*Morning hydration status*
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225On the mornings of the games, 47% of players awoke with a urine refractive index ≥ 700
226mOsmol/kg. No relationship was observed between previous days fluid intake during exercise and
227the following mornings urine refractive index (P = 0.116). Furthermore, no relationship was
228observed between morning urine refractive index and subsequent fluid intake during exercise, (r^2 =
2290.014; P = 0.307; Figure 4a). A significant relationship was observed between morning urine
230refractive index and percentage change in player BM during training/games (r^2 = 0.159; P = 0.001),
231Figure 4b. No between game differences in morning measures of hydration status were observed
232(Figure 6).

233

234
235*Fluid balance*

236Across the games, mean team fluid intake was 1.05 ± 0.16 L. After correction for amount of fluid
237ingested, estimated mean team fluid loss was 1.08 ± 0.18 L. A significant relationship was observed
238between fluid intake during exercise and estimated fluid (sweat) loss (r^2 = 0.0121; P = 0.001; Figure
2395). Fluid intake (L) was significantly lower during training sessions in comparison to games 2, 3
240and 4 (P < 0.001) (Figure 6). Between game comparisons indicated that players consumed
241significantly more fluid during game 4 than games 2 and 5. Larger volumes of fluids were also
242consumed during game 3 in comparison to game 5 (P < 0.001).

243

244Mean player sweat rates during games (1.02 ± 0.07 $L \cdot h^{-1}$) were significantly higher than those
245observed during training (0.56 ± 0.15 $L \cdot h^{-1}$) despite similar environmental temperatures (P < 0.05;
246Table 1). No between game differences were observed. A relationship was observed between
247individual player sweat rates ($L \cdot h^{-1}$) and time spent exercising at intensities >90% HR_{max} (r^2 = 0.181;
248P = 0.001). During games, 51% of players had a sweat rate < 1 $L \cdot h^{-1}$ and 47% had a sweat rate

between 1 and 2 $L \cdot h^{-1}$. Only on two occasions did player sweat rates exceed 2 $L \cdot h^{-1}$ during games. In contrast, all players had sweat rates < 1 $L \cdot h^{-1}$ during training. A large inter-individual variability was observed in sweating response between players (Figure 5).

On 56% of occasions, player fluid intake matched or exceeded fluid (sweat) loss. Sweat loss was significantly higher during game 4 than training sessions and game 2 ($P < 0.05$), game 3 and 5 ($P < 0.01$). Across the five games, 43% of players lost body mass (BM) as a result of exercise with most (36%) of the group recording losses of <1% BM. In turn, 7% of the player group recorded a loss of >1% BM and no player was dehydrated >2% of BM. Conversely, 6% of the players gained more than 1% in BM across the games. On average, 0.0 ± 0.1% and 0.1 ± 0.1% net fluid deficits were recorded following games and training respectively.

Electrolyte balance

The mean (4 site) sweat sodium, chloride and potassium concentrations were 38 ± 10 $mmol \cdot L^{-1}$, 27 ± 11 $mmol \cdot L^{-1}$ and 5 ± 1 $mmol \cdot L^{-1}$, respectively (Table 2). No significant effects for time (between games) were observed for sweat sodium, chloride or potassium content ($P > 0.05$). Mean sodium content of sweat in game 4 was significantly higher than game 5 ($P < 0.01$) while higher sweat chloride content was also observed in game 4 compared to game 2 and game 5 ($P = 0.03$). Sodium concentrations in the sweat patches of the 'back' torso were lower compared to all other sites ($P < 0.001$). Higher 'arm' chloride concentration was observed in comparison to values recorded from players 'back' and 'thigh' ($P < 0.001$). In turn, chest chloride concentrations were higher than both 'back' and 'thigh' samples ($P < 0.001$). Arm potassium concentrations were higher than back, chest and thigh concentrations while thigh concentrations were higher than back and chest ($P < 0.001$; Table 2). No association was observed between mean player sweat sodium concentration and sweat rate ($r^2 = 0.001$; $P = 0.57$).

A significant correlation was observed for total sodium loss (1.03 ± 0.43 g) and percentage of time spent by players exercising between 90-100% HR_{max} ($r^2 = 0.210$; $P = 0.016$).

Discussion

The aim of this investigation was to assess fluid balance behaviour and determine electrolyte losses across a competitive tournament in elite female handball players. Secondary aims were to assess physical demands and whether or not observed changes in the above variables could be explained from player exercise intensity data.

While a limited number of studies have documented intensity of exercise in elite men's handball, to date, only one study has been carried out in the elite women's game (Michalsik et al., 2013). Present data shows that female players exercise at a similar mean intensity (155 ± 14 bpm^{-1}; 80 ± 18% of HR_{max}) to their elite male counterparts (Chelly et al., 2011; Póvoas et al., 2012) and that the majority of time (64%) is spent performing movements at high >80% HR_{max} exercise intensities. As expected, mean exercise HR was considerably higher during competition in comparison to those observed in training sessions. This was due to the fact that the coaches used the training sessions with a tactical focus and structured lower intensity than normal to ensure players were not fatigued during the tournament. However, team HR values did not decrease during the second-half of games as observed previously in the elite men's game (Póvoas et al., 2012). In the latter study, modest average relative body weight losses (0.9 ± 0.3%) were recorded. This, combined with data from the current study (0.0 ± 0.1% fluid deficit), suggests that factors other than dehydration may have be responsible for any observed drop off in exercise intensity in Handball. Although dehydration due to sweat loss is a known factor which can impair exercise performance (Coyle, 2004); other factors such as game rotation strategy, score differential, physiological or physical factors may all

303contribute to game fatigue. It should also be noted that HR data only provides an indication of
304cardiovascular demands and time-motion characteristics should be studied to better understand how
305fatigue affects movement patterns.

306

307In the current study, player sweat rates (1.02 ± 0.07 $L \cdot h^{-1}$) were similar to the ones observed in
308female basketball players (Brandenburg & Gaetz, 2012), indoor male handball (1.1 ± 0.3 $L \cdot h^{-1}$) and
309volleyball (1.2 ± 0.3 $L.h^{-1}$) players but considerably lower than indoor soccer (1.8 ± 0.7 $L \cdot h^{-1}$)
310(Hamouti et al., 2010). However, a large inter-individual variability (range 0.26-2.1 L) was
311observed, as noted previously (Maughan et al., 2007b; Hamouti et al., 2010). This, taken together
312with the large variability in fluid intake across the investigation (range 0.25-2.74 L) highlights the
313need for individualized assessment of fluid balance strategies in a team sport environment
314(Shirreffs, Sawka & Stone, 2006). With respect to the current athlete group, it was noted that 6% of
315players gained more than 1% in body mass (BM) by the end of the games, suggesting that some
316players might consume excessive fluids. Overall, 56% of players either maintained or increased
317their BM at the end of the game and this was particularly evident for goalkeepers and rolling
318substitutes, some of whom consistently consumed more fluids than were lost in sweat. This was
319most likely due to increased time available on the bench and ease of access to drinking bottles.
320During handball competition, BM has to be moved against gravity and theoretically an increase in
321BM from resultant fluid intake might have negative effects on performance in particular when
322fatigue develops.

323

324It has been reported that as little as a 2% loss in BM due to dehydration affects many physiological
325factors thought to indirectly affect performance. These include elevations in HR, core temperature
326(Tc), rating of perceived exertion (Montain & Coyle, 1992; Buono & Wall, 2000) and muscle
327glycogenolysis (Logan-Sprenger et al., 2012). In this study, no player was dehydrated >2% BM

despite moderate to high sweat rates in some players. The most recent position stand from the American College of Sports Medicine (Sawka et al., 2007) has suggested that fluid intake during prolonged exercise should be sufficient to limit any BM loss to <2% of pre-exercise mass and that athletes should never drink so much that they gain BM during exercise. Therefore, the female handball players observed in this study appear to adequately replace fluids lost during exercise, and in some cases, education around the risks of overconsumption of fluids is warranted.

Across the six-day tournament, 54% of players awoke with a urine refractive index of <700 mOsmol/kg or a urine specific gravity <1.020, values previously suggestive of euhydration (Sawka et al. 2007). Research has reported that 91% of elite male athletes from a number of indoor team sports awoke in hypohydrated state before practice (Hamouti et al., 2010). Therefore, data is suggestive that fluid management following exercise appeared adequate in the current player group. Start time of training and games varied considerably between days and no difference in markers of hydration status was observed between sampling points. It is possible that given the elite nature of the current team and the tournament scenario, players were proactive in making sure to replace lost fluids during exercise. Alternatively, it is possible that fluid replacement strategies were altered by players as a result of been observed during the experimentation period. However, given that study experimenters were familiar with players and training environment prior to experimentation, the repeated observations and failure to identify trends in fluid intake across the study period; it does not appear that this was the case. It should be noted a small number of players (8%) consistently provided urine samples that were indicative of being very dehydrated (1.000-1.1290 mOsmol/kg; Armstrong et al., 2010) suggesting the need for targeted fluid replacement in such a player group. There is some evidence of a positive correlation between pre-training urine osmolality and the volume of fluid ingested during a training session where fluids are freely available (Maughan et al., 2005). Therefore, athletes who begin training with higher urine osmolality/specific gravity may be

likely to drink more due to a greater sensation of thirst. In the current study, no relationship was observed between morning urine measures and subsequent fluid intake. Such findings have been observed previously (Maughan et al., 2007b) and may be dependent on the time period from morning hydration assessment and exercise onset. In this study, some games started at 3 pm and so it is feasible that athletes had adequate time to correct any fluid imbalance through normal sensory means. Unlike previous findings (Maughan et al., 2007b; Hamouti et al., 2010), a modest but positive association was observed between the amount of sweat lost and volume of fluid consumed (Greenleaf, 1982), indicating that sensation of thirst and ample fluid breaks was capable of offsetting major disturbances in fluid balance. Further work should be carried out to determine the strength of this relationship in athlete groups whom display a wide range in sweat rates.

Data from tennis (Bergeron, 2003) and American football (Stofan et al., 2005; Horswill et al., 2009), have suggested that athletes who sweat profusely and have a high sweat sodium concentration may be more likely to experience muscle cramps. However, few if any studies have investigated whether or not electrolyte losses vary throughout a condensed tournament environment when replacement of salts lost in sweat may be of added importance to the recovery process. In the current study, mean sweat electrolyte concentrations were on the lower end of those reported previously (Maughan et al., 2005; Hamouti et al., 2010). Very few studies have investigated electrolyte losses in elite female athletes (Kilding et al., 2009) and it is possible that gender differences may account for observed differences. Although speculative, given that players in this study voluntarily chose to consume plain water during exercise, it is also possible that this may, in part, account for the lower mean sweat sodium (38 ± 10 mmol.L^{-1}) concentrations observed (Sigal & Dobson, 1968). A large inter-individual variation was observed for all sweat electrolyte concentration (Table 2.) as noted elsewhere (Maughan et al., 2007b). Given that players were rather homogenous in terms of fitness level (had similar daily exercise programs), dietary intake (same

food menus) and degree of acclimation, it's possible that genetic factors may underpin this finding. As observed previously, no relationships were found between whole body sweat rate and sweat concentration of any electrolyte (Maughan et al., 2007b; Kilding et al., 2009). This is in contrast to recent data in tennis which suggests sweat sodium concentration is related to an individual athletes sweat rate (Bergeron, 2014). Given that a significant correlation did exist between player sweat rates and time spent exercising at higher intensities, present data are suggestive that time exposure to intense exercise may, in part, have a role in overall electrolyte loss (Hamouti et al., 2011).

Regional differences in sweat electrolyte composition were observed as previously reported (Maughan et al., 2007b). In this study, lower back torso sodium concentrations were observed compared with other collection sites. These findings are in contrast to previous work carried out on male cyclists (Patterson, Galloway & Nimmo, 2000) and both male and female atheletes cycling in a heat chamber (Baker et al., 2009). Future studies should be carried out on female athletes undertaking differing exercise modes to explore further why such regional differences occur. While previous studies which have primarily focused on either once off sweat composition measurements, the current study assessed electrolyte losses across three games in a six-day tournament. Results showed that with the exception of game 4, electrolyte concentrations in sweat were consistent between games. With respect to game 4, both teams agreed to play across 3 x 30 minute period (90 minutes total) for technical development reasons. Although between game sweat rates did not differ in this study and stability of electrolyte concentration has been observed across varying exercise durations (Montain, Cheuvront, & Lukask., 2007), current data suggests that length of sweat patch sampling time does need to be taken into account when assessing electrolyte loss in athletes. A significant association was observed for total sodium deficit (1.03 ± 0.43 g) and per-cent change in BM. Although statistically significant, only a moderate correlation was observed ($r^2 = 0.367$) which, perhaps is not surprising given that salt losses through exercise are influenced by *both* sweat loss

and electrolyte concentration. Although moderate sodium deficits were incurred, a large variability in sodium loss was observed (range: 0.3-2.37 g). Given that the current player group refrained from consuming electrolytes during exercise, recommendations to a few select individuals (whom experienced high sodium losses) on consumption of electrolyte containing beverages during exercise to may be required reduced the sodium deficit and to better retain ingested fluid. This may be particularly relevant during a tournament scenario where players have a game every other day.

Present results indicate that moderate sweat and electrolyte losses occur during handball games played in temperate conditions and the magnitude of such losses appear related to time spent exercising at higher intensities. Furthermore, it seems evident that specific approaches need to be considered for certain individuals, not only to minimise fluids losses but also to avoid overconsumption of fluids which might have negative effects on performance. Lastly, our data seems to suggest that the cardiovascular demands of handball games in women are similar to those reported for men, albeit further studies are needed to quantify time-motion characteristics of elite women's handball.

References

Armstrong, L.E., Pumerantz, A.C., Fiala, K.A., Roti, M.W., Kavouras, S.A., Casa, D.J., & Maresh, C.M. (2010). Human hydration indices: acute and longitudinal reference values. *International Journal of Sports Nutrition and Exercise Metabolism*, *20*, 145-153.

Baker, L.B., Dougherty, K.A., Chow, M., & Kenney, W.L. (2007). Progressive dehydration causes a progressive decline in basketball skill performance. *Medicine and Science in Sports and Exercise, 39,* 1114-23.

Baker, L.B., Stofan, J.R., Hamilton, A.A., & Horswill, C.A. (2009). Comparison of regional patch collection vs. whole body washdown for measuring sweat sodium and potassium loss during exercise. *Journal of Applied Physiology, 107*, 887-895.

Bergeron, M.F. (2003). Heat cramps: fluid and electrolyte challenges during tennis in the heat. *Journal of Science and Medicine in Sport, 1,* 19-27.

Bergeron, M.F. (2014). Hydration and thermal strain during tennis in the heat. *British Journal of Sports Medicine, 48*, i12-i17.

Bradley, P.S., Mohr, M., Bendiksen, M., Randers, M.B., Flindt, M., Barnes, C., Hood, P., Gomez, A., Andersen, J,L., Di Mascio, M., Bangsbo, J., & Krustrup, P. (2011). Sub-maximal and maximal Yo-Yo intermittent endurance test level 2: heart rate response, reproducibility and application to elite soccer. *European Journal of Applied Physiology, 111,* 969-78.

Bradenburg, J.P., & Gaetz, M. (2012). Fluid balance of elite female basketball players before and during game play. *International Journal of Sport Nutrition and Exercise Metabolism, 22*, 347-352.

Buono, M. J., & Wall, A. J. (2000). Effect of hypohydration on core temperature during exercise in temperate and hot environments. *Pflugers Archive, 440,* 476–480.

Chelly, M.S., Hermassi, S., Aouadi, R., Khalifa, R., Van den Tillaar, R., Chamari, K., & Shephard, R.J. (2011). Match analysis of elite adolescent team handball players. *Journal of Strength and Conditioning Research, 25,* 2410-7.

Coyle, E.F. (2004). Fluid and fuel intake during exercise. *Journal of Sports Science, 22,* 39-55.

Greenleaf, J.E. (1982) Dehydration-induced drinking in humans. *Federation Proceedings, 41,* 4502509-14.

Hamouti, N., Del Coso, J., Estevez, E., & Mora-Rodriguez R. (2010). Dehydration and sodium deficit during indoor practice in elite European male team players. *European Journal of Sport Science, 10,* 329-336.

Hamouti N., Del Coso, J., Ortega, J.F., & Mora-Rodriguez R. (2011). Sweat sodium concentration during exercise in the heat in aerobically trained and untrained humans. *European Journal of Applied Physiology, 111,* 2873-81.

Horswill, C.A, Stofan, J.R, Lacambra, M., Toriscelli, T.A, Eichner, E.R., & Murray, R. (2009). Sodium balance during U. S. football training in the heat: cramp-prone vs. reference players. *International Journal of Sports Medicine, 30,* 789-94.

Kilding, A.E., Tunstall, H., Wraith, E., Good, M., Gammon, C., & Smith, C. (2009) Sweat rate and sweat electrolyte composition in international female soccer players during game specific training. *International Journal of Sports Medicine, 30,* 443-447.

Kurdak, S.S., Shirreffs, S.M., Maughan, R.J., Ozgünen, K.T., Zeren, C., Korkmaz, S., Yazici, Z., Ersöz, G., Binnet, M.S., & Dvorak, J. (2010). Hydration and sweating responses to hot-weather football competition. *Scandinavian Journal of Medicine and Science in Sports, 20,* 133-9.

Logan-Sprenger, H.M,, Heigenhauser, G JF., Jones, G.L., & Spriet, L.L. (2013). Progressive Dehydration during Cycling Increases Skeletal Muscle Glycogenolysis and Perceived Exertion in Hydrated Males. *International Journal of Sport Nutrition and Exercise Metabolism, 23,* 220-9.

Maughan, R.J., Shirreffs, S.M., Merson, S.J. & Horswill, C.A. (2005). Fluid and electrolyte balance in elite male football (soccer) players training in a cool environment. *Journal of Sports Sciences, 23,* 73–79.

473Maughan, R.J., & Shirreffs, S.M. (2008). Development of individual hydration strategies for 474athletes. *International Journal of Sport Nutrition and Exercise Metabolism, 18,* 457-72.

475Maughan, R.J., Shirreffs, S.M. & Leiper, J.B. (2007a). Errors in the estimation of hydration status 476from changes in body mass. *Journal of Sports Sciences, 25,* 797–804.

477Maughan, R.J., Watson, P., Evans, G.H., Broad, N., & Shirreffs, S.M. (2007b). Water balance and 478salt losses in competitive football. *International Journal of Sport Nutrition and Exercise* 479*Metabolism, 17,* 583-589.

480Michalsik LB, Madsen K, Aagaard P. (2014). Match Performance and Physiological Capacity of 481Female Elite Team Handball Players. *International Journal of Sports Medicine, 35*, 595-607.

482McGregor, S.J., Nicholas, C.W., Lakomy, H.K., & Williams, C. (1999). The influence of 483intermittent high-intensity shuttle running and fluid ingestion on the performance of a soccer skill. 484*Journal of Sports Sciences, 11,* 895-903.

485Montain, S.J., & Coyle, E.F. (1992). Influence of graded dehydration on hyperthermia and 486cardiovascular drift during exercise. *Journal of Applied Physiology, 73,* 1340–1350.

487Montain, S.J., Cheuvront, S.N., & Lukaski, H.C. (2007). Sweat mineral-element responses 488during 7 h of exercise-heat stress. *International Journal of Sport Nutrition and Exercise* 489*Metabolism, 17,* 574–582.

490Patterson, M.J., Galloway, S.D., & Nimmo, M.A. (2000). Variations in regional sweat composition 491in normal human males. *Experimental Physiology, 85*, 869-875.

492Póvoas, S.C., Seabra, A.F., Ascensão, A.A., Magalhães, J., Soares, J.M., & Rebelo, A.N. (2012). 493Physical and physiological demands of elite team handball. *Journal of Strength and Conditioning* 494*Research, 26,* 3365–3375.

495Sawka, M.N., Burke, L.M., Eichner, E.R., Maughan, R.J., Montain, S.J., & Stachenfeld, N.J.
496(2007). Exercise and fluid replacement. *Medicine and Science in Sports and Exercise, 39, 377–390.*

497Shirreffs, S.M. (2003). Markers of hydration status. *European Journal of Clinical Nutrition, 57:*
498S6–S9.

499Shirreffs, S.M., & Maughan, R.J. (1997). Whole body sweat collection in man: an improved
500method with preliminary data on electrolyte content. *Journal of Applied Physiology, 82,* 336-
501341.

502Shirreffs, S.M., Sawka, M.N., & Stone, M. (2006). Water and electrolyte needs for football training
503and match-play. *Journal of Sports Sciences, 24,* 699-707.

504Sigal, C.B., & Dobson, R.L. (1968). The effect of salt intake on sweat gland function. The
505*Journal of Investigative Dermatology, 50*, 451-455.

506Sparks, S.A., & Close, G.L. (2012). Validity of a portable urine refractometer: The effects of
507sample freezing. *Journal of Sports Sciences, 31*, 745-749.

508Stofan, J.R., Zachwieja, J.J, Horswill, C.A, Murray, R., Anderson, S.A, & Eichner, E.R. (2005).
509Sweat and sodium losses in NCAA football players: a precursor to heat cramps? *International*
510*Journal of Sport Nutrition and Exercise Metabolism*, 15, 641-52.

511

512Winter E.M. & Maughan R.J. (2009). Requirements for ethics approvals. *Journal of Sports*
513*Sciences, 27:* 985.

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Tables

Table 1. Environmental conditions: Temperature (°C) and relative humidity (%) taken at 15 min intervals during the games.

Table 2. Between game comparison of sweat electrolyte concentrations ($mmolxL^{-1}$) per collection site. *Significantly different from game 4, $P < 0.05$. # Significantly different from game 5, $P < 0.05$.

Figures

Figure 1. Testing schedule across the tournament.

Figure 2. Mean and maximal total and effective HR during the first and second halves and total match time. Data presented as absolute and relative to individual maximal HR values (means ± SD). * Significantly different from total HR ($P = 0.00$). Inset showing effective and total HR data recorded during training.

Figure 3. Percentage of effective match time spent at different interval percentages of player maximal HR in the first and second halves. Values are means ± SD.

Figure 4(a). Relationship between measured pre-exercise urine refractive index and measured fluid intake during training/games, $r^2 = 0.014$, $P = 0.307$.

Figure 4(b). Relationship between morning urine refractive index and percentage change in player body mass during training/games, $r^2 = 0.159$, $P = 0.001$.

Figure 5. Relationship between volume of fluid consumed during training/games and the amount of sweat loss (L), $r^2 = 0.121$, $P = 0.001$.

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555Figure 6. Rate of fluid intake and sweat loss in players across the tournament. * Significantly higher than training 556sessions ($P<0.05$). Morning urine refractive index values per game throughout tournament also provided. Values are 557mean ± SD.

Table 1. Environmental conditions: Temperature (°C) and relative humidity (%) taken at 15 min intervals during games

	Game 1	Game 2	Game 3	Game 4	Game 5	Training*
Temperature (°C)	21 ± 0	25 ± 0	24 ± 0	22 ± 2	21 ± 1	23 ± 2
Relative humidity (%)	30 ± 2	27 ± 1	30 ± 1	33 ± 2	27 ± 3	31 ± 0

Data are mean ± SD. *Averaged data from two training sessions at same venue.

Table 2. Between game comparison of sweat electrolyte concentrations (mmol·L⁻¹) per collection site. (SD): standard deviation.

	Game 2		Game 4	Game 5				
	Mean *(SD)*	Range		Mean *(SD)*	Range	Mean *(SD)*	Range	Mean *(SD)* Range
Mean Sodium	36 *(13)*	19-70		40 *(8)*	27-54	*35 *(7)*	24-47	38 *(10)* 23-57
Arm	38 *(14)*	16-60		44 *(12)*	29-67	39 *(13)*	18-61	41 *(13)* 16-66
Chest	40 *(14)*	16-55		46 *(11)*	23-64	*40 *(10)*	24-54	42 *(12)* 16-65
Back	31 *(12)*	14-60		33 *(10)*	17-46	*27 *(7)*	14-40	31 *(10)* 14-60
Thigh	36 *(15)*	19-75		42 *(11)*	26-70	38 *(8)*	24-48	39 *(12)* 19-75
Mean Chloride	*24 *(16)*	2-54		31 *(9)*	16-47	*25 *(8)*	15-37	27 *(11)* 11-46
Arm	*26 *(16)*	2-50		33 *(14)*	12-58	31 *(12)*	16-52	30 *(15)* 3-57
Chest	*29 *(18)*	2-55		38 *(11)*	19-53	*33 *(10)*	21-46	34 *(13)* 3-55
Back	20 *(13)*	2-27		24 *(10)*	8-40	20 *(9)*	9-35	22 *(11)* 2-47
Thigh	*20 *(15)*	6-52		27 *(11)*	10-46	*21 *(8)*	6-33	23 *(11)* 6-52
Mean Potassium	5 *(2)*	3-7		6 *(1)*	4-7	*5 *(1)*	3-7	5 *(1)* 3-7
Arm	*6 *(2)*	3-9		8 *(2)*	5-12	7 *(3)*	3-12	7 *(2)* 3-12
Chest	5 *(0)*	4-5		5 *(1)*	3-6	*4 *(1)*	2-5	4 *(1)* 2-6
Back	4 *(1)*	2-6		5 *(1)*	4-7	*5 *(1)*	3-6	5 *(1)* 2-7
Thigh	▪6 *(1)*	5-8		7 *(3)*	3-14	*5 *(1)*	3-6	6 *(2)* 3-14

*Significantly different from game 4, $P < 0.05$. ▪ Significantly different from game 5, $P < 0.05$.

Figure 1

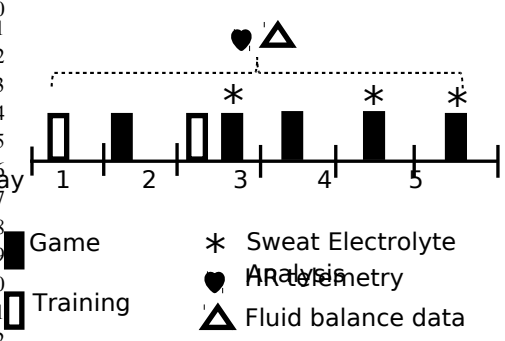

Figure 2

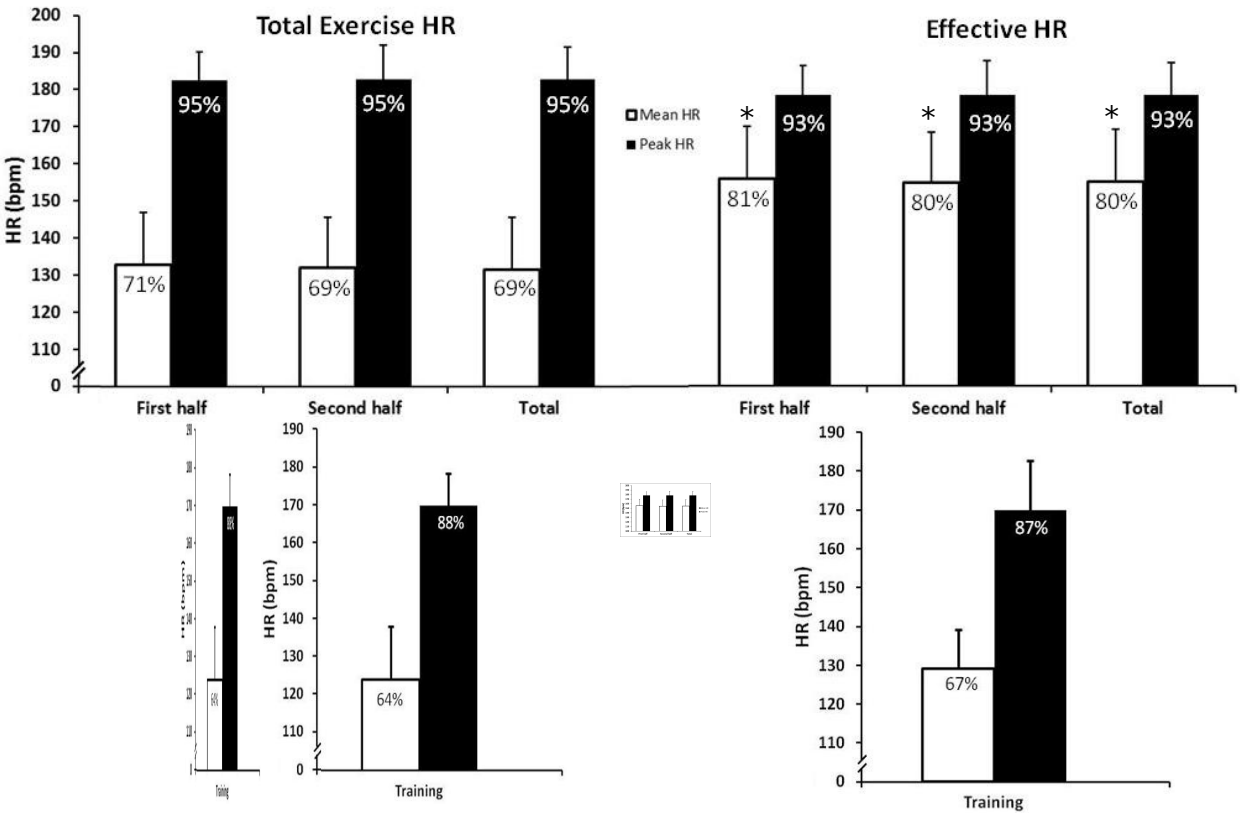

Figure 3

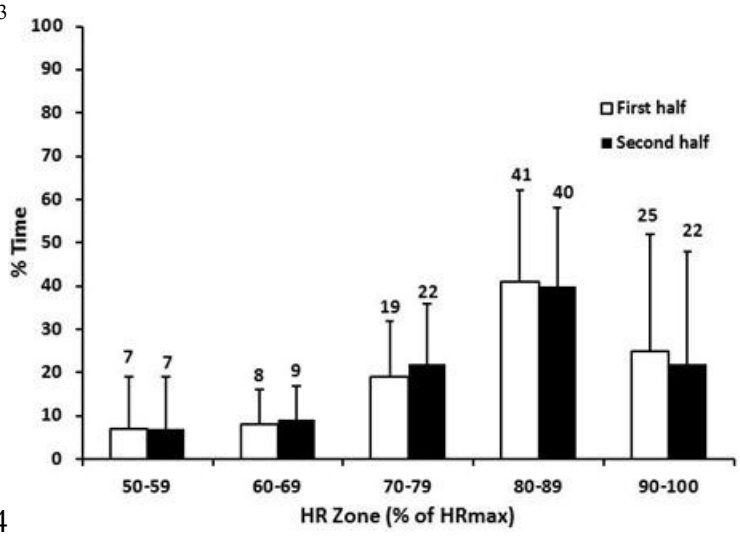

Figure 4

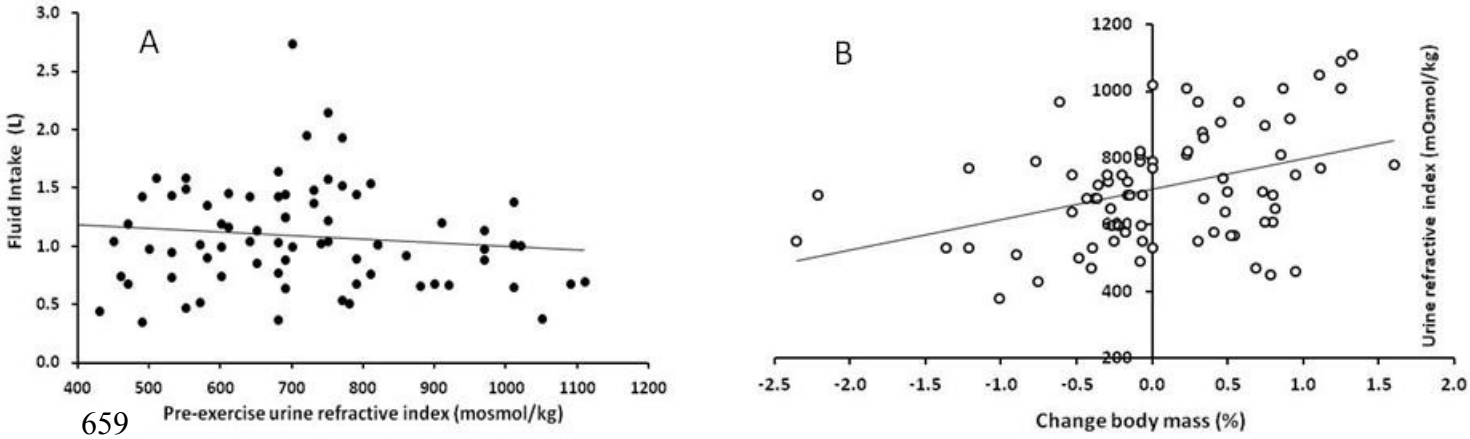

Figure 5

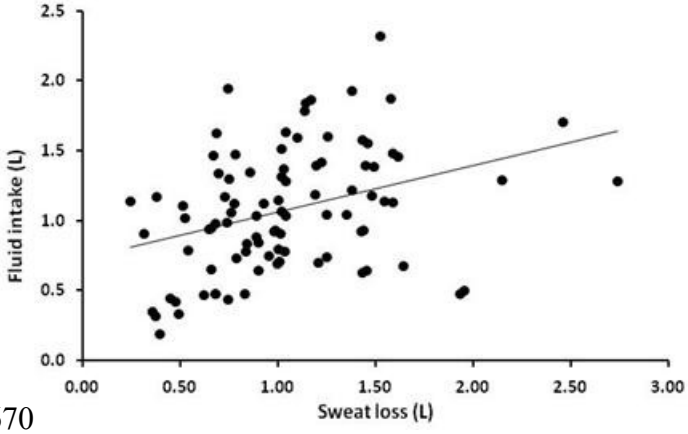

Figure 6