Proof of concept: hypovolemic hyponatremia may precede and augment creatine kinase elevations during an ultramarathon

Article *in* Arbeitsphysiologie · January 2016

DOI: 10.1007/s00421-015-3324-4

CITATIONS
3

READS
52

2 authors, including:

Tamara D Hew-Butler
Oakland University
131 PUBLICATIONS **2,208** CITATIONS

SEE PROFILE

Eur J Appl Physiol (2016) 116:647–655
DOI 10.1007/s00421-015-3324-4

ORIGINAL ARTICLE

Proof of concept: hypovolemic hyponatremia may precede and augment creatine kinase elevations during an ultramarathon

Ross S. Cairns[1] · **Tamara Hew-Butler**[2]

Received: 14 July 2015 / Accepted: 20 December 2015 / Published online: 8 January 2016

Abstract
Purpose It is not known if exercise-associated hyponatremia (EAH) is a cause or consequence of exertional rhabdomyolysis (ER).We hypothesized that osmotic stress (EAH) coupled with mechanical stress (running) potentiated muscle cell breakdown (ER). This concept would be supported if a nadir in serum sodium concentration ($[Na^+]$) temporally preceded peak creatine kinase levels (CK) during an ultramarathon run.
Methods Fifteen participants ran ≥104 km and had blood drawn: prior to start; 53; 104 km; and 24-h post run. Serum $[Na^+]$, CK, urea, creatinine and estimated glomerular filtration rate (eGFR) were measured from serial blood samples. Two-way repeated-measures ANOVA was used to examine differences regarding both race distance and natremia status.
Results Ten of 15 participants demonstrated EAH (serum $[Na^+]$ <135 mmol/L) at least once during serial testing. Participants were categorized post hoc into one of three natremia groups based on lowest recorded $[Na^+]$: (1) <129 mmol/L ($n = 3$; moderate EAH); (2) between 129 and 134 mmol/L ($n = 7$; mild EAH); and (3) >134 mmol/L ($n = 5$; normonatremia). Participants with lowest $[Na^+]$ demonstrated highest CK values at subsequent checkpoints. Significant natremia group differences noted at the 53 km point ($p = 0.0002$) for $[Na^+]$ while significant natremia group effect noted for CK seen at the 24-h post-finish testing point ($p = 0.02$). Significant natremia group effects noted for renal biomarkers, with the moderate EAH group documenting the lowest eGFR ($p = 0.005$), and highest serum urea ($p = 0.0006$) and creatinine ($p < 0.0001$) levels. Hyponatremic runners had lower post-race urine $[Na^+]$ than normonatremic runners (26 ± 15 vs. 89 ± 79 mmol/L; $p = 0.03$).
Conclusions Preliminary data support the possibility that transient hypovolemic EAH may precede and augment CK during an ultramarathon.

Keywords Acute kidney injury · Rhabdomyolysis · Dysnatremia

Abbreviations

AKI	Acute kidney injury
ANOVA	Analysis of variance
AVP	Arginine vasopressin
BUN	Blood urea nitrogen
CK	Creatine kinase
GFR	Glomerular filtration rate
EAH	Exercise-associated hyponatremia
ER	Exertional rhabdomyolysis
$[Na^+]$	Sodium concentration
NSAID	Non-steroidal anti-inflammatory drugs

Introduction

Exercise-associated hyponatremia (EAH: commonly defined as a serum sodium concentration below

Communicated by Fabio Fischetti.

Electronic supplementary material The online version of this article (doi:10.1007/s00421-015-3324-4) contains supplementary material, which is available to authorized users.

✉ Tamara Hew-Butler
hew@oakland.edu

[1] Newcastle Sports Medicine, New Lambton, New South Wales, Australia

[2] School of Health Science, 3157HHB, Oakland University, Rochester, MI 48309-4482, USA

135 mmol/L) (Hew-Butler et al. 2015) and exertional rhabdomyolysis (ER: commonly defined as a creatine kinase level above 10,000 U/L) (Clarkson et al. 2006) largely appear to be independent and transient laboratory features documented after an ultramarathon (Hoffman et al. 2013; Lebus et al. 2010). Both biochemical abnormalities have been reported in up to 50 % of convenience sampling cohorts (Hoffman et al. 2013; Lebus et al. 2010), with fatalities rare, but documented, in otherwise healthy marathon runners (Ayus et al. 2000; Lonka and Pedersen 1987). Most cases of clinically symptomatic EAH appear to be primarily related to overhydration states, with encephalopathy the most emergent medical complication of fluid overload (Ayus et al. 2000; Noakes et al. 2005). Conversely, symptomatic ER is more often associated with dehydration which may progress to acute renal failure if hypovolemia is not restored (Seedat et al. 1990). Thus, from a fluid balance perspective, the coexistence of symptomatic hyponatremia with rhabdomyolysis seems pathophysiologically unlikely because EAH and ER are associated with fluid imbalances at opposing ends of the spectrum.

With that said, three symptomatic (Ellis et al. 2009; Bruso et al. 2010; Boulter et al. 2011) and one asymptomatic (Chlibkova et al. 2015) case cluster of EAH with ER have been reported following ultramarathon footraces. In 2009, Ellis et al. described four hospitalized cases following a 153 km footrace (Ellis et al. 2009). Then, Bruso et al. described five cases after a 161 km mountain run, with one runner requiring dialysis with a peak CK of 785,250 U/L (Bruso et al. 2010). Boulter et al. then described four runners hospitalized with renal failure after an 89 km run in 2011, all of which were hyponatremic upon hospital admission (Boulter et al. 2011). Chlibkova recently documented 2/31 (6 %) cases of EAH with ER in ultramarathon runners but 0/82 (0 %) in ultradistance cyclists (Chlibkova et al. 2015). In all case clusters, it was unclear whether or not: (1) ER-mediated oliguria or non-osmotic arginine vasopressin (AVP) secretion preceded dilutional EAH or (2) EAH-mediated alterations in cellular size and/or intracellular cation imbalances (osmotic shock) augmented muscle cell breakdown (Adler 1980; Ellis et al. 2009; Martins et al. 2008). In previous reports detailing a hiker (Putterman et al. 1993) and a half-marathon runner (Glace and Murphy 2008) with severe EAH (serum [Na^+] <120 mmol/L) and increasing CK after normalization of natremia status, seizure activity may have complicated muscle cell breakdown following the return to normonatremia.

At present, the temporal relationship between EAH and ER has not been clearly established. A statistically significant increase in CK was noted in 12 ultramarathoners with mild EAH (54,583 ± 60,836 U/L) compared to 195 runners without EAH (30,335 ± 32,586 U/L) when tested at the finish line (Hoffman et al. 2013). A significant inverse relationship was also noted between post-race serum [Na^+] vs. CK in that observational study, although CK levels ranged between a few hundred to a few hundred thousand for the 12 runners finishing the race with a serum [Na^+] between 131 and 134 mmol/L (Hoffman et al. 2013). Data obtained from non-exercising patients, however, clearly document spontaneous rhabdomyolysis after correction of hyponatremia (Rizzieri 1995; Zaidi 2005) and characterize hyponatremia-induced myopathy (without seizures) as having either an acute or slowly progressive onset (Sasaki et al. 2007). Skeletal muscle cells are perfect osmometers (Overgaard-Steensen et al. 2010). Thus, hyponatremia sustained during exercise may exacerbate muscle cell breakdown by stretch-induced weakening of the sarcolemma which would then predispose it to rupture during vigorous or sustained physical activity. As such, the primary aim of this investigation was to assess the temporal relationship between EAH and ER in runners participating in an ultra-endurance mountain footrace. Secondary aims were to assess serial changes and relationships between in serum electrolytes and renal function biomarkers. We hypothesized that hyponatremia would both precede and augment CK levels following a mountain footrace. Such a finding would provide proof of concept, previously validated in clinical studies, that osmotic shock may be a precursor for significant skeletal muscle breakdown and subsequent renal compromise following prolonged endurance running.

Methods

These data represent the renal component of a previously published companion paper on non-osmotic arginine vasopressin (AVP) stimulation during prolonged endurance running (Cairns and Hew-Butler 2015).

Subjects All of the 120 entrants in the 'Great North Walk' (GNW 100s) ultra-endurance race, which includes two side-by-side races of approximately 100 km (104 km) and 100 miles (174 km), were invited to participate in this research trial. Athletes with diabetes, renal or cardiovascular disease or use of prescription medications which interfere with renal function were excluded from participation. Ethics approval was obtained from the Australian Institute of Sport Ethics Committee. All procedures performed were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants who were included in the study.

Serial blood sampling procedures Each athlete had either four (100 km entrants) or five (174 km/100 mile entrants) blood tests serially performed before, during and 24-h after finishing the race. These specific blood collection sites

were at: (1) race start (within 60 min of start); (2) 53 km; (3) 104 km; (4) 174 km; and (5) 24 h post-race. Samples were collected as close to 24 h post-race as possible (mean 23.1 h, range 12.3–37.3 h). The post-race sample was collected at the athlete's nearest Laverty Pathology Laboratory. Venesection stations were setup within 30 m of each checkpoint entrance. Athletes were immediately directed to the venesection station. Blood samples were obtained from the antecubital fossa in an upright seated position. Fifteen mLs of venous blood was collected into one 5 mL ethylenediaminetetraacetic acid (EDTA) plasma tube and two 5 mL serum clot activator tubes. The EDTA tube was centrifuged within 10 min of collection at 1500*g* for 10 min and then placed on dry ice. Clot activator tubes clotted for 30–60 min before being centrifuged at 1500*g* for 10 min and then placed on dry ice. All samples were kept on dry ice until they could be transferred to −80 °C freezers.

Urine collection and bodyweight Urine samples were provided at the time of the pre-race blood test and immediately post-race. The samples were kept on ice until they were transferred to clinical refrigerators. Athletes were weighed without shoes and socks on, on scales that were placed on a hard, flat surface.

Laboratory measurements Blood samples collected in serum clot activator tubes were analyzed for serum electrolytes, urea, creatinine and creatine kinase (CK). CK, serum electrolytes, urea, creatinine and urinary sodium were measured using 'Siemens Advia 2400' (Siemens Healthcare Diagnostics, Tarrytown, NY, US). Serum and urine electrolytes were measured using the Ion Selective Electrode method. Urea was measured using the Roch-Ramel enzymatic reaction. Creatinine was assessed using the picric acid reaction method. Glomerular filtration rate was estimated (eGFR) using the Cockroft-Gault equation: $\text{eGFR} = (140 - \text{age}) \times (\text{weight} \times 0.85(\text{if female}))/\text{creat}$ $(\text{mg/dL}) \times 72$. Urine osmolality was measured on the 'The Advanced Micro-Osmometer' (Advanced Instruments, Norwood, MA, US).

Statistical analyses A two-way repeated-measures ANOVA was used to examine potential differences for serial blood measurements with respect to both distance (start; 53; 104 km; and 24-h post finish) and natremia status (serum $[Na^+]$ <129 mmol/L; between 129 and 134 mmol/L; and >134 mmol/L). Only those distances with full data sets (start, 53, 104 km, and 24 h post; $N = 15$) were included. Data from the seven runners who completed the 174 km/100 mile race were not included in the two-way ANOVA, due to unequal sample size at this checkpoint. A repeated-measures ANOVA was also performed to examine potential pre- to post-race differences in urine $[Na^+]$ and osmolality. When necessary, a Bonferroni correction with unweighted means was performed post hoc to further identify specific differences between groups. T-tests were also performed at all checkpoints for all variables to assess potential differences between the following groups: Ever Hyponatremics vs. Never Hyponatremics; EAH race finish vs. Non-EAH at race finish; and NSAID ingestion vs. no NSAID ingestion. Statistical significance alpha level was set a priori at $p < 0.05$. All data presented as mean ± SD unless otherwise noted.

Results

Ten of fifteen runners (67 %) demonstrated biochemical EAH at least once during the serial testing time-points. Based on (post hoc) natremia status, the fifteen runners were then categorized into one of the three groups, based on a numerical classification system previously described by Noakes (Noakes et al. 2005): (1) Those runners with a serum $[Na^+]$ <129 mmol/L at any point during serial testing ($n = 3$; moderate hyponatremia); (2) Those runners with a serum $[Na^+]$ between 129 and 134 mmol/L ($n = 7$; mild hyponatremia); and (3) Those runners with a serum $[Na^+]$ >134 mmol/L at all testing points ($n = 5$; normonatremia). None of the runners demonstrated clinical symptomatology which required medical treatment at any time; thus we renamed our groups "moderate" instead of clinically significant and "mild" instead of biochemical hyponatremia to better reflect the absence of signs and symptoms in the present cohort. No statistically significant differences were noted with regards to age, pre-race bodyweight, fluid intake or race finish time between the three natremia groups (Table 1). Ambient temperatures ranged between 17.3 and 29.6 °C during the race (Cairns and Hew-Butler 2015).

For the two-way ANOVA analyses, a natremia effect represented a significant difference in the measured variable between serum $[Na^+]$ groups while a distance effect represented a significant change in the measured variable with respect to the distance covered. By design, there was a significant natremia effect with regards to serum $[Na^+]$ at the 53 km point, with the moderate hyponatremia group having a significantly lower serum $[Na^+]$ compared to both the mild hyponatremia and normonatremia groups $[F(2,48) = 10.53]$ (Fig. 1A). For CK, there was a significant natremia group effect $[F(2,48) = 4.15]$ and distance effect $[F(3,48) = 8.16]$ (Fig. 1B). There were no significant effects with regards to serum $[K^+]$, with the mean values of all groups within the normal physiological range (data not shown).

For eGFR, there was a significant natremia group effect $[F(2,48) = 5.83]$ and distance effect $[F(3,48) = 4.70]$ (Fig. 2A). For creatinine, there was a significant natremia group effect $[F(2,48) = 12.31]$ and distance effect $[F(3,48) = 11.67]$ (Fig. 2B). For urea, there was a significant natremia group effect $[F(2,48) = 8.69]$ and distance

Table 1 Average demographic, rate of fluid ingestion and finishing time of the 15 participants, categorized by the lowest serum [Na^+] recorded during any of the serial blood collection points (Natremia groupings of: moderate hyponatremia; mild hyponatremia; and normonatremia, respectively)

Variable (units)	Serum [Na^+] <129 mmol/L (n = 3: 1*F*; 2 M) Mean ± SD	Serum [Na^+] 129–134 mmol/L (n = 7: 2*F*; 5 M) Mean ± SD	Serum [Na^+] >134 mmol/L (n = 5: 5 M) Mean ± SD
Age (years)	50.6 ± 11.0	40.7 ± 10.3	43.8 ± 8.0
Pre-race weight (kg)	73.1 ± 10.9	68.8 ± 9.8	71.3 ± 5.9
Fluid intake (mL/h)	499.3 ± 191.5	501.3 ± 111.5	640.9 ± 251.0
Fluid intake (mL/h/kg)	6.8 ± 2.1	7.3 ± 1.5	8.8 ± 2.8
Race finish time (h)	24.6 ± 9.8	26.5 ± 8.3	24.2 ± 9.1

F female, *M* male

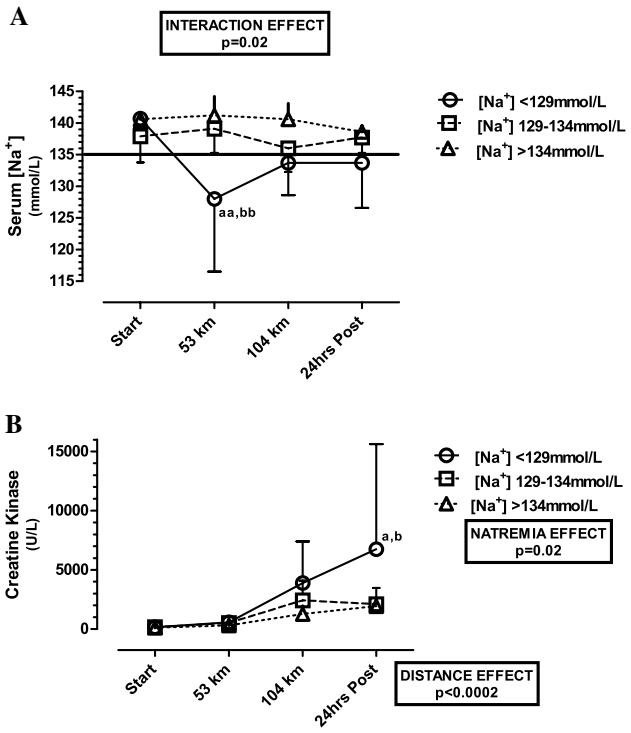

Fig. 1 Two-way repeated-measures ANOVA results for: **A** serum sodium ([Na^+]), and **B** creatine kinase; Race distance is represented on the *x*-axis and represents the temporal order in which the samples were taken. Natremia group is represented for each variable on the *y*-axis (moderate hyponatremia: [Na^+] <129 mmol/L; mild hyponatremia: [Na^+] 129–134 mmol/L; and normonatremia: [Na^+] >134 mmol/L). All data are represented by means (*symbols)* and standard deviation (*bars*). The standard deviation for the moderate hyponatremia group (*open circle*) is represented by the wide "T" and *solid line*; the mild hyponatremia group (*open square*) is represented by the more *narrow* "T" and *dashed line*; while the normonatremia group (*open triangle*) is represented by the bold "I" and *dotted line* to distinguish between the overlapping *error bars*. Significant differences from post hoc analyses are represented on the graphs as follows: *a* significantly different from mild hyponatremia group; *b* significantly different from normonatremia group (*a*, *b* = $p < 0.05$; *aa*, *bb* = $p < 0.01$; *aaa*, *bbb* = <0.001)

effect [F(3,48) = 17.99] (Fig. 2C). There were no significant effects for urine [Na^+] (Fig. 3A) but there was a significant distance effect for urine osmolality [F(1,22) = 5.37] (Fig. 3B). There were no significant effects with regards to bodyweight change (Fig. 3C).

The two runners with the lowest recorded serum [Na^+] values also demonstrated the highest CK values (Fig. 4). Both of these runners were male, with one (38 years old) participating in the 100 km (Fig. 4A, B) and the other (57 years old) participating in the 174 km (Fig. 4C, D) races. Both runners finished their respective races with normonatremia. Neither of these two runners ingested NSAID's prior to the documented nadir in serum [Na^+] at 53 km, with the 100 km runner (Fig. 4A, B) not ingesting any NSAID's while the 174 km runner (Fig. 4C, D) ingesting NSAID's between 104 and 174 km. The third runner (female, 57 years old) within the moderate hyponatremia group demonstrated a nadir serum [Na^+] value of 126 mmol/L 24 h after race finish. This runner ingested NSAID's between race start and 53 km, and subsequently recorded the lowest eGFR value and highest urea value at 104 km, compared with all other athletes tested (see supplements 1 and 2).

With regards to *t*-tests, when all the hyponatremic runners (both mild and moderate) were combined into a single group (Ever Hyponatremics), the only statistically significant difference between the Ever Hyponatremic (n = 10) vs. Never Hyponatremic groups (n = 5), was in serum [Na^+] at 104 km (135.3 ± 4.0 vs. 140.6 ± 2.4 mmol/L; p = 0.01) and in urine [Na^+] at race finish (25.8 ± 15.0 vs. 89.2 ± 78.9 mmol/L; p = 0.03). When the cohort was grouped into those runners with EAH at race finish (n = 4) vs. No-EAH at race finish (n = 11), the only statistically significant difference was found in serum [Na^+] at 104 km (131.8 ± 2.3 vs. 139.0 ± 3.0 mmol/L; p = 0.0009). When the cohort was categorized into those runners who ingested NSAID's (n = 7) vs. those who did not ingest NSAID's (n = 8), no statistically significant differences were noted at any checkpoint for any measured variable.

Individual trajectories for serum [Na^+], CK, creatinine, eGFR, urea and urine [Na^+] were plotted for all runners and all distances covered. In supplement 1, the Ever Hyponatremics were represented by solid lines while the Never Hyponatremics were represented by dashed lines. In supplement 2, those runners who ingested NSAID's (n = 7) were represented by solid lines while those runners who did not ingest NSAID's (n = 8) were represented by

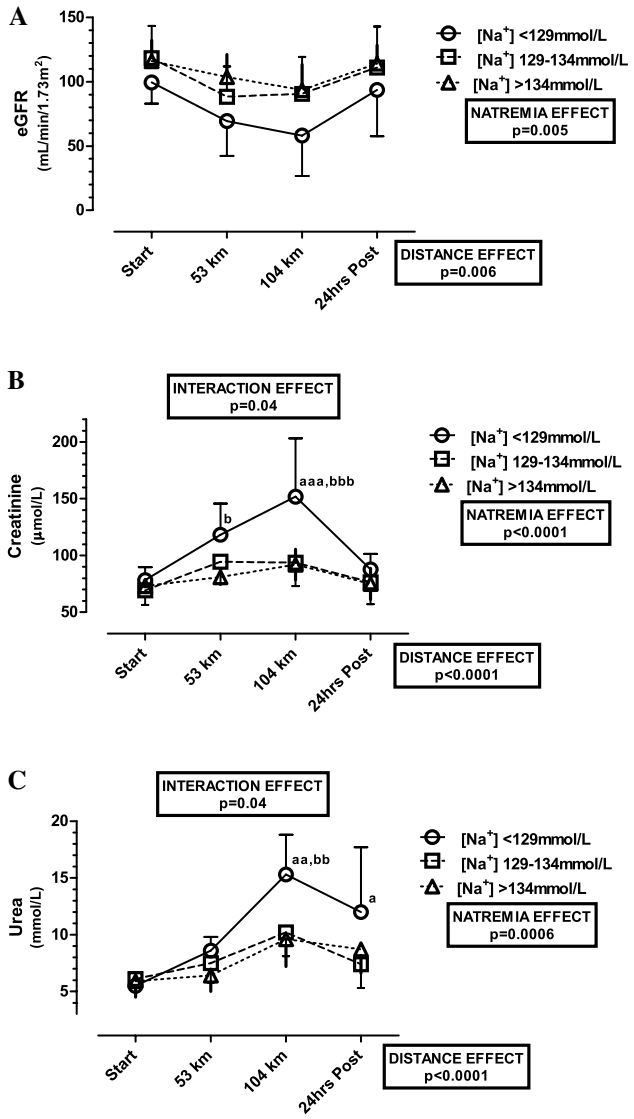

Fig. 2 Two-way repeated-measures ANOVA results for: **A** estimated glomerular filtration rate (eGFR); **B** serum creatinine concentration; **C** serum urea concentration. Race distance is represented on the *x*-axis and represents the temporal order in which the samples were taken. Natremia group is represented for each variable on the *y*-axis (moderate hyponatremia: $[Na^+]$ <129 mmol/L; mild hyponatremia: $[Na^+]$ 129–134 mmol/L; and normonatremia: $[Na^+]$ >134 mmol/L). All data are represented by means (*symbols*) and standard deviation (*bars*). The standard deviation for the moderate hyponatremia group (*open circle*) is represented by the wide "T" and *solid line*; the mild hyponatremia group (*open square*) is represented by the more narrow "T" and *dashed line*; while the normonatremia group (*open triangle*) is represented by the bold "I" and *dotted line* to distinguish between the overlapping *error bars*. Significant differences from post hoc analyses are represented on the graphs as follows: *a* significantly different from mild hyponatremia group; *b* significantly different from normonatremia group ($a, b = p < 0.05$; *aa, bb* $= p < 0.01$; *aaa, bbb* = <0.001)

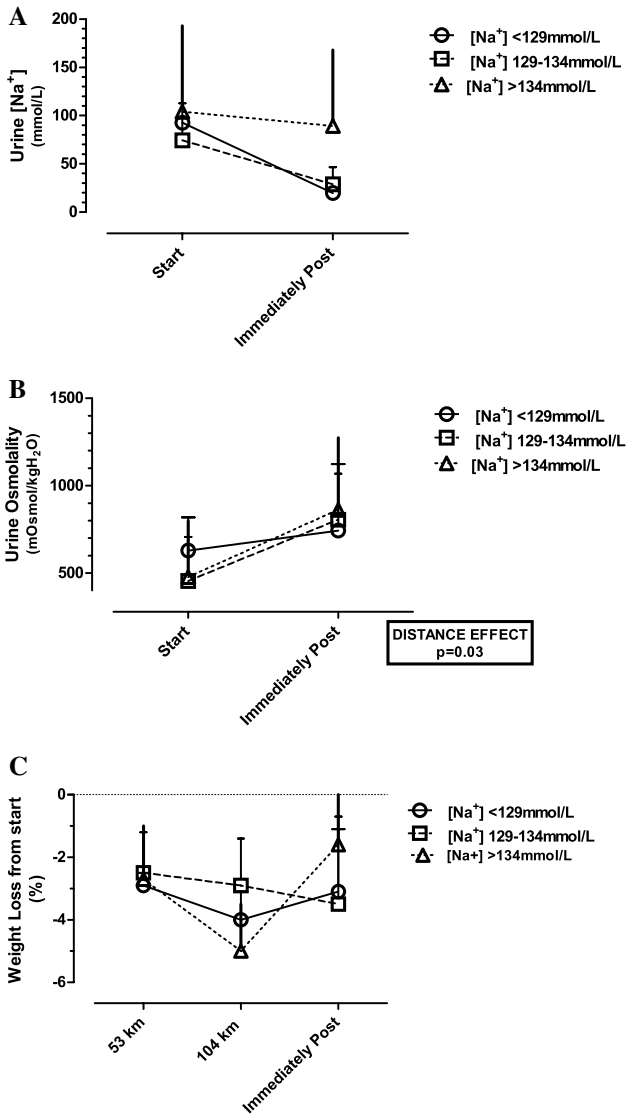

Fig. 3 Two-way repeated-measures ANOVA results for: **A** urine sodium concentration ($[Na^+]$); **B** urine osmolality; **C** body weight loss as a percentage of starting weight. Race distance is represented on the *x*-axis and represents the temporal order in which the samples were taken. Natremia group is represented for each variable on the *y*-axis (moderate hyponatremia: $[Na^+]$ <129 mmol/L; mild hyponatremia: $[Na^+]$ 129–134 mmol/L; and normonatremia: $[Na^+]$ >134 mmol/L). All data are represented by means (*symbols*) and standard deviation (*bars*). The standard deviation for the moderate hyponatremia group (*open circle*) is represented by the wide "T" and *solid line*; the mild hyponatremia group (*open square*) is represented by the more narrow "T" and *dashed line*; while the normonatremia group (*open triangle*) is represented by the bold "I" and *dotted line* to distinguish between the overlapping *error bars*

dashed lines. Of note, in supplement 2, the thicker portion of each solid line represents the individual trajectory after NSAID's were ingested.

Discussion

Transient hyponatremia appeared to precede significantly higher elevations in CK during a marathon footrace, in our limited sample size. The two runners with the lowest serum

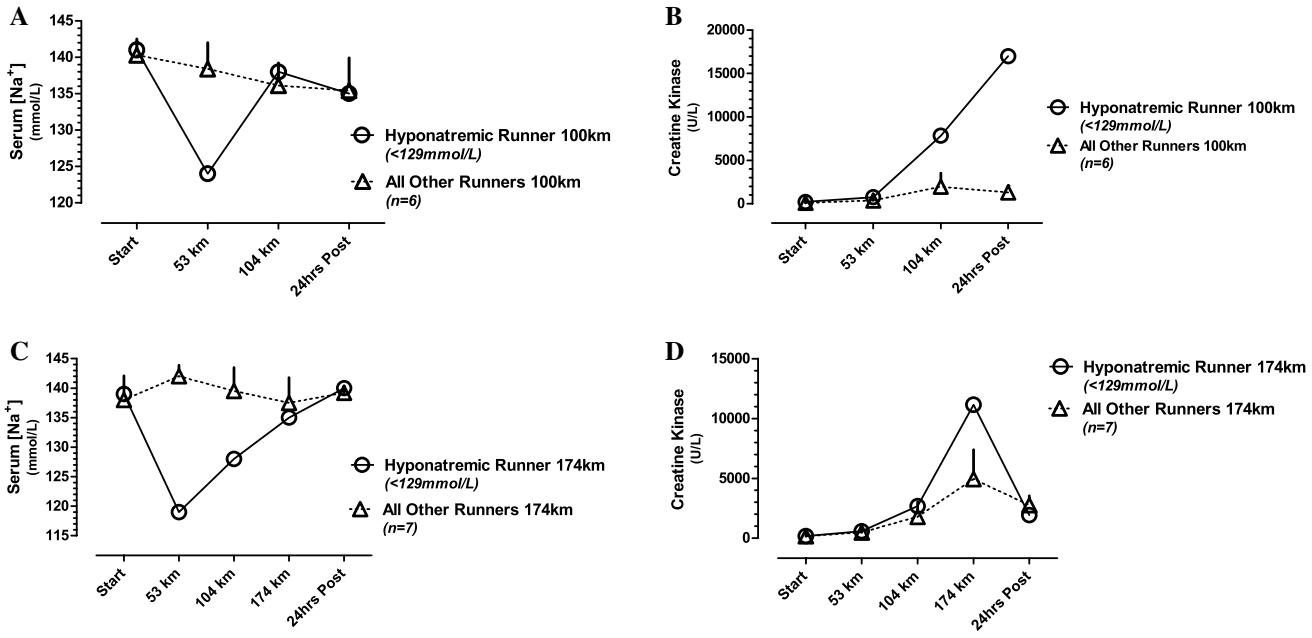

Fig. 4 The two participants with the lowest documented serum [Na^+] values (both at 53 km), also recorded the highest serum creatinine kinase values at subsequent blood collection points. The subject with the lowest serum [Na^+] and highest creatinine kinase level participating in the 100 km event is depicted next to the mean values of all other 100 km study participants in **A** and **B**, respectively. The subject with the lowest serum [Na^+] and highest creatinine kinase level participating in the 100 mile event is depicted next to the mean values of all other 100 mile study participants in **C** and **D**, respectively

[Na^+] demonstrated the highest CK values at subsequent checkpoints. At both the 104 and 174 km checkpoints, the mean elevations in CK appeared dose dependent; whereas those runners with the lowest serum [Na^+] had the highest CK values while those runners with the highest serum [Na^+] had the lowest CK values. Our preliminary results corroborate those findings obtained from hospitalized patients, which further suggest that all volemic variants of hyponatremia can precipitate muscle cell breakdown after serum [Na^+] correction is initiated (Korzets et al. 1996; Rizzieri 1995; Sasaki et al. 2007; Trimarchi et al. 1999; Zaidi 2005). The transient EAH and wide individual fluctuations in serum [Na^+]—which occurred most notably in the two asymptomatic runners at 53 km—remain unexplained and worthy of further critical evaluation with regards to both pathogenesis and clinical significance.

Although the exact mechanism is unclear, experiments performed on isolated mammalian skeletal muscle fibers suggest that mechanical stress generated by hypoosmotic cell swelling (osmotic shock) increases intracellular reactive oxygen species (ROS) which in turn triggers localized increase in cytosolic calcium (calcium sparks) (Martins et al. 2008). Calcium sparking—which can also be induced by strenuous exercise—then activates calcium-dependent proteases which may lead to skeletal muscle breakdown (Martins et al. 2008). Interestingly enough, it is the *return* to normonatremia (cell volume decrease following a hypoosmotic increase) which appears to initiate the pathological intracellular calcium sparking (Pickering et al. 2009). This temporal sequence of osmotically-mediated mechanical events is indirectly supported in our subjects with moderate, but transient, EAH and has also been confirmed in case studies involving psychogenic polydipsic patients (Rizzieri 1995; Zaidi 2005).

Rhabdomyolysis is a leading cause of acute renal failure, although ER appears to elevate the critical CK threshold before the onset of acute kidney injury (AKI) (de Meijer et al. 2003; MacSearraigh et al. 1979; Sinert et al. 1994). Skeletal muscle constitutes roughly 40 % of total body mass, 50 % of total body water and 70 % of total body potassium (MacSearraigh et al. 1979; Overgaard-Steensen et al. 2010). Any additional factor which may weaken the sarcolemma—in conjunction with the repetitive mechanical stressors which accompany intense or sustained physical activity—would hypothetically exacerbate the severity of ER. Hypoosmotic skeletal muscle swelling also weakens the cell membrane by stretching and thinning the mechanical barrier which separates intracellular from extracellular contents. Therefore, it seems plausible that even transient hyponatremia, as seen in our subjects, accelerates muscle cell breakdown via three cumulative pathological processes: (1) electrochemical disruptions associated with osmotic shock; (2) mechanical weakening of the cell membrane due to hypoosmotic cellular swelling; and (3) continued mechanical trauma from sustained endurance running.

Hypokalemia was not a contributing factor to ER in our subjects, as hypothesized elsewhere (MacSearraigh et al. 1979).

Non-osmotic AVP stimulation during exercise remains a strong pathophysiological factor in the development of EAH (Hew-Butler et al. 2008). However, decreases in glomerular filtration rate (GFR) and renal function may contribute similarly to fluid overload hyponatremia by limiting urinary excretion of any fluid excess. As such, the estimated GRF (eGFR) was lowest in our moderate EAH group at all distances tested, including baseline. Although the temporal changes in eGFR, creatinine and urea did not mirror the pattern of either serum [Na^+] or CK, the potential influence of reduced renal function in response to exercise has yet to be evaluated in relationship to natremic status during exercise.

The average percent decrease in eGFR seen in our subjects was dose dependent by natremia group, with the largest decreases noted in descending order of natremia status. As such, the reductions in eGFR from starting values at the 53, 104 km and 24-h post blood collection checkpoints were: −31, −43 and −8 % for the moderate natremia group; −26, −25 and −7 % for the mild natremia group; and −11, −20 and −1 % for the normonatremia group. The Risk, Injury, Failure, Loss of function, and End-stage renal disease (RIFLE) criteria defines a >25 % decrease in GFR from baseline into the category of AKI Risk (Ricci et al. 2011). According to this classification, both natremia groups had clinically relevant decreases in renal function. Whether or not these reductions in eGFR represent acute kidney injury is unlikely, however, as running has been shown to transiently reduce GFR immediately post-exercise with a return to pre-exercise levels noted within 2 h after a 30 min run at 80 % VO_2 max (Baker et al. 2005) and within 24 h after both a half-marathon (Lippi et al. 2008) and full marathon (McCullough et al. 2011). The lower eGFR's seen in the moderate hyponatremic group may reflect the older mean age of this particular cohort (Table 1) (Coresh et al. 2003). However, the contributions of reduced eGFR to pathologic water retention remain unclear, especially since fitness ameliorates exercise-induced reductions in both renal blood flow (RBF) and GFR (Baker et al. 2005). Of note, the third subject in the moderate EAH group ingested NSAID's between the start and 53 km and then demonstrated the lowest eGFR at 104 km (supplement 2); supporting the potential for NSAID-mediated reductions in RBF and GFR as described previously (Walker et al. 1994).

Serum creatinine and urea were also significantly elevated in the moderate hyponatremia group, compared to both the mild hyponatremia and normonatremia groups. Peak values for both of these variables were documented at the 104 km distance, after which the nadir in serum [Na^+] was detected, but not in parallel with temporal changes in CK. Serum creatinine is a metabolic product of creatine, so the lack of a parallel temporal response with CK suggests that elevations in creatinine were largely independent of skeletal muscle breakdown and perhaps more reflective of pre-renal azotemia.

Serum urea was negatively associated with serum [Na^+] and eGFR which would suggest that the EAH seen in this cohort was largely associated with a decrease in effective arterial blood volume. Hypovolemic hyponatremia can be further supported, in both the moderate and mild hyponatremic groups, by post-race urine [Na^+] values below 30 mmol/L, which would demonstrate maximal renal sodium conservation associated with volume depletion (Chung et al. 1987; Fenske et al. 2008). Urinary free water conservation occurred in all three natremic groups, as demonstrated by pre- to post-race increases in urine osmolality. Body weight loss was also present in all three natremia groups, with no discernible pattern temporally related to changes in either natremia status or renal function markers.

Although these results may support the proof of concept that transient EAH might be an amplifier of CK levels during exercise, we do not wish to minimize the well-known contributions of CK-induced renal failure to the subsequent development of dilutional hyponatremia from AKI-induced oliguria and anuria (Bosch et al. 2009; MacSearraigh et al. 1979). The strength of the present findings, however, highlights an under-recognized relationship between hypovolemic hyponatremia and rhabdomyolysis that may hypothetically be exacerbated by continued running.

The limitations of most, if not all, EAH research is enrolling enough athlete volunteers and collecting balanced data sets. The scientific hope of field-based data collection is to detect enough hyponatremia cases to detect meaningful interconnected pathophysiology. This particular venue offered us a rare window of insight, with 67 % of the cohort demonstrating serum [Na^+] values below the biochemical threshold of interest (serum [Na^+] <135 mmol/L). Thus, however uncontrolled and unbalanced field collection may appear, it is important to emphasize that EAH and ER are both potentially fatal medical conditions (Ayus et al. 2000; Lonka and Pedersen 1987) and the lessons learned from these serendipitous clusters are far beyond what we can ethically induce within our well-controlled laboratories. As such, we duly recognize the clear potential for artificial positives from a lack of normality and a Gaussian distribution when comparing sub-groups consisting of only three, five and seven subjects. However, choosing an alpha of 0.05 when conducting analyses between these artificial classifications of natremia status ameliorates the likelihood of false positives in what can alternatively be considered an observational case series (see supplements 1 and 2). Additional limitations include the large variability in NSAID ingestion, as this class of anti-inflammatory medications

has been previously associated with EAH, ER and renal insufficiency. Plasma volume was also not assessed in this trial, which may have particularly influenced urea (increased absorption from volume contraction) and serum [Na^+] (fluctuating total protein levels) values.

In conclusion, although these limited data cannot infer causality, they do provide support for the proof of concept that the hypovolemic variant of EAH may precede ER in some cases while ER may precede EAH in others. This circular relationship warrants further investigation with larger data sets in exercise settings. The influence of NSAID ingestion on the development of EAH, ER and renal compromise remains unclear.

Acknowledgments Funding of AU$2000 was received from the GNW100s race organization committee. The authors would like to thank Dr Vanessa Cairns BSc, MBBS for considerable help in formulation of data collection, data input and proof reading; Dr Jonathan Brown BM, MRCGP, FRACGP for help in data collection; Dr Malcolm Jonathan King MBChB, MSc, FRACGP, FACSP for help in data collection; Royal Prince Alfred Hospital, Camperdown, Sydney, NSW; Immunology and Biochemistry Laboratories for blood sample analysis; Laverty Pathology for blood sample analyses. The authors declare no conflicts of interest.

References

Adler S (1980) Hyponatremia and rhabdomyolysis: a possible relationship. South Med J 73:511–513

Ayus JC, Varon J, Arieff AI (2000) Hyponatremia, cerebral edema, and noncardiogenic pulmonary edema in marathon runners. Ann Intern Med 132:711–714

Baker J, Cotter JD, Gerrard DF, Bell ML, Walker RJ (2005) Effects of indomethacin and celecoxib on renal function in athletes. Med Sci Sports Exerc 37:712–717

Bosch X, Poch E, Grau JM (2009) Rhabdomyolysis and acute kidney injury. N Engl J Med 361:62–72

Boulter J, Noakes TD, Hew-Butler T (2011) Acute renal failure in four comrades marathon runners ingesting the same electrolyte supplement: coincidence or causation? S Afr Med J 101:876–878

Bruso JR, Hoffman MD, Rogers IR, Lee L, Towle G, Hew-Butler T (2010) Rhabdomyolysis and hyponatremia: a cluster of five cases at the 161-km 2009 Western States Endurance Run. Wilderness Environ Med 21:303–308

Cairns RS, Hew-Butler T (2015) Incidence of exercise-associated hyponatremia and its association with nonosmotic stimuli of arginine vasopressin in the GNW100s ultra-endurance marathon. Clin J Sport Med 25:347–354

Chlibkova D, Knechtle B, Rosemann T, Tomaskova I, Novotny J, Zakovska A, Uher T (2015) Rhabdomyolysis and exercise-associated hyponatremia in ultra-bikers and ultr-runners. J Int Soc Sports Nutr 12:29

Chung HM, Kluge R, Schrier RW, Anderson RJ (1987) Clinical assessment of extracellular fluid volume in hyponatremia. Am J Med 83:905–908

Clarkson PM, Kearns AK, Rouzier P, Rubin R, Thompson PD (2006) Serum creatine kinase levels and renal function measures in exertional muscle damage. Med Sci Sports Exerc 38:623–627

Coresh J, Astor BC, Greene T, Eknoyan G, Levey AS (2003) Prevalence of chronic kidney disease and decreased kidney function in the adult US population: third national health and nutrition examination survey. Am J Kidney Dis 41:1–12

de Meijer AR, Fikkers BG, de Keijzer MH, van Engelen BG, Drenth JP (2003) Serum creatine kinase as predictor of clinical course in rhabdomyolysis: a 5-year intensive care survey. Intensiv Care Med 29:1121–1125

Ellis C, Cuthill J, Hew-Butler T, George SM, Rosner MH (2009) Exercise-associated hyponatremia with rhabdomyolysis during endurance exercise. Phys Sportsmed 37:126–132

FenskeW, Stork S, Koschker A, Blechschmidt A, Lorenz D, Wortmann S, Allolio B (2008) Value of fractional uric acid excretion in differential diagnosis of hyponatremic patients on diuretics. J Clin Endocrinol Metab 93:2991–2997

Glace B, Murphy C (2008) Severe hyponatremia develops in a runner following a half-marathon. JAAPA 21:27–29

Hew-Butler T, Rosner MH, Fowkes-Godek S, Dugas JP, Hoffman MD, Lewis DP, Maughan RJ, Miller KC, Montain SJ, Rehrer NJ, Roberts WO, Rogers IR, Siegel AJ, Stuempfle KJ, Winger JM, Verbalis JG (2015) Statement of the third international exercise-associated hyponatremia consensus development conference, Carlsbad, CA, 2015. Clin J Sport Med 25:303–320

Hoffman MD, Fogard K, Winger J, Hew-Butler T, Stuempfle KJ (2013) Characteristics of those with exercise-associated hyponatremia after a 161-km run. Res Sports Med 21:164–175

Korzets A, Ori Y, Floro S, Ish-Tov E, Chagnac A, Weinstein T, Zevin D, Gruzman C (1996) Case report: severe hyponatremia after water intoxication: a potential cause of rhabdomyolysis. Am J Med Sci 312:92–94

Lebus DK, Casazza GA, Hoffman MD, Ganong AC, Van Loan M (2010) Can changes in body mass and total body water accurately predict hyponatremia following a 161-km running race? Clin J Sport Med 20:193–199

Lippi G, Schena F, Salvagno GL, Tarperi C, Montagnana M, Gelati M, Banfi G, Guidi GC (2008) Acute variation of estimated glomerular filtration rate following a half-marathon run. Int J Sports Med 29:948–951

Lonka L, Pedersen RS (1987) Fatal rhabdomyolysis in marathon runner. Lancet 8537:857–858

MacSearraigh ET, Kallmeyer JC, Schiff HB (1979) Acute renal failure in marathon runners. Nephron 24:236–240

Martins AV, Shkryl VM, Nowycky MC, Shirokova N (2008) Reactive oxygen species contribute to Ca2+ signals produced by osmotic stress in mouse skeletal muscle fibres. J Physiol 586:197–210

McCullough PA, Chinnaiyan KM, Gallagher MJ, Colar JM, Geddes T, Gold JM, Trivax JE (2011) Changes in renal markers and acute kidney injury after marathon running. Nephrology 16:194–199

Noakes TD, Sharwood K, Speedy D, Hew T, Reid S, Dugas J, Almond C, Wharam P, Weschler L (2005) Three independent biological mechanisms cause exercise-associated hyponatremia: evidence from 2135 weighed competitive athletic performances. Proc Natl Acad Sci 102:18550–18555

Overgaard-Steensen C, Stodkilde-Jorgensen H, Larsson A, Broch-Lips M, Tonnesen E, Frokiaer J, Ring T (2010) Regional differences in osmotic behavior in brain during acute hyponatremia: an in vivo MRI-study of brain and skeletal muscle in pigs. Am J Physiol Regul Integr Comp Physiol 299:R521–R532

Pickering JD, White E, Duke AM, Steele DS (2009) DHPR activation underlies SR Ca2+ release induced by osmotic stress in isolated rat skeletal muscle fibers. J Gen Physiol 133:511–524

Putterman C, Levy L, Rubinger D (1993) Transient exercise-induced water intoxication and rhabdomyolysis. Am J Kidney Dis 21:206–209

Ricci Z, Cruz DN, Ronco C (2011) Classification and staging of acute kidney injury: beyond the RIFLE and AKIN criteria. Nat Rev Nephrol 7:201–208

Rizzieri DA (1995) Rhabdomyolysis after correction of hyponatremia due to psychogenic polydipsia. Mayo Clin Proc 70:473–476

Sasaki M, Yuzawa M, Saito T, Ikoma A, Tamemoto H, Kawakami M, Ishikawa SE (2007) Clinical and laboratory features of hyponatremia-induced myopathy. Clin Exp Nephrol 11:283–286

Seedat YK, Aboo N, Naicker S, Parsoo I (1990) Acute renal failure in the "comrades marathon" runners. Ren Fail 11:209–212

Sinert R, Kohl L, Rainone T, Scalea T (1994) Exercise-induced rhabdomyolysis. Ann Emerg Med 23:1301–1306

Trimarchi H, Gonzalez J, Olivero J (1999) Hyponatremia-associated rhabdomyolysis. Nephron 82:274–277

Walker RJ, Fawcett JP, Flannery EM, Gerrard DF (1994) Indomethacin potentiates exercise-induced reduction in renal hemodynamics in athletes. Med Sci Sports Exerc 26:1302–1306

Zaidi AN (2005) Rhabdomyolysis after correction of hyponatremia in psychogenic polydipsia possibly complicated by ziprasidone. Ann Pharmacother 39:1726–1731