

# Appendices

## Reply on Baker's comments to Nolte and Noakes: "change in body mass accurately and reliably predicts change in body water after endurance exercise"

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To the Editor,

The title of the original article by Dr Baker and her colleagues was: "Change in body mass accurately and reliably predicts change in body water after endurance exercise". In her response to our letter Dr Baker reduces her certainty. She now concludes only that the change in body mass is a "reasonable method to estimate hydration status". This is a more reasonable conclusion that better reflects the published literature.

But the focus of our letter was not to show that change in body mass ( $\Delta$ BM) is an unreliable method for estimating hydration status. Why would we try to disprove a relationship that we believe exists (Nolte et al. 2010a, b; Tam et al. 2009)? There were two reasons for our letter. First, we concluded that the methods used by Baker et al. (2009) to measure total body water (TBW) may have been relatively imprecise since their study found a significant relationship between  $\Delta$ BM and the change in TBW ( $\Delta$ TBW) in a total of 62 measurements only when those data were analysed in a manner that, for the reasons we described in detail, we consider inappropriate. The potential methodological errors we identified might explain why their data did not show a relationship between  $\Delta$ BM and  $\Delta$ TBW when their four experiments were analysed separately.

Instead the real focus of our letter was to contest an apparent 1:1 relationship between the  $\Delta$ BM (in grams) and  $\Delta$ TBW (in millilitres) that the authors were able to extract from their data. We have not been able to show this relationship (Nolte et al. 2010a, b; Tam et al. 2009). Instead all our studies show an offset of at least 500 g in this relationship so that a  $\Delta$ BM of up to 1,000 g was required before it was possible to detect any  $\Delta$ TBW. In our letter, we explained why it is logical to assume that some of the  $\Delta$ BM during exercise is from sources other than water and which do not require replacement if the TBW is to be preserved. We are particularly interested in the hypothesis that a fluid reserve of up to 2 l, perhaps existing in the form of unabsorbed fluid in the intestine, may be retrieved as a fluid reserve when the rate of fluid loss from the body exceeds the immediate rate of fluid ingestion. There was substantial interest in this theory during the Second World War (Ladell 1947, 1955, 1965) but the issue was never resolved so that this possibility remains untested.

This unresolved questions is not without practical importance. After 1996, the idea took root that athletes should drink to stay ahead of thirst during exercise (Convertino et al. 1996; Armstrong et al. 1996). This was based on the theory that any  $\Delta$ BM during exercise is detrimental to both health and performance. Yet, when athletes drink to prevent any mass loss during exercise they usually develop a progressive hyponatremia as clearly shown in two separate studies by Dr Baker and her colleagues (Baker et al. 2005, 2008). We interpret this to mean that some body mass loss is essential during exercise if the serum sodium concentration is to be protected and exercise-associated hyponatremia is to be avoided (Noakes et al. 2005). Indeed changes in body mass alone explain almost all of the variance in the serum sodium concentrations during prolonged exercise (Noakes 2010). That is why it is important to

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determine the origin of the  $\Delta$ BM during exercise and the extent to which that mass loss reflects any  $\Delta$ TBW. Or conversely the extent to which other sources contributes to the  $\Delta$ BM during exercise.

Dr Baker's final paragraph indicates that she has not yet understood the point of our argument. Our data (Nolte et al. 2010a, b; Tam et al. 2009) indicate that there is indeed a relationship between the  $\Delta$ BM and the  $\Delta$ TBW so we fully agree that "the use of  $\Delta$ BM" is indeed "a reasonable method to estimate hydration status".

But that was not the main message that her original article sort to promote.

TD Noakes and HW Nolte.

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# Changes in Total Body Water Content During Running Races of 21.1 km and 56 km in Athletes Drinking Ad libitum

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**Objective:** To measure changes in body mass (BM), total body water (TBW), fluid intake, and blood biochemistry in athletes during 21.1-km and 56-km foot races.

**Design:** Observational study.

**Setting:** 2009 Two Oceans Marathon, South Africa.

**Participants:** Twenty-one (21.1 km) and 12 (56 km) participants were advised to drink according to thirst or their own race drink plan (ad libitum).

**Main Outcome Measures:** Body mass, TBW, plasma osmolality, plasma sodium ( $p[Na^+]$ ), and plasma total protein ([TP]) concentrations were measured before and after race. Fluid intake was recorded from recall after race.

**Results:** Significant BM loss occurred in both races (21.1 km;  $-1.4 \pm 0.6$  kg;  $P < 0.000$  and 56 km;  $-2.5 \pm 1.1$  kg;  $P < 0.000$ ). Total body water was reduced in the 56-km race ( $-1.4 \pm 1.1$  kg;  $P < 0.001$ ). A negative linear relationship was found between percentage change (% $\Delta$ ) in TBW and % $\Delta$  in BM in the 56-km runners ( $r = 0.6$ ;  $P < 0.01$ ). Plasma osmolality and [TP] increased significantly in the 56-km runners ( $6.8 \pm 8.2$  mOsm/kg  $H_2O$ ;  $P < 0.05$  and  $5.4 \pm 4.4$  g/L;  $P < 0.01$ , respectively), but all other biochemical measures were within the normal range.

**Conclusions:** Although TBW decreased in the 56-km race and was maintained in the 21.1-km race, the change in TBW over both races was less than the BM, suggesting that not all BM lost during endurance exercise is a result purely of an equivalent reduction in TBW. These findings support the interpretation that the body primarily defends

$p[Na^+]$  and not BM during exercise and that a reduction in BM can occur without an equivalent reduction in TBW during prolonged exercise. Furthermore, these data support that drinking without controlling for BM loss may allow athletes to complete these events.

**Key Words:** fluid balance, body mass loss, exercise, deuterium oxide  
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## INTRODUCTION

Recently, the American College of Sports Medicine (ACSM) revised its drinking guidelines from the proposal that athletes should drink “as much as tolerable during exercise” to advice that athletes should now drink according to the dictates of thirst provided any body mass (BM) loss during exercise does not exceed 2% of their starting BM.<sup>1,2</sup> In contrast, the drinking guidelines of the International Marathon Medical Directors Association propose that athletes should drink to thirst regardless of the extent of BM loss but should not drink more than 800 mL/h to reduce the risk that exercise-associated hyponatremia (EAH) will develop.<sup>3</sup>

Currently, the sole basis for the difference in these guidelines is the position of the ACSM that a BM loss in excess of 2% is associated with impaired exercise capacity.<sup>4</sup> Although the evidence to support this interpretation comes principally from laboratory-based studies, a number of field studies of competitive endurance events have failed to show that the best athletes always finish with BM loss less than 2%.<sup>4</sup> Rather, the evidence might be interpreted as proof for an opposite theory, namely, that BM loss during exercise may enhance performance especially in weight-bearing activities, particularly long-distance running.<sup>5–7</sup> Although the resolution of this debate requires additional studies of large numbers of competitors in out-of-laboratory exercise, a more direct question requiring an answer is the exact origin of the BM that is lost during exercise.

For example, Shephard and Kavanagh<sup>8</sup> proposed that BM loss might conceivably occur without any measurable reduction in total body water (TBW). This is because of irreversible substrate oxidation and the release of water complexed to glycogen (~2 kg) because the glycogen is metabolized during exercise. More recently, Pastene et al<sup>9</sup> and Maughan et al<sup>10</sup> have presented data to support this interpretation.

In contrast, Montain<sup>11</sup> and Baker et al<sup>12</sup> concluded the opposite. Baker et al suggest that all BM lost during exercise can be explained by an equivalent gram-for-milliliter reduction in TBW. But questions have been raised about their

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methodologies and statistical techniques and therefore its validity in supporting their conclusions.<sup>13</sup>

This debate has important practical implications for competitive athletes. If all the BM lost during exercise is because of a reduction in TBW alone and this TBW loss impairs exercise performance, then drinking to thirst alone is unlikely to be the optimal strategy. This is because participants who drink according to the dictates of thirst always lose BM during exercise, developing so-called *voluntary dehydration*. In contrast, if drinking to thirst prevents or minimizes any reduction in TBW, as some evidence suggests,<sup>14</sup> then this drinking strategy might be considered appropriate, in which case, the presence of thirst, not a reduced BM, might explain the impaired exercise performance in those who are forced to restrict their fluid intake during prolonged exercise.<sup>15</sup>

Indeed, there is no evidence to support the concept that during prolonged exercise, BM is a physiologically regulated parameter. Body mass regulation occurs chronically over months and years and is related to the regulation of fat and protein mass rather than to acute changes in fluid balance.<sup>16,17</sup>

We have recently shown that BM loss during exercise exceeded the reduction in TBW content in soldiers performing a 16-km route march in warm, dry conditions (−1 kg in BM and −0.5 kg in TBW).<sup>18</sup> To extend that analysis, we now report the findings of participants competing in 21.1-km and 56-km races, lasting from 1.5 to 7 hours. We hypothesized that BM loss would be greater in the 56-km race than in the 21.1-km race, but that in neither race would the change in BM accurately predict the change in TBW. We further hypothesized that fastest running performance would be associated with a greater degree of BM loss.

This study will have relevance to the theory that during real-life competition, athletes will drink to ensure plasma [Na<sup>+</sup>] maintenance irrespective of BM loss.

## METHODOLOGY

### Subjects

Entrants in the 2009 Two Oceans half marathon (21.1 km) and ultramarathon (56 km) were invited to participate in this study, which had been approved by the Human Research Ethics Committee of the University of Cape Town and conducted according to the Declaration of Helsinki (Seoul, South Korea, 2008). Twenty-one athletes gave written informed consent. All the athletes were encouraged to consume food and fluids ad libitum during the race.

### Setting

The Two Oceans Marathon is a 56-km ultradistance road running race around the Cape Peninsula, Cape Town, South Africa; the 21.1-km race is run on a part of the 56-km route. The event took place on a warm day with dry bulb temperatures ranging from 18°C to 24°C and 50% to 70% humidity. There was some cloud cover with a wind of 0.5 to 2 m/s. Cutoff time was 3 and 7 hours for the 21.1-km race and the 56-km race, respectively.

### Fluid Intake

Fluid intake was estimated from a food and fluid recall questionnaire after the race. The recall consisted of the amount of fluids (contained in sachets, cups, and bottles of known volume) consumed during the race. Fluids were available to the runners every 3 km and ~2 km on the 21.1-km route and the 56-km route, respectively.

### Measurement of Body Mass

Weight was acquired to the nearest 0.01 kg in racing attire without shoes on a calibrated digital scale [TCS-A300; Clover Scales (Pty), Ltd, Brackenfell, Cape Town, South Africa] that had been placed on a flat, solid, and stable surface after each athlete had emptied their bladder. Weighing took place 60 minutes before the race start (prerace) and immediately on completion of the race (postrace). Participants were dried with a towel immediately before the postrace reweighing.

### Urine Osmolality

Urine samples were collected before BM measurements when participants were requested to empty their bladder on the occasions described above. Time to urine collection after race was <15 minutes. Urine osmolality was measured in triplicate with the use of a portable refractometer (Osmocheck; Vitech Scientific, West Sussex, United Kingdom).

### Blood Biochemical Analyses

Ten milliliters of venous blood samples were collected from the antecubital vein into lithium–heparin Vacuette (Greiner Bio-one International AG, Kremmunster, Austria) containers after the participants were weighed before and after the race. During the blood drawing, participants were seated. Blood samples were centrifuged at 3000g for 10 minutes at 4°C. Plasma was extracted and then stored at −20°C until analysis for plasma total protein (Roche P-Module, Biuret, Basel, Switzerland) (coefficient of variation [CV] = <2%), sodium ([Na<sup>+</sup>]) and potassium ([K<sup>+</sup>]) (EasyLyte PLUS Na/K/Cl analyzer; Medica Corporation, Bedford, Massachusetts) concentrations, and plasma osmolality (POsm) (Osmomat 030 cryoscopic osmometer; Gonotec, Berlin, Germany) (CV = 2.86%). Subsequently, 2 postrace blood samples (1 subject from each distance) were unable to be obtained because of the inability to obtain a sample and blood hemolysis.

### Measurement of Total Body Water

Total body water was measured with the diluted isotope method using D<sub>2</sub>O.<sup>19</sup> The use of the stable isotope D<sub>2</sub>O allows for the most accurate quantification of changes in TBW during exercise.<sup>20</sup> This allows a more accurate determination if all the BM lost during exercise is because of a reduction in TBW.<sup>9,10,21–23</sup>

An initial approximately 1.5-mL saliva sample was collected to calculate baseline D<sub>2</sub>O abundance and repeated after 2-hour equilibration periods to determine TBW on race morning (prerace) and immediately after race completion (postrace). Saliva was collected by placing a cotton wool swab into the mouth, which was then saturated with saliva. The swab was then removed with tweezers and placed into a syringe barrel. Saliva was then extracted by compressing the cotton

swab against the head of the syringe; this allowed the saliva to flow directly into a cryotube which was then stored at  $-80^{\circ}\text{C}$ . Before analysis, samples were prepared by filtration through sterile syringe filters into 1-mL glass vials.

Each  $\text{D}_2\text{O}$  dose was dispensed from a 4% stock solution that was prepared by mixing appropriate amounts of 99%  $\text{D}_2\text{O}$  (Cambridge Isotope Laboratories, Inc, Amherst, Massachusetts) and water. Each participant received a dose of 0.05 g/kg BM with each dosage preweighed to the nearest 0.001 g. After each dose had been consumed, the dose bottle was reweighed to quantify the precise amount that had been ingested.

Food consumption was prohibited during the 2-hour equilibration period. For the first hour after the race, fluid ingestion was also restricted; if necessary,  $<100$  mL was permitted, which was measured and corrected for, and thereafter fluid abstinence was required. A 2-hour equilibration period after  $\text{D}_2\text{O}$  administration is known to be appropriate.<sup>19,23-33</sup> All urine excreted during the equilibration period was collected and analyzed to account for any isotope loss when calculating TBW.

Saliva  $\text{D}_2\text{O}$  enrichment was attained by pyrolysis in a Thermo Finnigan TC/EA with a SpectraSYSTEM AS3000 Autosampler, coupled via a Thermo Conflo III to a Thermo Delta XP stable light isotope mass spectrometer (Thermo Fisher Scientific, Inc, Waltham, Massachusetts). Samples were run against the internal laboratory standards and were measured at intervals throughout the run to ensure consistency. The results were normalized against and reported relative to the international standards (relative SD,  $<2\%$ ). Total body water was then calculated using the Halliday and Miller method<sup>34</sup> with a modified correction factor for nonaqueous hydrogen exchange at 1.04 (4%).<sup>28,34</sup>

**Calculations**

Equation 1: Percentage change ( $\% \Delta$ ) in BM over the race was calculated as follows:

$$\Delta\% \text{BM} = ((\text{BM}_{\text{post-race}} - \text{BM}_{\text{pre-race}}) / \text{BM}_{\text{pre-race}}) \times 100$$

Equation 2: Change in plasma volume (PV)<sup>35</sup> was calculated as follows:

$$\Delta \text{PV} (\%) = \left( \left[ \text{total protein}_{\text{post-race}} \right] - \left[ \text{total protein}_{\text{pre-race}} \right] \right) / \left[ \text{total protein}_{\text{pre-race}} \right] \times 100$$

Equation 3: The Davies method was used to calculate TBW<sup>36</sup> as follows:

$$\text{TBW (kg)} = [((T \times A / a) \times ((\text{Ea} - \text{Et}) / (\text{Es} - \text{Ep}))) / 1000] / 1.04$$

where  $A$  is the amount of dose solution drunk (g);  $a$ , amount of dose solution diluted in  $T$  (g);  $T$ , amount of water in which “ $a$ ” was diluted in (g);  $\text{Ea}$ , enrichment of diluted dose;  $\text{Et}$ , enrichment of water used to dilute the dose;  $\text{Ep}$ , enrichment of baseline sample;  $\text{Es}$ , enrichment of postdose sample; 1.04, correction factor for over estimation of TBW by the use of  $\text{D}_2\text{O}$ .<sup>37</sup>

Equation 4: Percentage change ( $\% \Delta$ ) in TBW was calculated as follows:

$$\% \Delta \text{TBW} = (\text{TBW}_{\text{post-race}} / \text{BM}_{\text{post-race}} \times 100) - (\text{TBW}_{\text{pre-race}} / \text{BM}_{\text{pre-race}} \times 100)$$

**Statistical Analysis**

Data were analyzed using the STATISTICA version 9 (StatSoft, Tulsa, Oklahoma) statistical program and Prism 3 (GraphPad Software, Inc, La Jolla, California) using Pearson correlations and the Students paired  $t$  test. Where applicable, all data are presented as means  $\pm$  SD, including the range of values. Statistical significance was accepted when  $P < 0.05$ .

**RESULTS**

All 21 runners (12 women and 9 men) successfully completed the 21.1-km race with a mean time of  $2.15 \pm 0.41$  hours (range, 1.5-3 hours) (Table 1). Of the 13 runners (3 women and 9 men), 12 completed the 56-km race with a mean time of  $5.7 \pm 1.1$  hours (range, 4.1-6.9 hours) (Table 1). One male runner failed to finish the 56-km race in the allotted time and was withdrawn from the study.

The mean fluid intake of the 21.1-km runners was  $326 \pm 180$  mL/h (range, 87-792 mL/h) and  $538 \pm 354$  mL/h (range, 99-1216 mL/h) for the 56-km runners (Table 1). Table 2 shows the increase in plasma total protein concentration (in both races), and that increase in POsm (in the 56-km cohort) was significant ( $P < 0.001$ ) (Table 2). No significant changes to plasma  $[\text{K}^+]$  and  $[\text{Na}^+]$  were observed during the races (Table 2). Body mass decreased by  $1.4 \pm 0.6$  kg and  $2.5 \pm 1.1$  kg in the 21.1-km race and the 56-km race ( $P < 0.000$ ), respectively. There were significant differences noted for both the absolute change ( $\Delta$ ) and  $\% \Delta$  in BM loss in the 2 races (Figure 1;  $P < 0.001$ ). There were no significant changes in  $\% \text{TBW}$  in runners at either distance. Absolute TBW was unchanged in 21.1-km runners but decreased significantly in 56-km runners.

**TABLE 1.** General Characteristics of Subjects Participating in the 21.1-km and 56-km Foot Races

	21.1 km (n = 21)			56 km (n = 12)		
	Mean ( $\pm$ SD)	Minimum	Maximum	Mean ( $\pm$ SD)	Minimum	Maximum
Age, y	31.7 $\pm$ 11.0	20	56	40.6 $\pm$ 10.6	28	54
Prerace body mass, kg	70.0 $\pm$ 8.2	57.1	89.4	69.9 $\pm$ 8.6	58.4	83.7
Body fat, %	18.1 $\pm$ 4.2	11.4	25.6	15.3 $\pm$ 4.6	8.2	24.4
Predicted race time, h	2.05 $\pm$ 0.38	1.5	2.8	5.32 $\pm$ 0.89	3.8	6.3
Actual race time, h	2.15 $\pm$ 0.41	1.5	3.0	5.66 $\pm$ 1.06	4.1	6.9
Fluid intake, mL	689.3 $\pm$ 365.6	150	1400	3186.3 $\pm$ 2254.3	400	7700
Drinking rate, mL/h	326.3 $\pm$ 180.0	87	792	538.4 $\pm$ 354.0	99	1216

**TABLE 2.** Body Mass, Total Body Water, and Blood Biochemical Measures in Runners Participating in the 21.1-km and 56-km Foot Races

Variable	21.1 km		56 km	
	PreRace	PostRace	PreRace	PostRace
Plasma [K <sup>+</sup> ], mmol/L	4.1 ± 0.3 (n = 20)	4.5 ± 0.4 (n = 20)	4.0 ± 0.5 (n = 11)	4.7 ± 1.9 (n = 11)
Plasma [Na <sup>+</sup> ], mmol/L	137.8 ± 3.6 (n = 20)	139.7 ± 2.5 (n = 20)	138.6 ± 2.0 (n = 11)	141.1 ± 3.3 (n = 11)
POsm, mOsm/kg H <sub>2</sub> O	289.4 ± 5.4 (n = 20)	287.7 ± 5.4 (n = 20)	284.6 ± 5.5 (n = 11)	291.1 ± 9.9 (n = 11)*
Plasma [TP], g/L	73.6 ± 3.3 (n = 20)	78.0 ± 3.8 (n = 20)^	76.1 ± 1.87 (n = 11)	81.5 ± 4.4 (n = 11)\$
Body mass, kg	70.0 ± 8.2 (n = 21)	68.6 ± 7.9 (n = 21)#	69.9 ± 8.6 (n = 12)	67.4 ± 8.7 (n = 12)#
Body mass, %	100 (n = 21)	98.05 ± 0.8 (n = 21)#	100 (n = 12)	96.5 ± 1.6 (n = 12)#
Total body water, kg	40.6 ± 7.2 (n = 21)	40.6 ± 7.0 (n = 21)	43.2 ± 7.4 (n = 12)	41.6 ± 7.3 (n = 12)^
Total body water, %	57.9 ± 6.8 (n = 21)	59.0 ± 6.8 (n = 21)	61.4 ± 5.4 (n = 12)	61.5 ± 6.5 (n = 12)

Values are presented as mean ± SD.

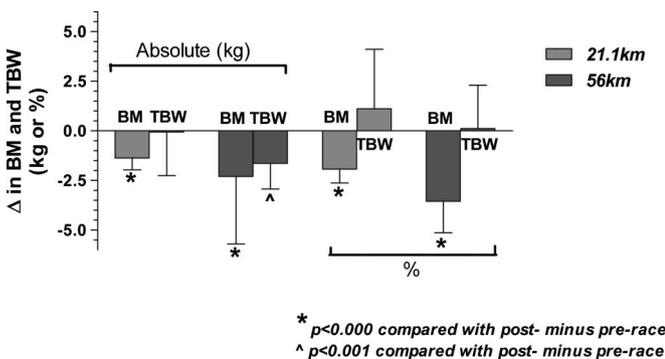
[K<sup>+</sup>], plasma potassium concentration; [Na<sup>+</sup>], plasma sodium concentration; POsm, plasma osmolality; [TP], total protein concentration.

#P < 0.000 versus prerace; \$P < 0.01 versus prerace; \*P < 0.05 versus prerace; ^P < 0.001 versus prerace.

There was no significant correlation between the change in TBW and in BM in either race (Figure 2). Interestingly, in the 21.1-km race, there was a wide variation in TBW changes (-3.9 to +3.3 kg) but a narrower range of changes in BM (-3.1 to -0.4 kg). In contrast, the range of BM losses was somewhat greater in the 56-km runners (-4.3 to +0.3 kg), whereas the change in TBW was much less (-2.9 to +0.2 kg).

Figure 3 shows that changes in %TBW and %BM ( $R^2 = 0.391$ ;  $P = 0.003$ ) significantly correlated in the 56-km race. No significant correlations were found between %Δ in BM and TBW and the postrace POsm and plasma [Na<sup>+</sup>] (Figure 4) in both races; however, the %Δ in BM significantly correlated to postrace [Na<sup>+</sup>] in the 56-km runners ( $R^2 = 0.408$ ;  $P = 0.034$ ). The dotted parallel lines signify the normal range limits for the 2 different measures (POsm, 280-296 mOsm/kg H<sub>2</sub>O and plasma [Na<sup>+</sup>], 135-145 mmol/L)<sup>38</sup> applicable for both Figures 4 and 5.

Similarly, the relationship between absolute change in BM and TBW to the postrace POsm and plasma [Na<sup>+</sup>] were investigated (Figure 5). A significant relationship between postrace plasma [Na<sup>+</sup>] and the ΔBM was found ( $R^2 = 0.457$ ;  $P = 0.023$ ) in the 56-km runners.



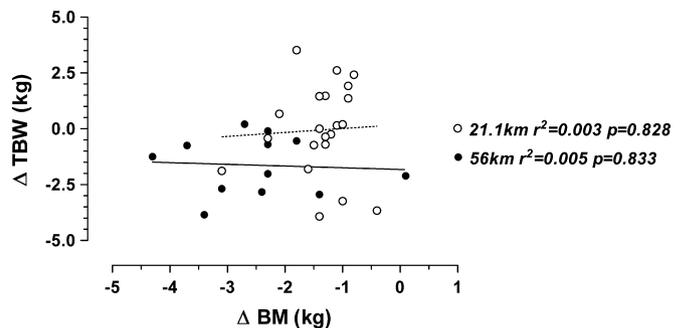
**FIGURE 1.** The prerace to postrace absolute and percentage changes in (%) body mass (BM) and total body water (TBW) for both 21-km and 56-km runners. \*P < 0.000 compared with postrace minus prerace. ^P < 0.001 compared with postrace minus prerace.

### DISCUSSION

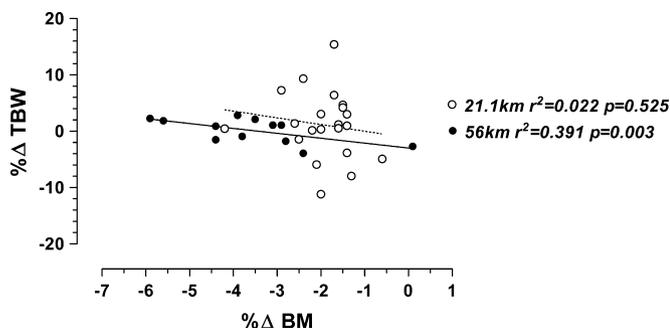
The first finding of this study was that participants who drank ad libitum during the 21.1-km race and 56-km race maintained their plasma [Na<sup>+</sup>] and [K<sup>+</sup>] within the normal range for athletes completing a standard marathon established by Kratz et al<sup>39</sup> and showed only relatively small change in POsm. This supports the EAH consensus view that abnormalities in fluid balance and not excessive loss of sodium chloride in sweat and urine explain variations beyond the normal range in blood [Na<sup>+</sup>] during exercise in the apparently normal population.<sup>40,41</sup>

Ad libitum drinking was associated with a significant reduction in BM in both races. This condition was termed “voluntary dehydration” by Adolph<sup>42</sup> on the assumption that this BM loss is because of water losses that cause a reduction in TBW. However, although ΔTBW was not actually measured, this descriptive term has been retained in the literature,<sup>43-45</sup> despite few attempts to measure concurrently exercise-induced changes in BM and TBW to determine if the condition of voluntary dehydration does indeed exist or whether it is another example of “christening by conjecture.”<sup>46</sup>

Accordingly, the second important finding of this study was that the changes in BM in both races exceeded the changes in TBW, indicating that water losses alone did not explain all the BM lost during the races in these participants. This finding is in keeping with the classic interpretation of the relationship



**FIGURE 2.** Nonsignificant correlation between absolute changes in (Δ) body mass (BM) (kg) and total body water (TBW) (kg) in both 21.1-km and 56-km runners.



**FIGURE 3.** Significant correlation between percentage changes in (%Δ) body mass (BM) and total body water (TBW) in the 56-km runners but not in the 21.1-km runners.

between BM and TBW loss suggested by the likes of Maughan et al<sup>10</sup> and Pastene et al.<sup>9</sup> Our findings disagree with those of Baker et al, who concluded that changes in TBW and BM tracked each other in a linear fashion.<sup>12</sup> Indeed, their data showed a wide scatter of changes in TBW for each level of BM change, quite similar to our findings for the 21.1-km race.<sup>13</sup>

Thus, we conclude that the changes in BM during exercise are not due purely to changes in TBW so that all BM loss during exercise is not due solely to water loss. Our other recent studies<sup>14,18</sup> support this interpretation, as indeed do the data of Baker et al when analyzed appropriately.<sup>12,13</sup>

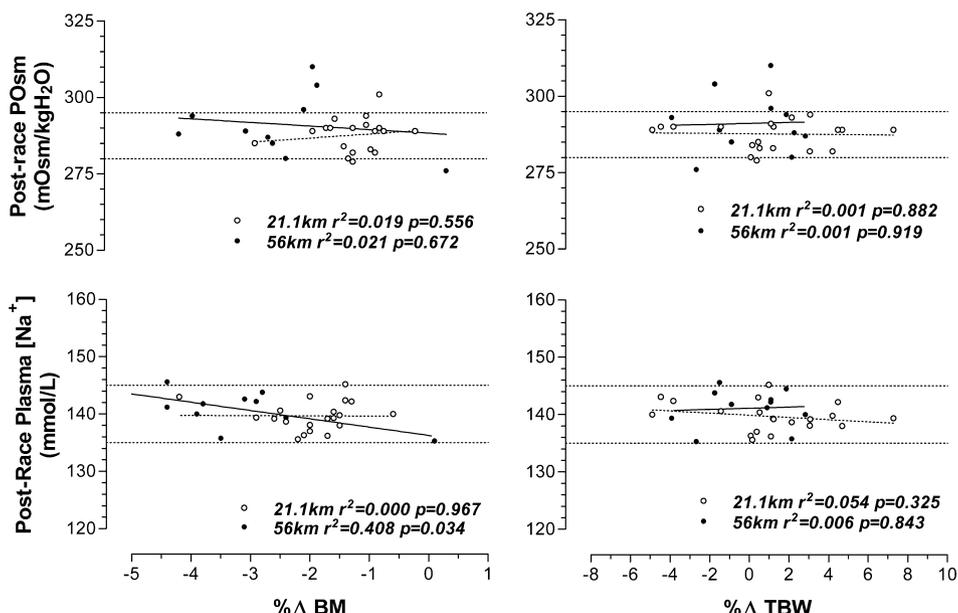
Interestingly, we found that although mean BM decreased ~2.5 kg (~3.5%) during the 56-km race, mean TBW actually increased ~1.6 kg (~0.12%). This finding is in agreement with those of Knechtle et al<sup>47</sup> who found an increase in %Δ in TBW over the course of a 1200-km multistage ultraendurance race, despite a BM loss of ~3 kg. This finding can be explained if the decrease in BM results from a loss of glycogen and fat mass with an increase in the body's water content of the remaining tissues at the end of exercise. Supporting this, other authors found similar findings

to ours over a 100-km running race with 11 female athletes, that is where TBW content was maintained, %TBW increased alongside a 1.5 kg decrease in BM.<sup>48</sup>

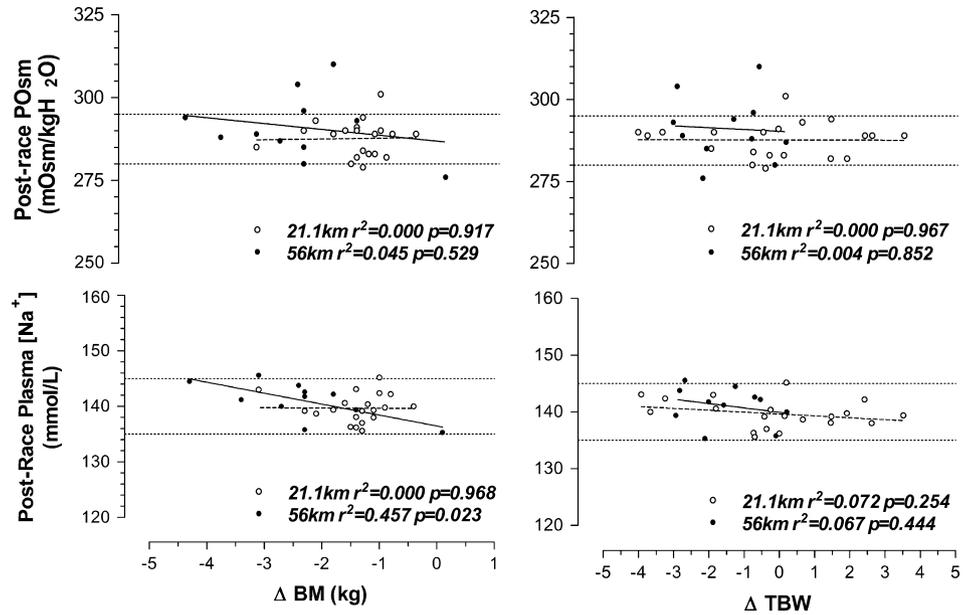
The origin of this added water might be the water originally bound to glycogen that is released as the glycogen is metabolized during exercise or possibly from fluid stored in the intestine at the start of exercise.<sup>10,21</sup> Thus, the findings of this study, combined with those of other recent studies, support the emerging viewpoint that changes in BM during exercise may not reflect exact changes in the body's hydration status.<sup>10,49</sup>

Furthermore, these results raise concern of the validity of fluid guidelines, which recommend that athletes must not incur a BM loss > 2% of their starting BM during exercise.<sup>2,50</sup> Although changes in BM may be useful to predict fluid homeostasis in certain clinical scenarios at rest,<sup>51</sup> during exercise the homeostatic controls that protect POsm may require a decrease in BM without any negative physiological consequences.<sup>3,52</sup> The failure of humans to replace 100% of their BM losses when they ingest fluid ad libitum during exercise has led to the recommendation that athletes must drink beyond the sensations of thirst to prevent “voluntary dehydration.”<sup>44,45</sup> However, our results confirm our previous findings that ad libitum drinking will maintain %TBW with a smaller decrease in absolute TBW when compared with the greater loss of BM. These data support our contention that BM is not the important physiologically regulated variable during exercise.<sup>53,54</sup> Strong evidence supporting the physiological regulation of BM has indeed been found in animals and humans in chronic conditions over months and years, but this relates more to the regulation of fat and protein mass rather than to acute changes in fluid balance.<sup>16,17</sup>

Others have reported a dissociation between BM loss and TBW. Colt et al<sup>55</sup> reported a 2.4% BM loss associated with a 2.4% increase in TBW in well-trained men running 16 km. These results agree directly with the results of our 21.1-km



**FIGURE 4.** The relationship between percentage changes in (%Δ) body mass (BM) and total body water (TBW) in relation to postrace plasma osmality (POsm) and plasma sodium concentrations ([Na<sup>+</sup>]) in both 21.1-km and 56-km runners.



**FIGURE 5.** The relationship between absolute changes ( $\Delta$ ) (kg) in body mass (BM) and total body water (TBW) in relation to postrace plasma osmolality (POsm) and plasma sodium concentrations ( $[Na^+]$ ) in both 21.1-km and 56-km runners.

runners, where we observed  $\sim 1.9\%$  BM loss with a  $1.1\%$  increase in TBW. Similarly, this trend is shown when comparing the changes of both absolute and  $\% \Delta$  in BM and TBW in both groups of our runners.

It is perhaps counterintuitive that there can be a reduction in BM with a maintained or even increased absolute or  $\%TBW$  in participants who drink less than they sweat during exercise and hence, in theory at least, must develop a reduction in body water reserves. But this excludes actual fluid ingestion and the possibility that there may be an internal water source, for example, free fluid in the gastrointestinal tract or water previously bound with the storage of glycogen, that can be liberated during exercise and which would explain unchanged blood biochemical parameters in association with an absolute BM loss.<sup>9,56,57</sup>

The only study that has critically assessed changes in BM, muscle glycogen content, and TBW was performed by Olsson and Saltin in 1970.<sup>21</sup> They demonstrated that 3 to 4 g of water may be complexed with each gram of glycogen stored in muscle (and the liver).<sup>21</sup> Thus, participants eating a “high” carbohydrate diet for 4 days showed an increase in BM concomitantly with an increase in TBW. A number of studies have recorded an increase in BM as a result of “carbohydrate loading” 2 to 3 days before exercise.<sup>14,21,58–60</sup> Because the magnitude of this BM gain exceeds the body’s storage capacity for glycogen, it may include some mass gain resulting also from water storage.

Although POsm rose significantly and certain values were above the “normal” clinical range in this study, they could be described as normal when placed in the adjusted norms established by Kratz et al.<sup>39</sup> Furthermore, none of the participants reported to the medical tent at the end of the race or showed symptoms of requiring medical assistance during the postrace testing period of this study ( $\sim 2$  hours after the race). This supports our hypothesis that athletes are able to regulate their plasma  $[Na^+]$  and osmolality, despite the loss of some TBW and a greater than  $2\%$  loss of their BM during prolonged exercise.<sup>61,62</sup>

Finally, it is important to acknowledge that the postrace TBW measurement might be prone to a slight overestimation of the TBW pool compared with the prerace measurement. Increased turnover of TBW could result in possible isotope loss during the equilibration period.<sup>63</sup> The isotope loss during the postrace equilibration period might be slightly higher compared with the prerace equilibration period because of increased metabolic rates after exercise. This could result in isotope loss through sweat, respiratory water loss (RWL), and dilution of the isotope because of an increased metabolic water production. Corrections for these variables remain approximates because of individual differences in RWL, metabolic rates, and sweat rates, as well as the influence of ambient weather conditions on RWL. However, isotope loss as a result of these mechanisms is likely to be minimal considering the duration of the equilibration period and is therefore unlikely to differ significantly from that occurring during the prerace measurements. Additional limitations were that the 56-km race sample size was small, fluid intake was estimated, and food intake was not recorded.

In summary, this study has demonstrated that a significant decrease [ $\sim 1.4$  kg ( $1.9\%$ ) and  $\sim 2.5$  kg ( $3.6\%$ ) for 21.1-km and 56-km races, respectively] in BM occurs in endurance athletes who drink according to the dictates of thirst and/or self-determined drinking strategy during prolonged exercise, but the changes in TBW were less. Thus, absolute TBW was maintained in the 21.1-km runners but decreased in 56-km runners. But adequate hydration in both groups was further confirmed with the maintenance of the blood biochemical markers of POsm and plasma  $[Na^+]$ .

## CONCLUSIONS

Therefore, the present study confirms our previous findings that the changes in TBW during prolonged endurance exercise were substantially less than the significant BM loss

measured in these runners. This finding further supports the conclusion<sup>3,49,61</sup> that the body primarily defends POsm and plasma [Na<sup>+</sup>]<sup>+</sup>—and not BM—during both short-duration (21.1 km) and prolonged (56 km) endurance exercises. In addition, the adoption of fluid intake rates of our athletes was adequate for them to complete their race, while maintaining their hydration status.

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# FLUID INTAKE AND CHANGES IN BLOOD BIOCHEMISTRY, RUNNING SPEED AND BODY MASS DURING AN 80 KM MOUNTAIN TRAIL RACE

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## Abstract

**Introduction:** To measure changes in body mass, total body water, running performance, fluid intake and blood biochemical variables in an ultra-marathon mountain race.

**Methods:** 9 subjects (44.0 ± 9.2 years; 72.2 ± 9.0 kg) were measured 2-days before the race, immediately pre-race and post-race for body mass (BM), total body water (TBW), plasma osmolality, plasma sodium ([Na<sup>+</sup>]), plasma potassium ([K<sup>+</sup>]) and plasma total protein concentrations. Fluid intake and rating of perceived exertion (RPE) were also measured over the entire race.

**Results:** Significant BM loss occurred from 2-days pre-race to post-race (-3.1 ± 1.2 kg; *P*<0.05) and pre-race to post-race (-3.7 ± 2.7 kg; *P*<0.01). A positive linear relationship was found between fluid intake and changes in BM during the race (*r* = 0.7; *P*<0.05). Rates of fluid intake (321-628 ml/hr) and total body mass loss varied substantially between individuals. Plasma osmolality and [Na<sup>+</sup>] concentrations were well regulated and did not change significantly. There was a non-significant correlation between changes in body mass and race performance.

**Conclusion:** We conclude that drinking rates vary substantially in athletes drinking *ad libitum* during an 80 km mountain race whereas plasma osmolality and [Na<sup>+</sup>] are well regulated despite large changes in BM. Therefore, drinking *ad libitum* during prolonged endurance running seems to be an appropriate method to maintain fluid homeostasis during ultra-marathon mountain races.

**Key words:** fluid balance, plasma sodium, plasma osmolality, exercise

## Introduction

Current fluid replacement guidelines advise athletes to consume enough fluid in order to prevent a >2% decrease in body mass during exercise (1). These guidelines are usually applied to athletes participating in events such as road running, triathlons, tennis, soccer and American football. There is less information about rates of fluid intake and body mass losses in ultra-distance events like the Ironman triathlon or ultra-marathon races, in which some successful athletes can incur body mass losses > 6-8% without apparent detriment to their health or performance (2-4). It is usually difficult to measure fluid intakes in these races since competitors cover large geographical distances and the events last for prolonged periods making careful observation impractical.

A local 80km trail race provided an opportunity to carefully monitor rates of fluid intake in athletes during exercise. At the same time we were able to measure changes in body mass, running speed, rating

of perceived exertion and various blood biochemical measures.

We hypothesized that rates of fluid intake amongst the competitors would vary as would the extent to which they lost body mass during the race. In contrast blood electrolyte measures especially plasma sodium concentrations and plasma osmolality would vary less since these are the biological variables that are homeostatically regulated during exercise (5). Finally we seek to re-enforce the observation that athletes losing the most body mass during the race will exhibit the best performance whilst drinking and eating *ad libitum* during an 80km off-road running race.

## Materials and Methods

**Subjects:** All entrants of the 2007 Peninsula Ultra Fun Run (PUFfeR) 80km trail race were invited to participate in this study. Approval for this study was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape

Town and was carried out according to the Declaration of Helsinki (Seoul, 2008). Ten athletes (Table 1) gave written informed consent to participate in this project from a field limited to 125 runners (8% of the entire field). All food and fluid were consumed *ad libitum* during the race.

**Setting:** The PUFFeR is an 80km trail race starting at Cape Point (Cape Town, South Africa) which traverses the Cape Peninsula for 80km (highest elevation of 1080m) over the Table Mountain National Park with 13 checkpoints dotted at intervals of 6 km (Figure 2). Ambient temperature (dry bulb) fluctuated between 8-20°C on race day (South African National Weather Bureau) on a clear and calm day.

**Measurement of Body Mass(BM):** Subjects were weighed in racing attire without shoes after emptying their bladder on an electronic digital scale (Beurer GS32) (to the nearest 0.1 kg) on three occasions: 1. race briefing (2-days pre-race), 2. 60 minutes prior to race start (pre-race) and 3. immediately upon completion of the race (post-race).

**Blood Biochemical Analyses:** 10 ml venous blood samples were collected on the three occasions described, into lithium- heparin Vacutainer (Becton Dickinson, Rutherford, NJ) tubes. During the blood drawing subjects were seated following measurements of their body mass. Samples were stored on ice until centrifugation. Blood samples were centrifuged at 3000g for 10 minutes at 4°C. Plasma was extracted and stored at -20°C until analysed for: plasma sodium ( $[Na^+]$ ) and potassium ( $[K^+]$ ) (EasyLyte PLUS Na/K/Cl analyzer, Medica Corporation) concentrations; plasma osmolality (POsm) (Osmomat 030 Cryoscopic osmometer, Gonotec) (CV- 2.86%); and plasma protein concentrations (Roche P-Module, Biuret) (CV- 2.1%) (Table 2).

**Fluid Intake:** Fluid intakes were measured and recorded by researchers stationed at 13 mandatory checkpoints throughout the 80 km route. Researchers recorded the number and size of cups (175 ml or 350 ml) consumed by the athletes at the checkpoints. They also questioned each athlete about the volume of fluid consumed between checkpoints with a fluid recall method. As a result subjects needed to recall only the additional volumes of fluid consumed during the previous ~6 km. The data from the 13 checkpoints was then summed to calculate the total volume (L) and rates of fluid intake (ml/hr) for the individual athletes (Table 3).

The exploratory measurement of total body water took place 2-days before; immediately before and after the race with the use of deuterium oxide dosage.

**Measurement of Total Body Water (TBW):** TBW was calculated with the diluted isotope method using deuterium oxide. A ~1.5 ml saliva sample was used to calculate natural deuterium abundance (as well as increased deuterium abundance due to prior doses) and after a 2-hour equilibration period to determine TBW on three separate occasions: race briefing (2-days pre-race), race morning (pre-race) and immediately after race completion (post-race). Saliva was collected by placing a sterile cotton wool swab into each subject's mouth with sterile tweezers. Each subject then was asked to moisten the cotton swab with saliva by rolling the swab around the mouth until the swab was fully saturated. The cotton swab was then removed with tweezers and immediately placed into the barrel of a 10 ml sterile syringe. All saliva was then extracted by compressing the cotton swab against the head of the syringe and which allowed all collected fluid to flow directly into a cryotube. Samples were immediately sealed and frozen (-20°C) until further analysis could be performed. Food and fluids consumption was prohibited during the 2-hour equilibration. During the post-race setting fluid ingestion was restricted. However some subjects required to drink. In which case the volume ingested was measured (to the nearest 0.001g) and corrected for during the first hour of equilibration. No further ingestion was allowed during the final hour during the 2-hour equilibration period which followed baseline saliva sampling. A 2-hour equilibration period following deuterium administration has been seen to be appropriate (6-17). All urine produced during this equilibration period was collected to account for any isotope loss. The deuterium enrichment and volume of these urine samples were accounted for in subsequent TBW calculations.

The dose of deuterium used for total body water measurement was administered as individualized doses immediately following baseline saliva sampling. Each deuterium dose was dispensed from a 4% weight-to-weight stock solution that was created by mixing required amounts of 99% Deuterium Oxide (Cambridge Isotope Laboratories Inc., MA.) and distilled water. The pre-mixed deuterium stock solution was poured into an airtight container and sealed with duct tape to prevent fractionation during storage. Each participant received a dose of approximately 0.05 g/kg body weight with each dosage pre-weighed to the nearest 0.001g. After each dosage had been consumed, the dose bottle was immediately re-weighed to quantify the exact amount that had been ingested. TBW determination was obtained through measurement of deuterium enrichment in saliva samples, as measured by continuous flow isotope ratio mass spectrometry (Europa Scientific ANCA-GSL and Geo 20-20 IRMS, Iso-Analytical Inc, UK)(relative standard deviation <2%). Total body water was calculated utilizing the Halliday and Miller method (18) with a

modified correction factor for non-aqueous hydrogen exchange at 1.04 (4 %)(12;18).

### Calculations:

Equation 1:

The Davies method was used to calculate the total body water (TBW) in kilograms (kg)(19):

$$\text{TBW (kg)} = \left[ \left( \frac{T \cdot A}{a} \right) \times \left( \frac{E_a - E_t}{E_s - E_p} \right) \right] / 1000 / 1.04$$

Whereas: **A** = amount of dose solution drunk (g); **a** = amount of dose solution diluted in T (g); **T** = amount of tap water 'a' was diluted in; **E<sub>a</sub>** = enrichment of diluted dose; **E<sub>t</sub>** = enrichment of tap water used to dilute the dose; **E<sub>p</sub>** = enrichment of baseline sample; **E<sub>s</sub>** = enrichment of post dose sample; **1.04** = correction factor for over estimation of TBW by the use of deuterium (20).

Equation 2:

Total body water (kg) was attained and percentages of total body water (TBW) were calculated as:

$$\% \Delta \text{ TBW} = \left[ \frac{\text{TBW (post-race)} - \text{TBW (pre-race)}}{\text{TBW (post-race)}} \right] \times 100$$

Equation 3:

Percent of body mass (% $\Delta$  BM) lost or gained during the race was calculated as the difference between the starting weight (either 2-day pre-race or pre-race) and the finishing weight (post-race) divided by the start weight and multiplied by 100.

Equation 4:

Change in plasma volume (PV) was calculated (21):

$$\Delta \text{ PV (\%)} = \left[ \frac{\text{total protein (post)} - \text{total protein (pre)}}{\text{total protein (pre)}} \right]$$

Equation 5:

**Performance Analysis:** Running speed (km/hr) was calculated as an average over the entire race and over each leg between twelve checkpoints:

$$\text{Running Speed (km/hr)} = \frac{\text{Distance (km)}}{\text{Race Time (hrs)}}$$

**Rating of Perceived Exertion (RPE):** RPE was noted using the Borg (6-20) category ratio scale(22). RPE was noted at each checkpoint, it was mandatory for subjects to stop and check in.

### Statistical analysis

Data were analyzed using the STATISTICA version 8 (StatSoft Tulsa, OK) statistical program using cor-

relations, repeated measures ANOVAs and post-hoc analysis with Tukeys HSD. Pearson's correlations were calculated through the use of Prism 3 (GraphPad Software, Inc). Where applicable, all data are presented as means  $\pm$  standard deviations (SD) including the range of values. Statistical significance was accepted when  $p < 0.05$ .

### Results

Eight of the runners (seven males and one female) successfully completed the 80 km mountain race with a mean finishing time of  $691.4 \pm 51.8$  minutes (range: 583.5 – 769.2) (Table 1). One female runner withdrew prior to race start and all her baseline data collected were excluded entirely from the subsequent analysis. One male runner dropped out at ~60 km, but completed bodymass measurements after being transported to the finish line. Thus his data were included in the study since he did complete ultra-distance. The mean age of the cohort was  $44.0 \pm 9.2$  years (range: 33 - 61) with a mean baseline weight (2-days pre) of  $72.2 \pm 9.0$  kg (range: 61.3 - 88.6) (Table 1).

The individual data for body mass measurements including the relative and absolute changes during the course of the experiment are detailed in Table 1. Body mass increased ~1.05 kg ( $\pm 1.99$ ) from 2-days pre-race to immediately pre-race but decreased ~4 kg ( $\pm 2.55$ ) (5% BM) during the course of the race ( $P < 0.05$ ).

Plasma potassium ( $K^+$ ), sodium ( $Na^+$ ) and protein concentrations did not change significantly during the race, nor did the plasma osmolality (Table 2). Furthermore, no significant correlations existed between percent change in body mass with either post-race plasma sodium concentrations or plasma osmolality (Figure 1). The dotted lines indicate the normal range of plasma osmolality (280–296 mOsm/kgH<sub>2</sub>O) while the solid lines denote the normal range of plasma sodium (135–145 mmol/L) (23). Only one plasma sodium concentration fell outside the normal range whereas three plasma osmolalities were above the normal range.

Table 3 provides a breakdown of the fluid intakes of the subjects denoted as water intake and the intake of 3 different carbohydrate containing drinks (CHO A, B and C). The column noted as "other" is the sum of different fluid types other than those already described. Total volumes of fluid ingested during the race varied from 2.11–6.11 liters (mean 4.50 liters). The rates of fluid intake varied from 327 to 628 ml/hr (mean 422 ml/hr).

The data obtained resulted in only two complete sets of data for all three TBW measurements (Table 4). However we did acquire 3 data sets of changes in the period between 2-days pre-race to immediately pre-race (A-B); 5 data sets of changes from 2-days pre-race to post-race (A-C) and 4 data sets from immediately pre-race to post-race (B-C).

Table 1. *Body mass changes in runners participating in an 80km ultra endurance trail race*

Subject	Sex	RT (hrs)	BM (kg)			Δ BM (kg)			%Δ BM (kg)		
			A	B	C	A-B	A-C	B-C	A-B	A-C	B-C
1	M	12.2	75.0	76.4	71.4	1.4	-3.6	-5.0	1.9	-4.8	-6.5
2	M	12.82	61.3	60.7	59.9	-0.6	-1.4	-0.8	-1.0	-2.3	-1.3
3	M	10.98	78.1	80.7	74.1	2.6	-4.0	-6.6	3.3	-5.1	-8.2
4	M	9.72	74.5	74.8	72.1	0.3	-2.4	-2.7	0.4	-3.2	-3.6
5	M	10.92	70.2	67.2	67.4	-3.0	-2.8	0.2	-4.3	-4.0	0.3
6	M	12.2	88.6	89.9	83.6	1.3	-5.0	-6.3	1.5	-5.6	-7.0
7	M	11.77	-	74.2	73.3	-	-	-0.9	-	-	-1.2
8	M	-	68.7	71.7	65.4	3.0	-3.3	-6.3	4.4	-4.8	-8.8
9	F	11.57	61.3	64.7	59.4	3.4	-1.9	-5.3	5.5	-3.1	-8.2
<b>mean</b>		<b>11.5</b>	<b>72.2</b>	<b>73.4</b>	<b>69.6*#</b>	<b>1.1</b>	<b>-3.1</b>	<b>-3.7</b>	<b>1.5</b>	<b>-4.1<sup>s</sup></b>	<b>-5.0<sup>s</sup></b>
<b>±SD</b>		<b>1.0</b>	<b>9.0</b>	<b>8.8</b>	<b>7.6</b>	<b>2.1</b>	<b>1.2</b>	<b>2.7</b>	<b>3.1</b>	<b>1.2</b>	<b>3.5</b>

RT- Race Time; BM- Body mass; %Δ- Percentage change; A- 2 Days Pre-race; B- Pre-race; C- Post-race

\*P<0.05 when compared to A; #P<0.001 when compared to B; <sup>s</sup>P<0.05 when compared to A-B; (-) - Unavailable data.

Table 2. *Haematological measures of runners participating in an 80km ultra endurance trail race (Mean ± SD)*

	2-Days Pre-race	Pre-race	Post-race
<b>Plasma [K<sup>+</sup>] (mmol/L)</b>	5.0 ± 0.5 (n= 8)	4.9 ± 0.6 (n= 9)	5.1 ± 0.7 (n= 8)
<b>Plasma [Na<sup>+</sup>] (mmol/L)</b>	140.0 ± 2.0 (n= 8)	137.3 ± 2.2 (n= 9)	138.5 ± 4.3 (n= 8)
<b>Plasma Osmolality (mosm/kgH<sub>2</sub>O)</b>	286.1 ± 9.7 (n= 8)	298.2 ± 7.4 (n= 9)	292.9 ± 8.5 (n= 8)
<b>Plasma Protein (g/L)</b>	71.0 ± 3.3 (n= 8)	73.0 ± 2.8 (n= 8)	75.0 ± 5.1 (n= 8)

Table 3. *Fluid intake of runners participating in an 80km ultra endurance trail race*

Subject	Fluid Intake (ml)							Total (L)	Rate (ml/hr)
	Fluid	CHO A	CHO B	Cola	CHO C	Other			
1	965	690	350	1980	-	-	3.99	327	
2	1050	2500	1025	300	-	170	5.05	394	
3	800	200	175	2160	-	200	3.54	322	
4	2720	1800	700	885	-	-	6.11	628	
5	2290	1050	850	875	-	200	5.27	482	
6	2610	375	1025	835	-	300	5.15	422	
7	2500	650	1400	-	500	385	5.44	462	
8	910	125	175	350	-	550	2.11	-	
9	1430	125	450	1325	-	575	3.91	338	
<b>mean</b>	<b>1697</b>	<b>835</b>	<b>683</b>	<b>1089</b>	<b>500</b>	<b>340</b>	<b>4.50</b>	<b>422</b>	
<b>±SD</b>	<b>816</b>	<b>823</b>	<b>427</b>	<b>688</b>		<b>169</b>	<b>1.23</b>	<b>103</b>	

Subject 8 = did not complete the race, retired at 60 km. L - Liter; CHO A contains 7g/100ml carbohydrates & 30mg/100ml sodium; CHO B contains 7.5g/100ml carbohydrate & 21mg/100ml sodium; CHO C contains 20g/100ml carbohydrates & 47.5mg/100ml sodium

Table 4. *Total body water changes of participants in an 80km ultra-endurance trail race*

Subject	TBW (kg)			Δ TBW (kg)			% TBW relative to BM			%ΔTBW relative to BM		
	A	B	C	A-B	A-C	B-C	A	B	C	A-B	A-C	B-C
1	44.3	-	-	-	-	-	59.1	-	-	-	-	-
2	37.7	37.8	35.1	-0.1	-2.6	-2.7	61.5	62.3	58.6	1.4	-4.7	-6.0
3	-	40.6	37.5	-	-	-3.2	-	50.4	50.6	-	-	0.4
4	43.6	-	41.9	-	-1.6	-	58.5	-	58.2	-	-0.6	-
5	40.1	39.4	-	-0.7	-	-	57.1	58.6	-	2.6	-	-
6	53.8	51.5	50.2	-2.3	-3.6	-1.4	60.7	57.3	60.0	-5.6	-1.2	4.7
7	-	48.6	45.8	-	-	-2.8	-	65.5	62.5	-	-	-4.7
8	40.7	-	37.6	-	-3.0	-	59.2	-	57.6	-	-2.8	-
9	39.7	-	38.2	-	-1.5	-	64.7	-	64.3	-	-0.7	-
<b>mean</b>	<b>42.8</b>	<b>43.6</b>	<b>40.9</b>	<b>-1.0</b>	<b>-2.5</b>	<b>-2.5</b>	<b>60.1</b>	<b>58.8</b>	<b>58.8</b>	<b>-0.6</b>	<b>-2.0</b>	<b>-1.4</b>
<b>±SD</b>	<b>5.35</b>	<b>6.09</b>	<b>5.40</b>	<b>1.11</b>	<b>0.91</b>	<b>0.80</b>	<b>2.48</b>	<b>5.72</b>	<b>4.37</b>	<b>4.44</b>	<b>1.76</b>	<b>4.92</b>

TBW- Total body water; A- 2 Days Pre-race; B- Pre-race; C- Post-race; Δ-change; (-) - data unavailable

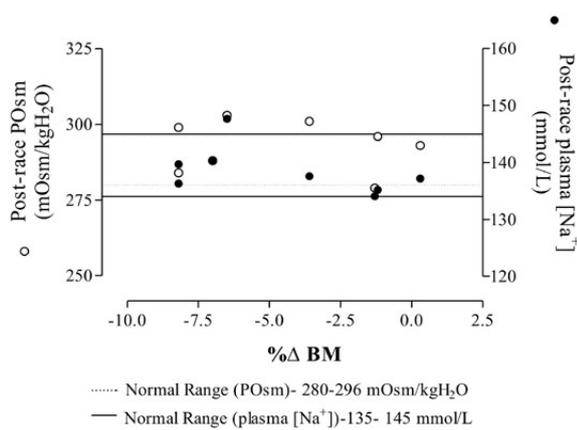


Fig. 1. Percentage change (%Δ) in body mass (BM) in relation to post-race plasma osmolality (POsm) and sodium concentrations ( $[Na^+]$ )

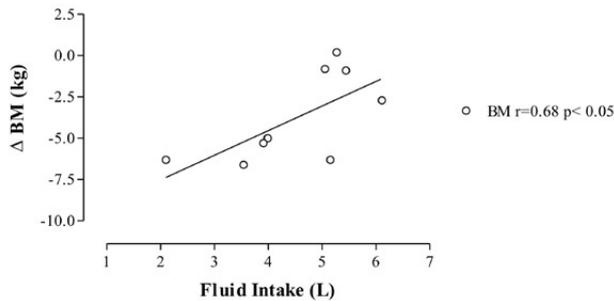


Fig. 3. Changes ( $\Delta$ ) in body mass (B-C) (BM) in relation to total fluid intake (liters)

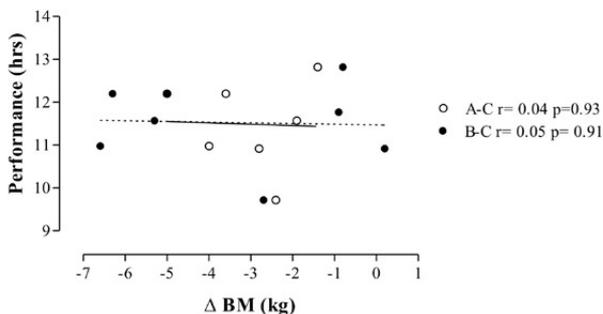


Fig. 4. Non-significant correlation between change in body mass ( $\Delta$ BM) and performance (race time) (hours) for both 2-days pre-race minus post-race (A-C) and immediately pre-race minus post-race (B-C) ( $n = 8$ )

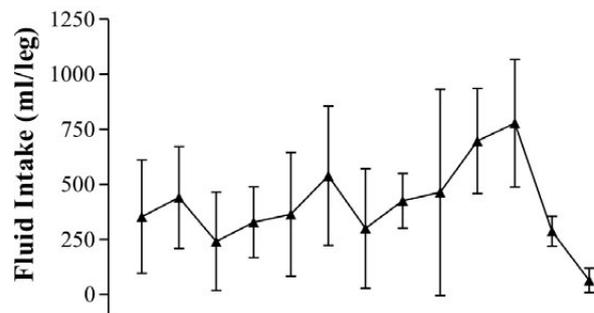
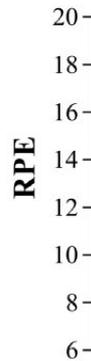
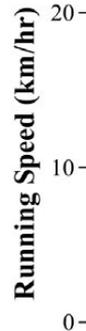
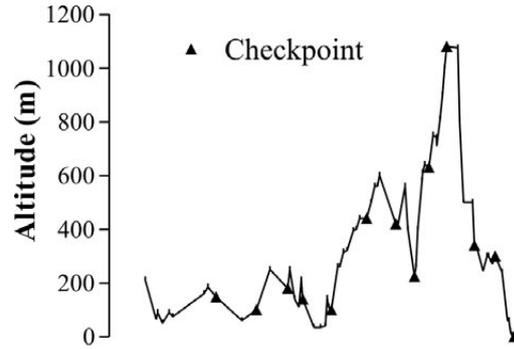


Fig. 2. The race profile in distance (checkpoints) set against change altitude (m); subject's running speed (km/hr); their ratings of perceived exertion (RPE) and fluid intake from the last checkpoint to the specified checkpoint (ml/leg)

Figure 2 illustrates the race profile and elevation (altitude - meters); average running speed of the group between checkpoints (km/hrs); average RPE and average fluid intake of the group at each checkpoint with bars indicating standard deviation.

There was a significant correlation between percentage change in body mass and fluid intake so that

those athletes who ingested the most fluid had the smallest reductions in body mass during the race and vice versa (Figure 3).

There was also no correlation between performance and change in body mass from 2-days pre-race to post-race and immediately pre-race to post-race (Figure 4).

## Discussion

The first relevant finding of this study was that despite quite large individual differences in body mass loss, subjects completed the race without significant changes in plasma sodium or potassium concentrations or in plasma osmolality. Although there were certain biochemical markers out of the normal ranges, Kratz et al. noted in a group of athletes that there was an increase in range of biochemical values in those who finished a marathon without any clinical complications (24). Ad libitum rates of fluid intake were relatively similar between subjects and were at the lower range of the current American College of Sports Medicine (1) and USA Track and Field/International Marathon Medical Directors Association guidelines (25).

These data therefore confirm that moderate drinking according to the dictates of thirst appropriately protects the homeostasis of plasma electrolyte concentrations and plasma osmolality (26). Importantly no athlete drank to excess, thereby avoiding a significant gain in body mass and developing exercise-associated hyponatremic encephalopathy as can occur when athletes are encouraged to drink to “stay ahead of thirst” or “as much as tolerable” and demonstrates that fluid restriction is not necessary to prevent exercise-associated hyponatremia (27). It seems suggestive that overdrinking is seems to be associative with that of the inexperienced runner, this rationalized since any athlete entering this race need to demonstrate some pedigree in both ultra-distance running on both trail and the road (qualify with a ultra-distance race run within in the year and have showed some sort of trail running history). Hence it seems these seasoned athletes indeed are in touch with their bodily needs in regards to both fluid and nutrition and thus have consumed enough to finish in good health.

The second important finding was that plasma sodium concentrations and plasma osmolality were regulated within the adjusted normal range (established by Kratz et al.) even in subjects who lost > 2% of body mass during the race (Figure 1; Table 2). As previously shown by us (27) these variables are homeostatically regulated within the normal range despite quite large differences in percentage body mass loss (26,28). This suggests that athletes are able to regulate their plasma osmolality even when they lose greater than 2% of their body mass during prolonged exercise (5).

All finishers were asymptomatic despite body mass losses greater than 2% in six subjects and > 6% in five subjects. This again confirms our previous findings that athletes may complete ultra-distance races with a body mass loss > 6%, yet be completely asymptomatic (3,4,29). This conflicts with the theory that any body mass loss greater than 2% is inevitably injurious to health or performance during exercise (1). The recent study of Kao et al. (4) reported that athletes completing

12- and 24-hour ultra-marathons can finish without adverse effects on their health even when the body mass loss in some exceeded 7%. Furthermore there was a significant relationship between percentage body mass loss and performance so that athletes who lost the greatest mass completed the greatest distance in the 24 hour race. This is the opposite of the expected outcome if a body mass loss >2% causes a progressive impairment in running performance (1). In contrast we found no such relationship although we noted that regardless of >2% body mass loss that subjects were able to complete the race without experiencing severe dehydration. However the 2 fastest runners lost between 2-3% of their body mass which again exceeds the recommended optimum weight loss according to currently accepted blanket drinking guidelines (Figure 4).

This suggests that the body's hydration state is not regulated solely by the volume of fluid ingested during exercise and that this regulation is highly individualized. While changes in body mass may be useful to predict fluid homeostasis in certain clinical scenarios at rest and over short episodes of exercise (<2 hours) (30), during exercise the protection of plasma osmolality and the plasma sodium concentrations may in some cases require a concomitant decrease in body water content and hence in body mass without causing any apparent disruption of bodily function or athletic performance.

Limited data obtained from total body water measurement by deuterium analysis revealed a small but highly variable (range 1.5L – 3.6L) decrease in TBW content from 2-days pre-race to post-race (Table 4). This is interesting to note because a majority of the athletes finished adequately hydrated (best indicated by plasma osmolality) and the athletes concerned in the data set did not complete the race with significant clinical signs of dehydration. The same could be noted with the data set obtained from immediately pre-race to post-race (B-C).

These small observations lead us to believe that TBW is more appropriately physiologically regulated rather than body mass, which encourages us to further assess the relationship between body mass and TBW loss during ultra-endurance exercise and exercise of a short duration (<3 hours). This brief data set was not in agreement with findings of Baker et al. (31) who ran subjects intermittently for a period of 2 hours in total for each running session. Although we do not feel 2 hours of intermittent exercise is sufficient in length and structure (exercising to certain body mass loss percentages) to observe such findings as we have found with some our subjects data sets but do feel it would comparable in the exercise of a short duration (e.g. half-marathon, football matches etc.). Further investigation into the discrepancy in this respect to field versus laboratory setting.

In summary, this study found that ad libitum drinking rates between 300–650 ml/hr were associated with protection of plasma osmolality and plasma electrolyte homeostasis despite a range of body mass losses. The maintenance of water and solute homeostasis was likely regulated by fluid regulatory hormones arginine vasopressin and aldosterone although these hormones were not measured in this study. This data set also reveals the “reality” of drinking behavior in the field rather than the unrealistic forced drinking and eventual skewed data from laboratory studies on which many fluid intake guidelines are set. The mean change in body mass loss during the race was 5%. Despite changes in body mass exceeding 2%, no subject developed medical complications such as severe dehydration or fluid balance associated disorders. Finally these data support the adoption of ad libitum drinking guidelines during exercise since performance is not negatively affected (25,32).

### Strengths and weaknesses of the study

The strengths of this study include the use of deuterium to quantify changes in total body water. Furthermore, our ability to accurately measure and quantify fluid intake is an improvement over the commonly utilised – but less exact – fluid recall method. The weaknesses of this study include our very limited small sample size (8% participation out of the entire field) with further loss of deuterium data due to methodological difficulties pre-race. Furthermore, only one woman finished the study which may have biased our limited results, since sex-hormones may influence fluid balance parameters particularly during the luteal phase of the menstrual cycle. However, the numbers of individuals – male or female – who are capable of finishing gruelling mountain ultra-endurance races provide a very limited subject pool to draw upon.

### Future directions

A larger cohort of ultra-endurance athletes will be investigated in future, to make these preliminary findings more robust. Furthermore, to better understand fluid balance despite body mass loss in athletes participating in ultra-endurance exercise, fluid regulatory hormones such as arginine vasopressin, aldosterone and natriuretic peptides will be evaluated along with measurement of total body water using the deuterium dilution method. Hopefully with a bigger cohort, we can document associations between changes in total body water versus the maintenance of plasma osmolality and sodium concentrations.

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C – Statistical Analysis

D – Data Interpretation

E – Manuscript Preparation

F – Literature Search

G – Funds Collection