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Fluid, electrolyte and thermoregulatory responses to *ad libitum* water replacement during prolonged exercise

by

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This thesis is dedicated to my wife and best friend, Kim.

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Chapter 1

General Introduction

1.1 Fluid requirements and replacement strategies within a military context

The Research and Technology Organisation (RTO) of the North Atlantic Treaty Organisation (NATO) states in the executive summary of their Human Factors and Medicine Panel Specialists' meeting held in Boston 2003 that: "There is a need to provide more precise estimates of fluid requirements to lessen the loads that the soldier might have to carry and reduce costs associated with water transport and re-supply" (RTO-MP-HFM-086).

The extent to which humans need to replace their fluid losses during exercise remains contentious despite more than 60 years of focused research (Adolph 1947; Ladell 1947; Ladell 1955; Ladell 1965; Montain et al. 2001; Noakes 2003; Passe et al. 2007; Sawka and Noakes 2007; Sawka et al. 2007; Gonzalez et al. 2009). While it is now accepted that exercisers should not be encouraged to drink "as much as tolerable" there is still no consensus of the optimum rate of fluid ingestion during exercise.

The recently modified ACSM guidelines advise that exercisers should drink sufficiently to ensure that their body mass loss during exercise is less than 2% (Sawka et al. 2007). More recently the US Army Research Institute of Environmental Medicine (USARIEM) proposed sweat loss prediction equations ranging from ~575 g/hr to ~1092 g/hr (based on an individual with a body surface area of 1.9 m^2) for various workloads, environmental and clothing configurations in order to more accurately predict fluid replacement volumes during work and exercise (Gonzalez et

al. 2009). Others (Noakes 2003; Noakes 2007) argue that drinking to the dictates of thirst is the biologically appropriate behaviour that optimises performance and is unrelated to heat illness regardless of the exact level of dehydration that develops during exercise.

This debate has special relevance for the military since soldiers ingesting fluid *ad libitum* will drink less that those who are forced to drink in order to lose less than 2 % of their body mass during exercise. Many soldiers drinking *ad libitum* according to the dictates of their thirst will drink less, develop "voluntary dehydration" and will therefore need to carry less water during military operations. In order to investigate these possibilities, there is a need for field studies to establish the optimum rates at which soldiers should ingest fluid during exercise.

1.2   Statement of the problem

Knowledge of a soldier's water needs is vital in ensuring the health, safety and performance of military forces especially in hot arid conditions (Adolph, 1947) in which the provision of water is the critical factor determining ability to sustain military operations. Furthermore accurate knowledge of the fluid needs of military personnel provides much needed information to be applied within the logistics of the transport and provision of water to the front during military operations. The logistical burden of providing water during military operations is second in magnitude only to the supply of battery power to drive military equipment and systems.

Unfortunately providing an excess of water contributes to the burden of the payloads imposed on the dismounted foot soldier, whereas the provision of too little water could have detrimental health consequences. By determining the optimal water requirements of soldiers, one can ensure their safety and physiological comfort while optimising their payload burdens. The correct water replacement strategy will provide safe hydration levels without affecting soldier performance, while potentially reducing the payload burden imposed on the modern soldier.

1.3   Aims and objectives

The aim of the research was to determine the optimal rates of fluid ingestion by military personnel. In turn this could reduce the mass, in the form of water, soldiers might need to carry on military missions.

Accordingly we posed the following questions:

1. What are the rates of fluid ingestion freely chosen by soldiers during exercise?
2. Are these freely chosen (*ad libitum*) rates of fluid ingestion sufficient to protect against major fluid and electrolyte imbalances as evaluated by?
   - Total body water (TBW),
   - Serum sodium concentration [Na^+]; and
   - Plasma osmolality (POsm)
3. Are these freely chosen (*ad libitum*) rates of fluid ingestion sufficient to maintain safe thermoregulation during exercise?

4. Can changes in body mass be used as an accurate surrogate measure for changes in TBW during prolonged exercise?

1.4 Abstract

Herewith follows an abstract of the initial and subsequent chapters of the thesis. Where the applicable chapter consisted of a scientific manuscript the abstract of the manuscript is presented below. Note that the formats of these abstracts are as determined by the scientific journal to which the manuscript was submitted.

Chapter 1

Briefly describes fluid replacement within the military context as well as general introduction and structure of the thesis.

Chapter 2

Knowledge of a soldier's water needs is vital to ensure the health, safety and performance of military forces. Water also contributes to the burden of payloads imposed on the dismounted foot soldier. By determining the optimal water requirements of soldiers, it is possible to ensure safe hydration while decreasing payload burdens. The primary objective of this study was to evaluate the effect of *ad libitum* vs. restricted fluid replacement on selected hydration status markers and performance in three military tasks that evaluated handling, aiming and trigger control during shooting, observation skills and fine motor skills. The secondary objective was to determine if a restricted intake of 300 ml/hr could be considered a safe minimum recommended fluid intake. Data was collected during a field study involving 4 hours of exercise, simulating a

route march over 16 km. Fifty seven subjects participated in the study; the average age of the subjects was 29.5 ± 1.3 (SD) years. The mean pre-exercise body mass of the *ad libitum* group (N = 29) was 70.4 ± 13.3 kg compared to 69.3 ± 8.9 kg in the restricted group (N =28). The mean environmental dry bulb temperature during the study was 24.6°C with a range of 21.0°C to 28.2°C. The mean total fluid intake of the *ad libitum* group was 2.1 ± 0.9 litres compared to 1.2 ± 0.0 litres in the restricted group. There were no significant differences between or within groups for either urine specific gravity (USG) or urine osmolality (UOsm) before or after the exercise period. The *ad libitum* and restricted intake groups respectively lost a mean of 1.05 kg ± 0.77 (1.5%) ($p<0.05$) and 1.34 kg ± 0.37 (1.9%) ($p<0.05$) during the march. Predicted sweat rate was 608 ml/hr ± 93 compared to 762 ml/hr ± 162 in the *ad libitum* group ($p<0.05$). Apart from a non-significant ($p>0.05$) weak ($r^2=0.25$) and medium ($r^2=0.67$) correlation between the post-exercise USG and UOsm for the restricted intake and *ad libitum* groups respectively, there were no other significant medium or strong correlations between either USG or UOsm and any other variable, including total fluid intake and body mass loss during exercise.

Compared to their pre-exercise scores the restricted intake group produced better scores in their post exercise performance measures for all five variables of the three military tasks whereas the *ad libitum* group improved their scores in three of the variables with a decreased performance in two variables. Thus 300 ml/h intake of the restricted group could be considered a current safe minimum hourly water intake for soldiers of similar mass under conditions similar to those of the study

(moderate, dry climate over mixed terrain for similar exercise durations) at similar exercise intensities.

Although variables including soldier functional performance and urinary markers of hydration were measured in this study, fluid balance as determined by changes in total body water (TBW) was not. The lack of correlation between urinary markers and hydration status posed questions regarding the accuracy and validity of these markers to assess fluid balance during exercise. This led to the investigation and application of the measure of TBW though the diluted isotope method in the subsequent studies. Additional measures to evaluate electrolyte balance (serum sodium concentration and plasma osmolality) and thermoregulation (peak exercise core temperature) was added to the methods of the subsequent studies.

Chapter 3

This chapter provide a concise literature summary of the measurement of TBW through the diluted isotope method. This serves as background information describing the technique and methods as applied during the studies presented in Chapters 4, 5 and 6. The introductions, results and discussions of all the subsequent chapters incorporate comprehensive literature on recommendations for fluid replacement during exercise and military training.

Chapter 4

Opportunities to determine optimal rates of fluid ingestion could reduce the mass soldiers might need to carry on military missions. The first objective was to evaluate the effects of an *ad libitum* fluid replacement strategy on total body water (TBW), core temperature, serum sodium concentrations [Na^+] and plasma osmolality (POsm). The second objective was to determine if an *ad libitum* water intake was sufficient to maintain these variables during exercise. A third objective was to determine whether changes in body mass are an accurate measure of changes in TBW. A field study was conducted with 15 soldiers performing a 16.4 km route march. The average age of the subjects was 27 ± 4.6 (SD) years. Their mean hourly *ad libitum* fluid intake was 383 ± 150 ml. Predicted sweat rate was 626 ± 122 ml/hr. Despite an average body mass loss of 1.0 kg ± 0.50 TBW, POsm and serum [Na^+] did not change significantly during exercise. There was a significant ($p < 0.05$) linear relationship with a negative slope between post-exercise serum [Na^+] and changes in both body mass and % TBW. Post-exercise POsm and serum [Na^+] were significantly related ($p < 0.05$). Higher post-exercise % TBW was associated with lower post-exercise POsm and serum [Na^+] levels. There was no relation between % body mass loss and peak exercise core temperature (38.1 ± 0.6°C). Conclusion: A mean *ad libitum* water intake of 383 ml/h, replacing approximately 61% of body mass losses during 4 hours of exercise maintained TBW, peak exercise core temperature, POsm and serum [Na^+] despite a 1.4% body mass loss. A reduction in body mass of 1.4% (1.0 kg) was not associated with a reduction in TBW.

Chapter 5

The extent to which humans need to replace fluid losses during exercise remains contentious despite years of focused research. The primary objective was to evaluate *ad libitum* drinking on hydration status to determine whether body mass loss can be used as an accurate surrogate for changes in total body water during exercise. Data was collected during a 14.6 km route march (WBGT of 14.1). 18 subjects with an average age of 26 ± 2.5 (SD) years participated. Their mean *ad libitum* total fluid intake was 2.1 ± 1.4 litres during the exercise. Predicted sweat rate was 1.289 ± 0.530 L/hr. There were no significant changes ($p > 0.05$) in total body water, urine specific gravity or urine osmolality despite an average body mass loss ($p < 0.05$) of 1.3 kg ± 0.45 during the march. Core temperature rose as a function of marching speed and was unrelated to the % change in body mass. This suggests that changes in mass do not accurately predict changes in total body water ($r = -0.16$) either because the body mass loss during exercise includes losses other than water or because there is an endogenous body water source that is released during exercise which does not require replacement during exercise, or both. *Ad libitum* water replacement between 65% and 70% of sweat losses maintained safe levels of hydration during the experiment. The finding that total body water was protected by *ad libitum* drinking despite ~2% body mass loss suggests that the concept of "voluntary dehydration" may require revision.

Chapter 6

The guidelines to establish safe environmental conditions for exercise are based in part on the concept of a thermal prescriptive zone in which humans exercising at an externally-regulated (fixed) work rate (exercise intensity) are unable to reach thermal equilibrium. This data suggest that it is dangerous for humans to exercise competitively at ambient temperatures in excess of 34°C, especially when the air humidity is increased. Yet there are isolated reports of unusual self-paced human athletic performances in extreme heat. As part of a series of experiments to determine the minimal fluid requirements of soldiers during route marches, we were granted the opportunity to study a group of 18 exceptionally well conditioned and heat-adapted members of the South African National Defence Force. The soldiers participated in an individually-timed, competitive 25 km route march while wearing full battle dress and carrying 26 kg in a dry bulb temperature that reached 44.3°C. The average age of the subjects was 26 ± 3.7 (SD) years. Their mean hourly *ad libitum* water intake was 1264 ± 229 ml. Predicted sweat rate was 1789 ± 267 ml/hr. Despite an average body mass loss of 2.73 ± 0.98 kg, plasma osmolality and serum sodium concentrations did not change significantly during exercise. Total body water (TBW) fell 1.47 kg (2.0%) during exercise (p<0.05). However, the change in body mass did not accurately predict the changes in TBW as a 1:1 ratio. There was a significant (p<0.05) linear relationship with a negative slope between post-exercise serum [Na$^+$] and changes in both body mass and % TBW. Higher post-exercise % TBW was associated with lower post-exercise POsm and serum [Na$^+$] levels. There was no relation between % body mass loss and peak exercise core temperature (40.3 ± 0.9°C) or time

taken to finish the march. Subjects maintained low core body temperatures in part by behaviour modifications which included resting in the shade. We conclude that these subjects maintained plasma osmolality, serum [Na$^+$] and safe core body temperatures by (i) adopting a pacing strategy which included intermittent rest in the shade; (ii) very high rates of *ad libitum* water intake and (iii) by allowing a small reduction in TBW to maintain serum [Na$^+$] despite probable sweat sodium losses of > 200 mmol. Our findings support the hypothesis that humans are the mammals with the greatest capacity for exercising in extreme heat, an adaptation that may have special significance for the evolution of the Homo sapiens.

Chapter 7

A manuscript by Baker et al. (2009) was published in the European Journal of Applied Physiology stating that changes in body mass accurately and reliably predicts changes in TBW and could therefore by used as an indication of hydration status during prolonged exercise.

Since the results of Baker and associates were in direct conflict with the results of the studies presented in this thesis document, a thorough review of their methods and results were performed. A letter was addressed to the editor of the European Journal of Applied Physiology in order to raise concerns over the results and the manner in which they were calculated.

Chapter 8

Chapter 8, provide a summary, conclusion of the interpretations on the findings, and indications for further research.

Summary

The extent to which humans need to replace their fluid losses during exercise remains contentious despite more than 60 years of focused research. Unfortunately, apart from the inherent physiological risk associated with "under" or "over" hydration, providing an excess of water contributes to the burden of the payloads imposed on the dismounted foot soldier. The correct water replacement strategy will provide safe hydration levels without affecting soldier performance, while potentially reducing the payload burden imposed on the modern soldier. This debate has special relevance for the military since soldiers ingesting fluid *ad libitum* will drink less that those who are forced to drink in order to lose less than 2 % of their body mass during exercise.

The main findings of this research effort are that the mean *ad libitum* fluid (water) intake of soldiers ranged from 383 ml/hr to 1264 ml/hr and that these *ad libitum* rates of fluid ingestion were sufficient to protect against major fluid, electrolyte and thermoregulatory imbalances. Furthermore the findings suggest that changes in body mass can not be used as an accurate surrogate measure for changes in TBW during prolonged exercise.

Current position stands on fluid replacement proposes that the mass loss during exercise should not exceed 2% of the starting body mass.

The findings of this research effort do not support this prescription and indicates that large changes in body mass did not cause deleterious physiological changes during exercise. The study raises questions about the validity of the term "voluntary dehydration" that was first coined more than 60 years ago. Indeed this study invites a more thorough interrogation of the use of the term "dehydration" which should be used only when there is a proven reduction in TBW and not, as this study shows, merely a reduction in body mass during exercise.

1.5   Publications and presentations

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Chapter 2

Effect of *ad libitum* and restricted fluid replacement strategies during prolonged exercise on various hydration markers and performance of selected military tasks in soldiers

Article:

Nolte, H.W., Noakes, T.D. and van Vuuren, B. 2009. Effect of *ad libitum* and restricted fluid replacement strategies during prolonged exercise on various hydration markers and performance of selected military tasks in soldiers.

2.1 Introduction

Water is the largest single constituent of the human body and is essential for cellular homeostasis and life. Knowledge of a soldier's water needs is vital in ensuring the health, safety and performance of military forces especially in arid conditions (Adolph, 1947) in which the provision of water is the critical factor determining ability to sustain military operations.

Unfortunately providing an excess of water contributes to the burden of the payloads imposed on the dismounted foot soldier, whereas the provision of too little water could have detrimental health consequences. By determining the optimal water requirements of soldiers, one can ensure their safety and physiological comfort while optimising their payload burdens. The correct water replacement strategy will provide safe hydration levels without affecting soldier performance, while potentially reducing the payload burden imposed on the modern soldier.

Foot soldiers can not usually be expected to carry masses in excess of 50% of their own body mass. These masses have a negative impact on soldier endurance, situation awareness and the ability to respond quickly and accurately to threat. In arid environments water comprises a significant amount of this mass. For example, during deployment in Afghanistan, United States soldiers often carried water supplies for missions lasting between 1-3 days representing 9-10 kg or in excess of 30% of their fighting load (RTO-MP-HFM-086). However few studies have attempted to establish a safe minimum level of replacement for soldiers during operational activities. Such minimal values would reduce

payload requirements while maintaining optimal fluid levels and contribute to overall system performance and mission success.

Thus the primary objective of the study was to evaluate the effect of *ad libitum* vs. restricted fluid replacement protocol (fluid intake limited to exactly 300 ml/hour) on selected hydration status markers and military performance measures in a group of 57 dismounted infantry soldiers during a simulated route march of 16km lasting 4 hours.

The secondary objective was to determine if the volume of 300 ml/h could be considered a safe minimum recommended fluid intake without negatively affecting either the hydration status or performance of this group of dismounted soldiers during the march.

Subject selection

Ethical clearance for this study was obtained from the Research Ethics Committee of the South African Military Health Services (SAMHS) of the SANDF. A request for subjects was put forward to the SANDF in order to identify soldiers that were experienced and conditioned to route marches with payloads of up to 35 kg. All the subjects were medically fit to participate in the study and none was suffering from any musculoskeletal injuries. All subjects had recently passed the Chief of the Army Fitness Test. Subjects were told that they could terminate their participation at any stage without any consequences to their careers. Subjects were required to voluntarily sign an informed consent form before they participated in the study. The subjects were asked to provide basic demographic information for record purposes. Anthropometric

measurements were included to predict fat and muscle percentage distributions using the Drinkwater and Ross method (Ross and Marfell-Jones, 1991). Two days prior to the field study the subjects performed a multi staged shuttle run test (Lèger and Lambert, 1982) to determine predicted maximal oxygen consumption (VO_2max). A qualified exercise scientist facilitated a warm-up and stretching session with the subjects prior to the performance of the test.

2.2   Materials and methods

Performance Measures

On the day of the study performance was recorded during selected military tasks in order to compare the results prior to and upon completion of the 4 hour exercise intervention. Shooting skills were evaluated on an Electronic Learning Aiming Correction System (ELACS) shooting simulator. All of the subjects were familiar with the use of this simulator. The simulator graded the subjects on three variables of their shooting skills namely handling, aiming and trigger control during the task.

An observation task required the subjects to observe, spot and identify military related objects that were positioned in the operational area. They needed to identify various objects and report back their location regarding position and distance from where they were standing using standard military protocol. These objects were rotated or replaced with alternatives for the post-exercise observation task. The objects were placed to evaluate near, far and peripheral vision of the soldiers. A plastic weapon magazine and dummy metal rounds were used in a task

which evaluated fine motor skills. The subjects had to unload and reload a magazine in the fastest possible time. The time was measured from the moment the soldier picked up the loaded magazine until he had completed the unloading and reloading and placed the magazine back on the table.

Exercise intervention

Subjects wore standard issue combat dress with military boots, battle jacket and bush hat. The battle jackets of each of the subjects were similarly packed with mass to the amount of 20 kg. One member of each group carried a Global Positioning System (GPS) and radio to ensure communication and tracking of the groups in terms of speed and direction. The route was 4.0 km and was repeated four times for a total march distance of 16 km which took 4 hours to complete. Subjects were randomly assigned to either the restricted (300 ml/h) or *ad libitum* intake group. Subjects in the *ad libitum* group were instructed to drink as desired while the restricted group were instructed to finish 300 ml but no more within each hour of the four hour intervention. Neither of the two groups was allowed to eat during the study. The ambient temperature, wind speed, relative humidity, solar radiation and barometric pressure were recorded for the duration of the study.

Hydration markers

Select measures to identify acute changes in hydration status were measured including body mass, urine specific gravity (USG) and urine osmolality (UOsm). Sweat rates were predicted from body mass

changes as well as fluid intake and urine output during the exercise period. Sweat rates were estimate as follows:

Sweat rate = ((pre body mass – post body mass) + (fluid intake – urine produced))/exercise time

Sweat rates so calculated are not corrected for respiratory water loss as well as CO_2 loss and O_2 gain. Nude body mass was recorded prior and upon completion of the route march with an electronic scale (accurate to the nearest 100 grams). Subjects were provided with towels to dry excess perspiration prior to weighing. Subjects were required to empty their bladders prior to each weighing; these voids were used for USG and UOsm analyses prior to and upon completion of the route march.

Statistical analyses

In order to determine which statistical test would be most suited for the comparisons of the *ad libitum* and restricted intake groups, the differences (paired differences) between the group results were calculated. The distributions (in the form of histograms) of paired differences of all the results were plotted with the number of classes as calculated according to the Rule of Sturge. The normality of this distribution was tested by means of the Shapiro-Wilks' W test. The statistical Rule of Sturge states that the number of classes equals N x 1.4 + 1 (Where: N = sample size). T-tests were used to compare *ad libitum* and restricted intake results where the distribution of the paired differences was normal. Where the distribution of the paired differences was not normal, the non-parametric alternative to the T-test, the

Wilcoxon Rank Sum Test was used to compare *ad libitum* and restricted intake results. A Pearson's product moment correlation coefficient was used to determine relationships between appropriate variables. Statistical significant differences between the *ad libitum* and restricted intake results were indicated by a p-value of less than 0.05. The statistical analyses were completed using the STATISTICA© software package (Statsoft, 2000).

2.3   Results

Fifty seven subjects that met the selection criteria volunteered for the study. Table 1 presents the military experience; body mass, stature, predicted VO$_2$max as well as predicted percentage body fat and muscle for the two groups. There were no statistically significant differences between any of these variables for the two groups. Results for the ambient temperature, wind speed, relative humidity, solar radiation and barometric pressure for the duration of the study are presented in Table 2.

Table 1: Subject information for both groups

		Minimum	Mean (±SD)	Maximum
Ad Libitum Group (N = 29)	Years military service	6.0	8.1 (1.0)	10.0
	Body mass (kg)	50.4	70.6 (13.4)	103.2
	Stature (mm)	1594	1724 (81)	1895
	Predicted VO_2 max (ml/kg/min)	24.9	36.7 (6.8)	50.2
	Body fat (%)	6.3	12.1 (4.2)	20.8
	Muscle (%)	36.7	44.6 (3.7)	50.1
Restricted Group (N = 28)	Years military service	6.0	8.2 (1.1)	11.0
	Body mass (kg)	55.2	69.3 (8.8)	88.4
	Stature (mm)	1600	1723 (71)	1895
	Predicted VO_2 max (ml/kg/min)	26.8	37.3 (4.4)	46.8
	Body fat (%)	5.9	11.5 (3.9)	22.9
	Muscle (%)	38.7	44.2 (2.5)	48.1

Table 2: Prevailing environmental conditions during the study

	Minimum	Mean (±SD)	Maximum
Dry bulb temperature [°C]	21.0	24.6	28.2
Solar radiation [W/sq.m]	257.0	599.2	781.0
Barometric pressure [mbar]	640.0	641.0	644.2
Wind speed [km/h]	1.1	1.9	2.7
Relative humidity [%]	16.0	20.7	47.0

Table 3 presents the mean hourly water intake of the *ad libitum* group; the restricted group drank 300 ml/hr. Considering the mean total fluid intake during the 4 hour exercise period the *ad libitum* group consumed 2.1 litres compared to the 1.2 litres of the restricted group, a difference of 224 ml/hr during the 4 hours or a total of 900ml. Table 4 presents the results for pre- and post-exercise values and changes in mean USG for both groups. There were no significant differences between or within groups for USG before or after the exercise period. Table 5 presents the results for pre- and post-exercise values and changes in UOsm for both groups. There were no significant differences between or within groups for UOsm before or after the exercise.

Table 3: Mean fluid intake of *ad libitum* group

	Minimum	Mean (±SD)	Maximum
Water intake (ml/hr)	228	524 (227)	1000

Table 4: USG results for both groups pre- and post-exercise

		Minimum	Mean (±SD)	Maximum
Restricted Group **(N = 28)**	USG (pre-exercise)	1.010	1.020 (0.00)	1.025
	USG (post-exercise)	1.015	1.021 (0.00)	1.025
	% Change		0.11	
***Ad Libitum* Group** **(N = 29)**	USG (pre-exercise)	1.010	1.019 (0.00)	1.030
	USG (post-exercise)	1.005	1.019 (0.00)	1.025
	% Change		-0.07	

Table 5: UOsm results for both groups pre- and post-exercise

		Minimum	Mean (±SD)	Maximum
Restricted Group (N = 28)	**UOsm (pre-exercise) [mmol/kg]**	356	893.2 (170)	1183
	UOsm (post-exercise) [mmol/kg]	599	878.5 (130)	1141
	% Change		-1.65	
***Ad Libitum* Group (N = 29)**	**UOsm (pre-exercise) [mmol/kg]**	429	868.2 (193)	1242
	UOsm (post-exercise) [mmol/kg]	84	805.6 (290)	1173
	% Change		-7.2	

Table 6 presents the body mass changes and calculated sweat rates of both the *ad libitum* and restricted intake groups. The differences in the sweat rates between the groups were statistically significant while the decrease in body mass were significant for both groups but not different between groups. Figures 1 and 2 respectively present the relationship between the total volume consumed and the post exercise UOsm and USG for the *ad libitum* intake group. Figures 3 and 4 respectively present the relationship between the change in body mass and UOsm and USG for the *ad libitum* group. Figures 5 and 6 respectively present the relationship between the change in body mass and UOsm and USG for the restricted intake group.

Table 6: Body mass loss and sweat rate prediction results for both groups pre- and post-exercise (* = p<0.05)

		Minimum	Mean (±SD)	Maximum
Restricted Group **(N = 28)**	**Mass (pre-exercise) [kg]**	54.20	69.33 (8.9)	88.00
	Mass (post-exercise) [kg]	53.70	67.99 (8.7)	86.40
	Body mass loss [kg]	0.50	1.34* (0.37)	1.60
	Body mass loss [%]	0.9	1.9	2.60
	Sweat Rate [ml/hr]	395	608* (93)	0.755
***Ad Libitum* Group** **(N = 29)**	**Mass (pre-exercise) [kg]**	50.40	70.41 (13.3)	102.00
	Mass (post-exercise) [kg]	49.30	69.36 (13.0)	99.90
	Body mass loss [kg]	0.50	1.05* (0.77)	2.10
	Body mass loss [%]	0.9	1.5	2.0
	Sweat Rate [ml/hr]	0.489	762* (162)	1.189
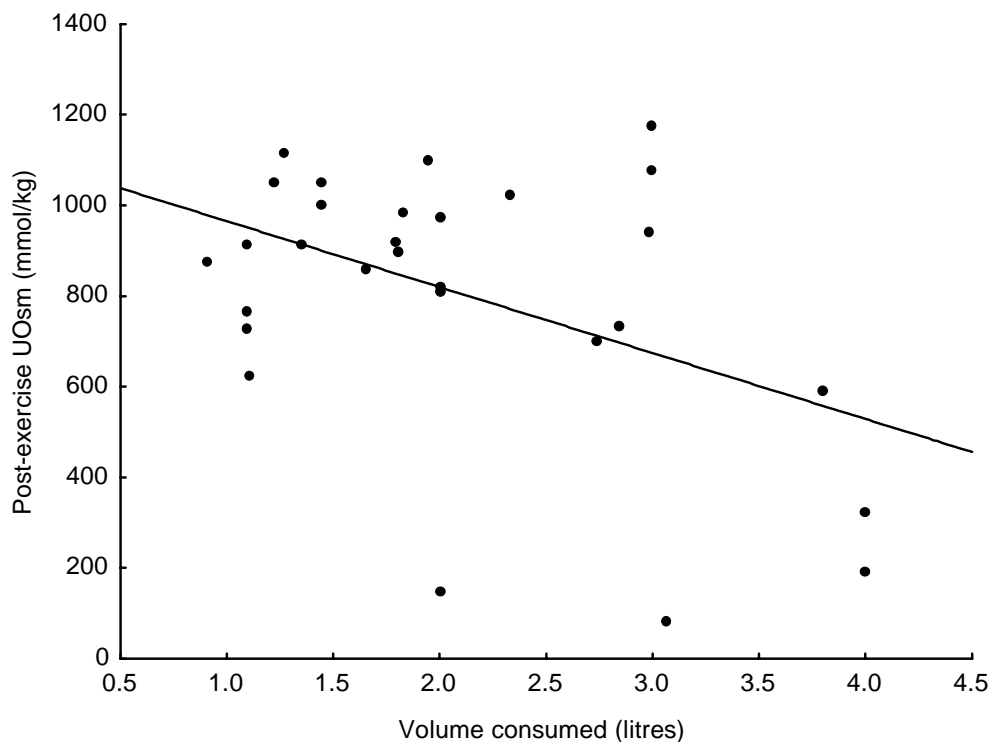
Figure 1: The relationship between total water intake and urine osmolality for the *ad libitum* intake group (N =29) (r = -0.45, p>0.05)
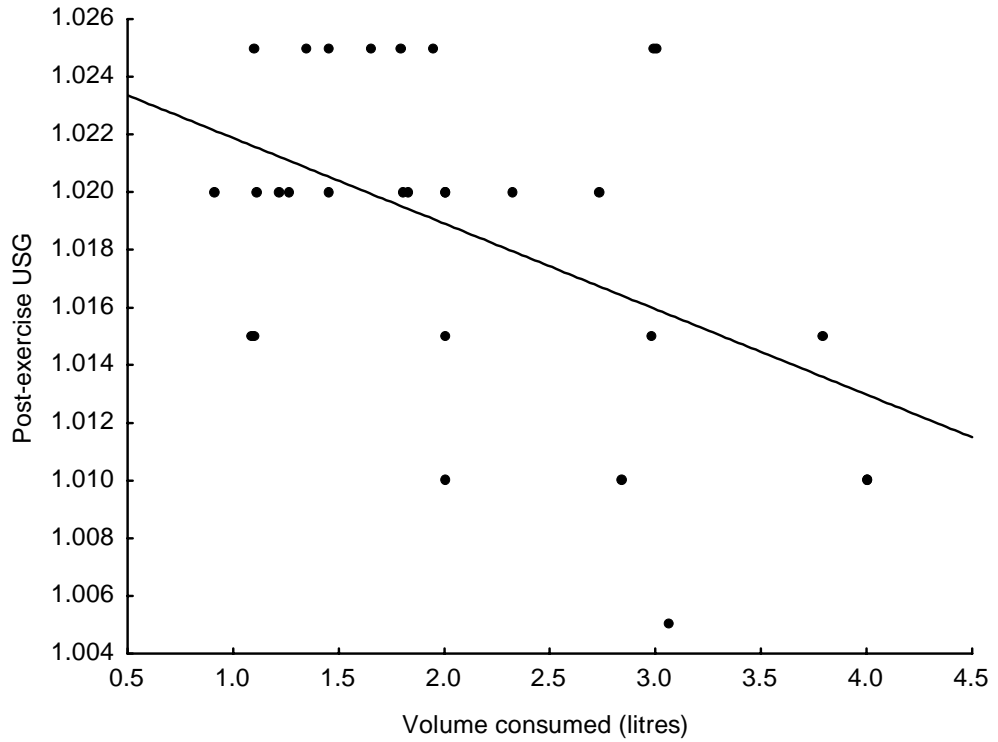
Figure 2: The relationship between total water intake and urine specific gravity for the *ad libitum* intake group (N =29) (r = -0.48, p>0.05)
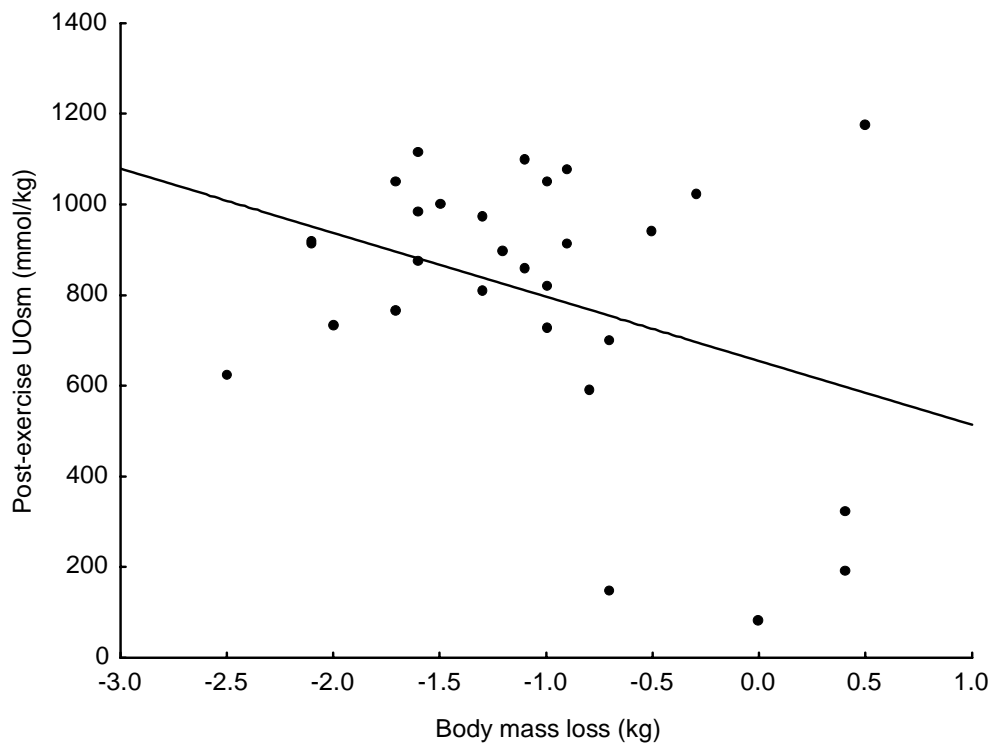
Figure 3: The relationship between body mass loss and urine osmolality for the *ad libitum* intake group (N =29) (r = -0.37, p>0.05)
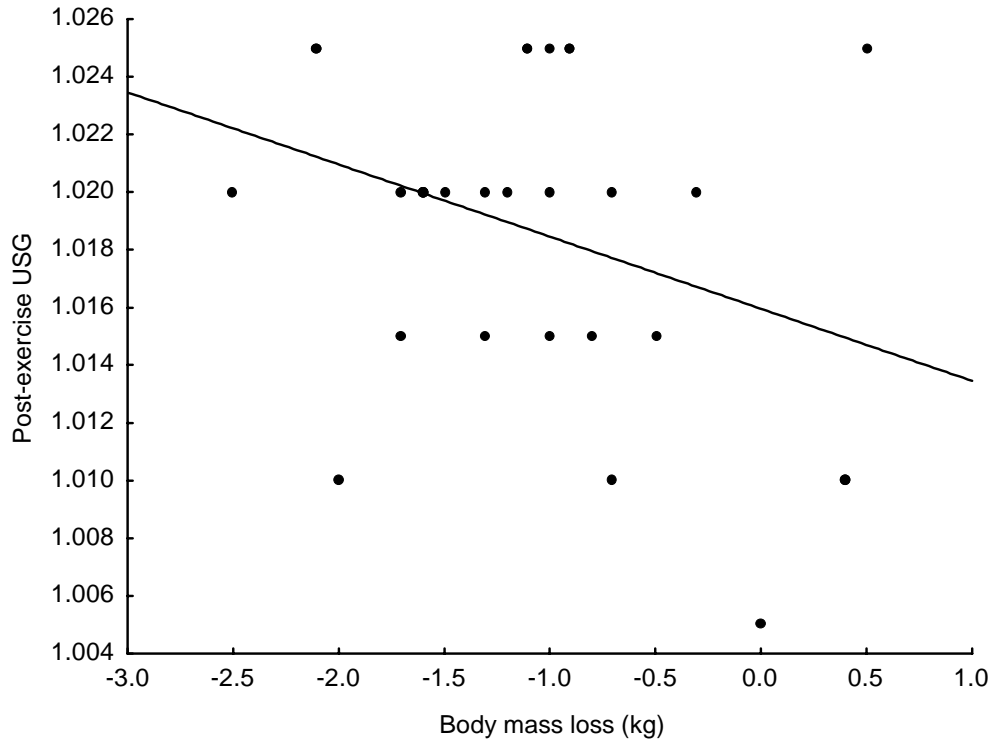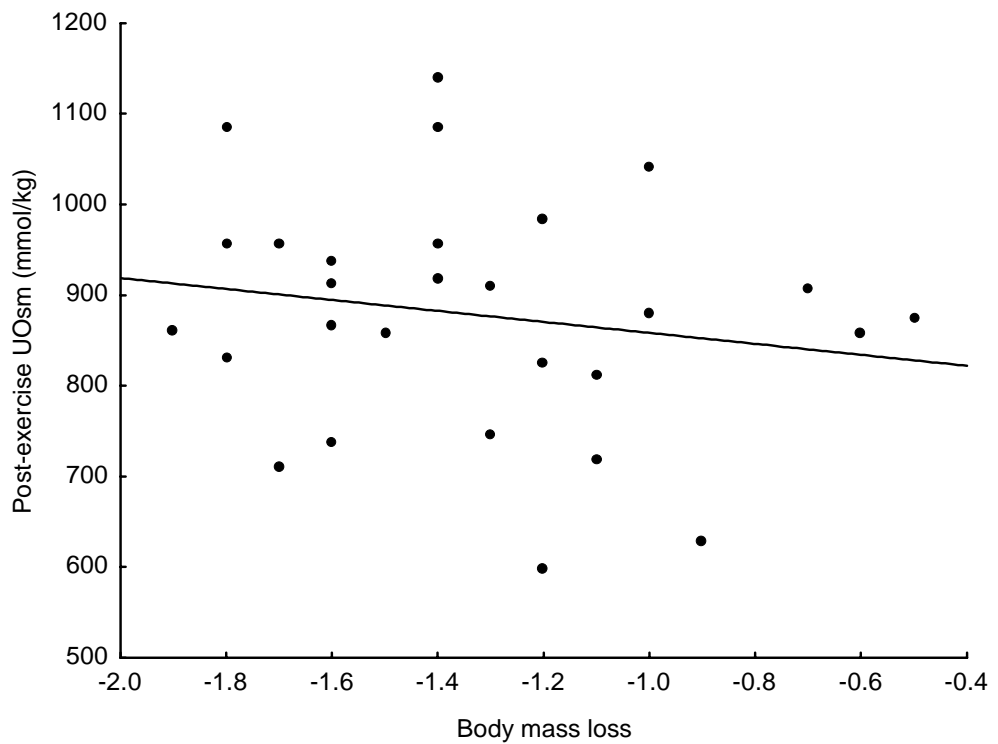
Figure 4: The relationship between body mass loss and urine specific gravity for the *ad libitum* intake group (N =29) (r = -0.34, p>0.05)
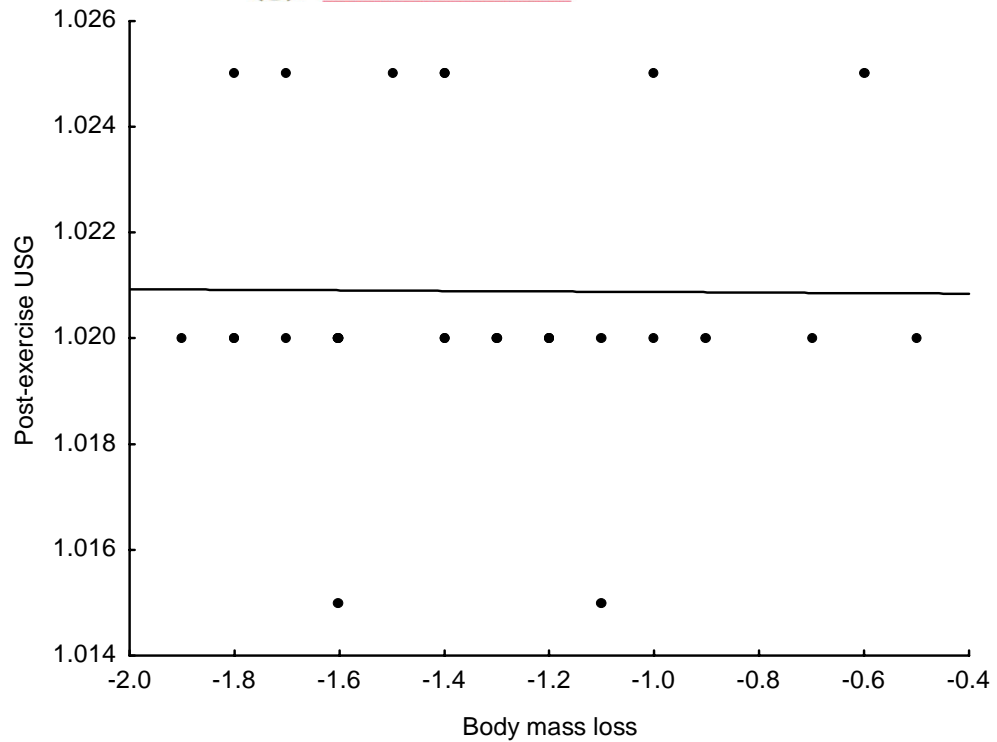

Figure 5: The relationship between body mass loss and urine osmolality for the restricted intake group (N =29) (r = -0.17, p>0.05)

Figure 6: The relationship between body mass loss and urine specific gravity for the restricted intake group (N =29) (r = -0.07, p>0.05)

Table 7 presents the mean handling performance scores for the shooting simulator task before and after the march. Although not statistically significant, the restricted group performed better than the *ad libitum* group after the exercise and presented with a larger within group improvement. Table 8 presents the mean aiming performance scores for the shooting simulator task pre- and post-exercise. Table 9 presents the mean trigger control performance scores for the shooting simulator task pre- and post-exercise. The *ad libitum* group showed statistically significantly better scores during the pre route march task despite the random assignment of each group. The restricted group improved their

performance significantly after the route march while the *ad libitum* group showed a non-significant decrease in their performance of this task.

Table 10 presents the observation performance scores for both groups pre- and post-exercise. There may well be contributing factors in the improvement in performance such as changes in light contrast due to time of day testing. However performance did not deteriorate despite the exercise intervention and different fluid replacement strategies and the completion of a 4 hour route march. Table 11 presents the fine motor skill scores for both groups pre- and post-exercise. Although not statistically significant, the *ad libitum* group performed better than the restricted intake group after the exercise and presented with a larger within group improvement.

Table 7: Shooting simulator handling results for both groups

		Minimum	Mean (±SD)	Maximum
Restricted Group (N = 28)	**Handling results (pre-exercise)**	15.00	60.70 (24.2)	88.00
	Handling results (post-exercise)	15.00	68.04 (19.9)	89.00
	% Change		12.08	
Ad Libitum Group (N = 29)	**Handling results (pre-exercise)**	16.00	66.48 (26.0)	90.00
	Handling results (post-exercise)	16.00	67.26 (19.8)	90.00
	% Change		4.18	

Table 8: Shooting simulator aiming results for both groups

		Minimum	Mean (±SD)	Maximum
Restricted Group **(N = 28)**	**Aiming results (pre-exercise)**	7.00	30.37 (17.0)	69.00
	Aiming results (post-exercise)	5.00	33.22 (14.4)	63.00
	% Change		9.39	
***Ad Libitum* Group** **(N = 29)**	**Aiming results (pre-exercise)**	7.00	35.78 (17.3)	67.00
	Aiming results (post-exercise)	6.00	35.48 (15.4)	64.00
	% Change		-0.83	

Table 9: Shooting simulator trigger control time results for both groups

(* = $p < 0.05$)

		Minimum	Mean (±SD)	Maximum
Restricted **Group (N =28)**	**Trigger control time score (pre-exercise)**	8.00	41.33* (28.1)	86.00
	Trigger control time score (post-exercise)	15.00	54.08 (23.1)	83.00
	% Change		30.84	
***Ad Libitum* Group (N = 29)**	**Trigger control time score (pre-exercise)**	17.00	62.48* (24.2)	86.00
	Trigger control time score (post-exercise)	8.00	60.63 (22.6)	86.00
	% Change		-2.96	

Table 10: Observation skill results for both groups (* = p<0.05)

		Minimum	Mean (±SD)	Maximum
Restricted Group **(N = 28)**	**Number of objects identified out of possible 8 (pre-exercise)**	3	5.96 (1.6)	8
	Number of objects identified out of possible 8 (post-exercise)	4	7.04 (1.1)	8
	% Change		17.96*	
Ad Libitum Group **(N = 29)**	**Number of objects identified out of possible 8 (pre-exercise)**	1	6.21 (1.5)	8
	Number of objects identified out of possible 8 (post-exercise)	4	7.07 (1.1)	8
	% Change		13.89*	

Table 11: Fine motor skills results for both groups

		Minimum	Mean (±SD)	Maximum
Restricted Group **(N = 28)**	**Time (pre-exercise)**	01:03	01:23 (00:19)	02:44
	Time (post-exercise)	00:57	01:18 (00:12)	01:38
	% Change		-4.19% (improvement)	
Ad Libitum Group **(N = 29)**	**Time (pre-exercise)**	00:58	01:20 (00:19)	02:16
	Time (post-exercise)	00:52	01:15 (00:15)	01:53
	% Change		-7% (improvement)	

2.4   Discussion

Our first relevant finding of this study was that despite a significant ($p<0.05$) body mass loss presented by the restricted intake group during exercise (1.9% of pre-exercise body mass) the only significant difference between the two groups was the predicted sweat rate during exercise. The sweat rate was significantly higher in the ad libitum group at 762 ml/hr vs. 608 ml/hr ($p<0.01$).

The differences in sweat rates might be attributed to the differences in the water volumes consumed during the march since the *ad libitum* group ingested on average 900 ml more water during the four hour route march. Other than this difference, there were no significant differences between the two groups for any of the post-exercise hydration variables that were measured despite differences in body mass loss and total fluid intake.

Our second relevant finding was the lack of any significant correlations between the urinary indices and hydration status (Figures 1 to 6). Apart from a non-significant, weak ($r^2=0.25$) and medium strong ($r^2=0.67$) correlation between the post-exercise USG and the post-exercise UOsm for the restricted intake and *ad libitum* groups respectively, there were no other significant medium or strong correlation between either USG or UOsm and any other variable, including total fluid intake and body mass loss (Figures 3 to 6) during the exercise period. The duration in the renal response to exercise induced dehydration will result in significant delays in urinary electrolyte changes so that these measures are of little value to assess fluid balance status.   Laursen et al. (2006) also reported that

changes in body mass were unrelated to core temperature, serum sodium concentration and urine specific gravity in athletes completing a 226 km Ironman Triathlon. The lack of an apparent correlation between these urinary markers and body mass loss and fluid intake should caution against the use of these markers when accurate hydration status assessments are required.

Both USG and UOsm are often used to monitor hydration status in field settings due to the non-invasive and cost effective nature of these methods. According to Armstrong et al. (1994) and Armstrong (2005) USG and UOsm will both increase with dehydration and are strongly correlated. Previously Armstrong et al. (1994) presented correlation values of 0.96 (r^2). However, others argue that urine osmolality is not a good indicator of changes in total body water (TBW) (Hackney et al., 1995, Ruby et al., 2003 and Kavouras, 2002). For a "normally" hydrated (euhydrated) person, USG values range from 1.010 to 1.030. It has generally been accepted that a USG of less or equal to 1.020 represents euhydration and a USG greater than 1.030 represents dehydration (Popowski et al., 2001). Normal urine osmolality should be between 300 – 900 mmol/kg.

In the setting of such variability, there may be no single threshold at which the urine osmolality accurately predicted the hydration status. In addition, urine osmolality is increased when osmotically active solutes are excreted, such as glucose, in patients with uncontrolled diabetes mellitus. For these reasons of high variability; dependence on solute excretion and lack of correlation with TBW changes, UOsm is not

considered a good indicator of hydration status (Institute of Medicine of the National Academies, 2004). Further limitations regarding UOsm may include accuracy issues when used immediately after exercise and large inter-cultural differences as evident by mean differences between Germans (860 mmol/kg) and Poles (392 mmol/kg) (Armstrong, 2005 and Manz and Wentz, 2003).

None of the subjects in any of the groups presented with USG values in excess of 1.030 considered indicative of dehydration (Popowski et al., 2001).

Results of an intra-group comparison indicates that the restricted intake group presented with better scores in their post-exercise performance measures for all five variables while the *ad libitum* group presented with better scores for three ($p > 0.05$) of the variables and a decrease performance in two. The within group increase of 31% for the trigger control measure and 18% for the observational task of the restricted intake group were both statistically significant ($p < 0.05$). Furthermore the restricted intake group presented with better between group performance scores post-exercise in four of the possible five variables. However none of these differences were statistically significant.

The improvements present in the current study may be attributed to a warm-up effect on coordination as described by Adam et al. (2008), but it must be noted that performance did not deteriorate for the restricted intake group. But these results also suggest that aerobic exercise improves some aspects of military task performance since performance

in the shooting, observational and fine motor tasks improved after the exercise bout.

Comparison of these results with other published studies is difficult since these tests form part of a custom designed soldier task measurement tool. Recently Adam et al. (2008) showed that moderate hypohydration of 3% did not effect cognitive or psychomotor performance in cold or temperate environments. Serwah and Marino (2006) investigated the combined effects of hydration and exercise heat stress on choice reaction time. They found that different levels of dehydration produced by different drinking regimes during up to 90 minutes of exercise in warm, humid conditions did not compromise choice reaction time. Similarly Szinnai et al. (2005) showed that dehydration of up to 2.6% body mass did not alter cognitive-motor function in healthy young subjects.

In surprising contrast Baker et al. (2007) found that vigilance-related attention of male basketball players was impaired by dehydration levels ranging between 1-4% concluding that fluid replacement is essential to prevent a decline in vigilance that occurs with dehydration in highly technically demanding sports. Were this finding universally true, we would have expected that performance in one or more of the tests that we performed should have been impaired.

Conclusion

The aim of this study was to compare the effects of an *ad libitum* and a restricted fluid replacement strategy on selected hydration markers and soldier performance in selected military tasks. We were unable to detect

any superiority of the *ad libitum* drinking regime (525 ml/hr) compared to the restricted regime. Thus we conclude that a fluid ingestion rate of 300 ml/hr could be regarded as a safe minimum rate of fluid ingestion for male soldiers exercising under conditions similar to those in this study namely moderate, dry climate for similar exercise durations at equivalent exercise intensity. However, drinking *ad libitum* is probably the more appropriate response even though there was no measurable benefit associated with this slightly higher rate of fluid intake. Finally we show that urinary measures are poorly related to hydration status and recommend that the diluted isotope technique be applied during future research in order to accurately assess changes in total body water during exercise. This technique would be able to relate body mass change to actual body water changes and could provide significant insight into the efficacy of *ad libitum* fluid replacement strategies in maintaining safe fluid levels during exercise.

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Chapter 3

Overview of assessing total body water using the diluted isotope (deuterium oxide) technique

3.1 Introduction

While the results obtained during the first study (Chapter 2) provided valuable information regarding soldier performance following either *ad libitum* or restricted fluid replacement strategies, it also highlighted the poor relationship between urinary markers and hydration status. Thus the recommendations that the diluted isotope technique be applied during future research in order to accurately assess changes in total body water during exercise. This technique enables the comparison of changes in total body water with that of body mass and provides significant insight into the efficacy of *ad libitum* fluid replacement strategies to maintain safe fluid levels during exercise.

This chapter will provide an overview of the measurement of TBW through the diluted isotope method. This serves as background information describing the technique as applied during the studies as presented in Chapters 4, 5 and 6. The overview will not discuss deuterium abundance analysis techniques such as isotope ratio mass spectrometry (IRMS) in detail.

3.2 General overview

The Research and Technology Organisation (RTO) of the North Atlantic Treaty Organisation (NATO) states in the executive summary of their Human Factors and Medicine Panel Specialists' meeting held in Boston 2003 that: "There is a need to provide more precise estimates of fluid requirements to lessen the loads that the soldier might have to carry and

reduce costs associated with water transport and re-supply" (RTO-MP-HFM-086).

TBW together with plasma osmolality (POsm) is widely regarded as the golden standard when it comes to assessing hydration changes in humans. The accuracy of the deuterium technique closely approximates values measured by desiccation. The total error of measuring TBW with tracer dilution is as low as 1%, thus allowing measurement of fairly small changes in body fluids (Ritz, 1998). Determining TBW through the diluted isotope technique remains the most reliable method currently available, producing lower coefficient of variation values than methods such a bioelectrical impedance (Schoeller et al., 1985).

Total body water (TBW), which consists of extracellular fluid (ECF) and intracellular fluid (ICF), averages approximately 60% of body mass. The TBW range has been reported from approximately 45 to 75 percent (Altman and Dittmer, 1961) of body mass. The ICF and ECF contain 65% and 35% of the TBW respectively. The ECF is further divided into the interstitial and plasma spaces. An average adult male weighing 70kg has approximately 42L of TBW, 28L of ICF and 14L of ECF, with the ECF consisting of approximately 3L of plasma and 11L of interstitial fluid. These volumes are dynamic and ensure fluid exchange with varying turnover rates between the compartments (Guyton and Hall, 2000).

The interpretation of fluctuations in body mass and changes in the state of hydration requires the knowledge of the total amount of water in an organism. The ideal substance for TBW determination should be "tracer water" which is diffusible into all the fluid compartments of the body within a short time, reaching a stable uniform equilibrium at which its concentration can be measured. It should not be selectively stored, secreted or metabolised and should be completely exchangeable with water. As pointed out by Hevesy and Hofer (1934), the stable hydrogen isotope deuterium (D_2O) would be ideally suitable for the above application. Deuterium oxide (heavy water) forms an ideal solution with water and evidence suggests that it is not affected selectively by any of the bodily secretory or metabolic processes in the dilute solutions used (Schloerb et al., 1950). Heavy water (D_2O) is almost identical to regular water (H_2O), except that a heavier, non-radioactive, form of hydrogen called deuterium replaces the hydrogen part of the water molecule.

3.3 Corrections and TBW calculations

Diluted isotope methods designed to measure TBW at the time of isotope administration are subject to systematic errors from water entering the body between the time of dosing and the sample collection (Schoeller et al. 1985). Corrections should be made for the ingestion of the isotope dose, metabolic water production and water added to the TBW pool through exchange with atmospheric moisture. The technique also requires correction for isotope exchange with non-aqueous hydrogen within the body which will lead to a slight overestimation of TBW (Schoeller et al., 1985).

Total body water was calculated using the preferred method of Halliday and Miller (1977) according to the following equation:

TBW (Kg) = ((((T*A)/a)*((Ea-Et)/(Es-Ep)))/1000) / 1.04, in which

A = amount of dose solution drunk (grams)

a = amount of dose solution diluted in T (grams)

T = amount of tap water 'a' was diluted in

Ea = enrichment of diluted dose

Et = enrichment of tap water used to dilute the dose

Ep = enrichment of baseline sample.

Es = enrichment of post dose sample

1.04 = correction factor for over estimation due to exchange with non-aqueous hydrogen

3.4 Isotope administration

The importance of the isotope dosing procedure cannot be over emphasised. All dosing procedures should be carefully controlled by the researcher and as far as possible not be left to the subjects themselves. According to Prentice (1990) the main dosing requirements are:

- Subjects may be asked to be fasting for a period of 4 hours prior to dose administration; although not compulsory. Early protocols insisted that subjects should remain fasted during the equilibration period. The theoretical concern about excessive fluid ingestion during the equilibration period is that it will slightly expand the dilution space. However, the converse can be argued if no fluid is allowed, and recent studies have often permitted moderate food and fluid intake. It is important to record these intakes to be able to correct for them if need be;

- A pre-dose sample (urine, saliva or plasma) must be collected to determine the subject's background enrichment. This also ensures that the subjects have voided prior to consuming the dose;

- An accurate body mass (corrected to nude mass) should be measured;

- Avoid any isotope dose spillage;

- The dose containers should be kept sealed for as long as possible to avoid evaporation and isotopic fractionation;

- The exact quantity of dose delivered must be obtained by weighing the dose container before and after administration (accuracy of ± 0.2% or better is desirable);

- A sample of the dose should be reserved for dilution and analysis; and

- Collection and analysis of all urine voided during the equilibration period should be performed in order to correct the estimate of dose given by subtracting the dose lost through voiding. These corrections are usually of minor importance, and even omitted in

many studies, but it remains prudent to collect the information for correction purposes if need be.

3.5 Choice of physiological fluid as sample medium

Isotopic enrichments can be determined in any physiological fluid (plasma, urine and saliva) provided that the same fluid is sampled throughout the duration of the study. Saliva was chosen for the studies performed due to the ease and non-invasive nature of its collection, as well as the fact that saliva has been proven as a valid and convenient sampling medium for determining TBW through the diluted isotope technique (Halliday and Miller 1977; Schoeller et al. 1985; Wong et al. 1988). Furthermore it has been documented that enrichments of deuterium oxide in saliva and plasma samples were identical and reached a 2 hour plateau after administration of an oral dose of the tracer. However, care should be taken when sampling saliva as not to collect samples that could have been contaminated by the intake of food or fluids prior to the collection of the sample.

3.6 Collection and storage of samples

Collection of saliva could be performed by providing cotton wool to be moistened in the mouth and then the saliva be expressed into aliquots via a syringe. This is performed by taking the moistened cotton wool with tweezers and placing into the syringe by removing the plunger. The plunger is then replaced and depressed in order to express the saliva from the cotton wool into the aliquots. All sampling and storage procedures should observe the following rules to avoid isotopic fractionation:

- The sample should only be exposed to the atmosphere for the minimal possible time;

- Containers should be absolutely airtight;

- There should be a minimum of air-space above the sample inside the container to minimise the possibility of isotopic exchange with any trapped atmospheric moisture;

- Although some mass spectrometer procedures only require micro-litre samples it is prudent to collect several millilitres of urine and at least 0.5 ml of saliva in order to minimise chances of significant fractionation during pre-analytical manipulations; and

- Samples can be stored indefinitely and should preferably be frozen although this is not an absolute requirement under difficult field conditions. There is no objection to samples being frozen, defrosted during transit and then refrozen (Schoeller, 1988).

3.7   Summary of TBW method as applied during studies presented in chapters 4, 5 and 6

Prior to the exercise intervention each subject emptied his/her bladder and provided a saliva sample for analysis of deuterium abundance.

Subjects were weighed wearing only their underclothing.

Deuterium oxide (99%) was used to prepare a 4% (weight to weight) solution with water. This solution was then used to prepare the individual deuterium oxide doses according to individual body mass (± 0.05 g/kg body mass).

Prior to dose consumption subjects were requested to shake the dose bottle to ensure proper mixing of the content. Appropriate weighing of the dose bottle (to the nearest 0.1g) was performed in order to determine the exact dose consumed by each participant. Three aliquots (2 ml each) of the pre-prepared dose mixture were frozen and stored to be analysed with the subsequent samples.

After a two hour equilibration period (Bunt et al. 1989; Chumlea et al. 1999; Culebras et al. 1977; Davies et al. 2001; Ellis and Wong 1998; Jankowski et al. 2004; Khaled et al. 1987; Lukaski and Johnson 1985; Schloerb et al. 1950; Shimamoto and Komiya 2003; Spanel and Smith 2005; Wong et al. 1988; Tam et al. 2009) a second saliva sample was collected in order to determine the pre-exercise TBW.

At the completion of the exercise each participant was provided with a towel to dry excess perspiration prior to re-weighing. A third saliva sample was collected and used for the determination of post-exercise deuterium abundance. The participants then received a post-exercise deuterium oxide dose followed by another two hour equilibration period. Urine voided during equilibration periods were recorded for correction of isotope loss. No food or fluids were allowed during either the pre- or post-exercise equilibration periods. A final saliva sample was collected and body mass measurement performed in order to calculate post-exercise TBW. Care was taken not to collect saliva samples for at least 45 minutes after any food or fluid was consumed.

All samples were pipetted into a 2ml cryo-eppendorf tube. Care was taken to fill the tubes to their maximal capacity in order to limit the possibility of evaporation and fractionation during storage. Samples were frozen and all efforts were made to keep the samples frozen until shipment to the laboratory for analysis. Samples were shipped in an appropriate container including "ice-bricks" to keep the samples frozen and cool for as long as possible.

3.8 Deuterium abundance analysis

All deuterium abundance analysis was performed by Iso Analytical Limited, a commercial laboratory specialising in stable isotope analysis for research purposes. This laboratory is based in the United Kingdom. An abstract from a laboratory report is presented below to provide an overview of their methods.

Samples were measured in duplicate with all results given and presented in per mil (‰) notation. Samples were pipetted into septum sealed containers (in duplicate where possible). Platinum (5%) on Alumina catalyst, in insert vials, was then added to the containers. The containers were sealed and the headspace flushed with pure hydrogen. Reference waters (including a quality control standard) were prepared in the same manner. Once all containers were flushed, they were left for a period of three days to ensure complete equilibration of the water with the hydrogen. The samples and references were then analysed by continuous flow – isotope ratio mass spectrometry using a Europa Scientific ANCA-GSL and Geo 20-20 IRMS. The deuterium enrichments of the samples were calibrated against two laboratory reference waters

IA-R018 [-56.5 ‰ δ^2H vs. V-SMOW (Vienna-Standard Mean Ocean Water)] and IA-R020 (+1089.2 ‰ δ^2H vs. V-SMOW). The accuracy of the analyses was controlled by measuring laboratory standard water IA-R019 (+522.3 ‰ δ^2H vs. V-SMOW) as a check standard in each batch of samples. All of these references are traceable to the primary references V-SMOW and SLAP (Standard Light Antarctic Precipitation). An accurately weighed (to 4 decimal places) aliquot of the dose sample was diluted to 100 mL with tap water in a volumetric flask. The weight of the dose and results of the isotope analysis of the diluted dose and the tap water were supplied with each report (Iso Analytical).

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Chapter 4

Ad libitum fluid replacement maintains total body water, plasma osmolality and serum sodium concentrations in military personnel during a 4 hour route march

Article:

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4.1 Introduction

Soldiers are expected to carry heavy loads to ensure mission success. Every kilogram added to these loads increases the physiological burden. Concerted efforts are being made to minimise the soldier's load by optimising every component contributing to this load. The Research and Technology Organisation (RTO) of the North Atlantic Treaty Organization (NATO) reports instances in which United States soldiers deployed in Afghanistan carried in excess of their body mass in mountainous terrain at altitudes approaching 3000 m (32).

Water carriage contributes significantly to the soldier's load. For example, during deployment in Afghanistan, United States soldiers often carried water supplies for missions lasting between 1-3 days representing 9-10 kg or in excess of 30% of their fighting load (32). One of the most hotly debated topics in both military medicine (12) and the exercise sciences (33) is the volume of water that persons exercising in hot, arid environments need to ingest to optimise their performance and maintain their health.

In the early 1990s new drinking guidelines were adopted by the U.S. military which encouraged high rates of fluid ingestion. The goal of these new guidelines was to optimise performance and to reduce the risk of "heat injury". Adoption of these drinking guidelines led to an increased number of cases of exercise-associated hyponatremia (EAH) in the US military (11). The incidence of EAH fell rapidly in the US Army (6) and elsewhere (39) with the adoption of more conservative drinking guidelines (26) which specifically mandated against the over-

consumption of fluids, either water or a sports drink, during exercise (29). These guidelines superseded the 1996 American College of Sports Medicine (ACSM) guidelines which advocated that athletes should drink "as much as tolerable" during exercise (8). The modern emphasis is now on individualised drinking behaviours, the goal of which is to limit body water losses to <2% of body mass during exercise (34).

This altered emphasis has provided the opportunity to determine the optimal rates of fluid ingestion by military personnel. In turn, this could reduce the mass in the form of water, soldiers might need to carry on military missions. Accordingly we posed the following questions: What are the rates of fluid ingestion freely chosen by soldiers during a 4 hour route march? Are these freely chosen (*ad libitum*) rates of fluid ingestion sufficient to protect against major fluid and electrolyte imbalances?

Thus the objective of the field study was to evaluate the effects of *ad libitum* fluid replacement on total body water (TBW), core temperature, serum sodium concentration [Na^+] and plasma osmolality (POsm) during a simulated 4 hour route march in professional soldiers. We wished to determine whether *ad libitum* water intake during a typical military exercise is sufficient to maintain these variables within the homeostatic range. Since *ad libitum* drinking is usually 30-50% of the volume ingested when drinking "as much as tolerable" and substantially less than the 1000-1800 ml/hr originally proposed for US military personnel in the 1990s (25), such a drinking regime could produce significant mass savings for military personnel.

4.2　　　　Methods

Subject Selection

Ethical clearance for this study was obtained from 1 Military Hospital Research Ethics Committee within the South African Military Health Services (SAMHS) of the South African National Defence Force (SANDF). Twenty Operational Emergency Care Practitioners (OECPs) were identified and invited to volunteer for this study. All were experienced and conditioned to route marches with payloads of up to 35 kg, medically fit to participate in the study and without any musculoskeletal injuries. Subjects were told that they could terminate their participation at any stage without any consequences to their careers. All were required voluntarily to sign an informed consent form before they were accepted for participation in the study. The subjects were asked to provide basic demographic information for record purposes.

Exercise intervention

The route march was of 16.4 km, comprising four laps of 4.1 km each. Subjects were asked to pace themselves with a GPS at an average speed of 5 km/h. Each participant carried a minimum mass of 17 kg plus 2 litres of water. All backpacks were packed in a similar configuration and weighed prior to the start of the exercise. Water was available for replenishment if required at the start of each lap. Soldiers were instructed to drink *ad libitum* during the march. Their core body temperatures were measured at one minute intervals with a CorTemp ^{TM}2000 ambulatory remote sensing system (HQ Inc, USA). The wet bulb globe temperature (WBGT) index, relative humidity and wind speed were

monitored for the duration of the exercise (Davis Health Environmental Monitor and Questemp, Quest Technologies, South Africa).

Hydration markers

Prior to the exercise intervention each subject emptied his/her bladder and provided a saliva sample to be analysed for background deuterium enrichment. Due to ease and non-invasive nature of collection, saliva has been proven as a valid sampling medium for determining TBW through the diluted isotopes technique (14,35,41). Furthermore it has been documented that enrichments of deuterium oxide in saliva and plasma samples were identical and reached a 2 hour plateau after administration of an oral dose of the tracer. Determining TBW through the diluted isotope technique remains the most reliable method currently available, producing lower coefficient of variation values than methods such a bioelectrical impedance (35). A pre-exercise blood sample (5 ml) was collected from the antecubital vein to determine serum [Na^+] and plasma osmolality. The samples were collected with the subject in a seated position after being seated for 45 minutes. Subjects were weighed wearing only their underpants (to the nearest 0.1 kg). Deuterium oxide doses (± 0.05 g/kg body mass) were pre-mixed from 99% deuterium oxide. Appropriate weighing of the dose bottle (to the nearest 0.1 g) was performed in order to determine the exact dose consumed. After a two hour equilibration period (4,9,17,23,36,38,40,41) a second saliva sample was collected in order to determine the pre-exercise total body water (TBW). At the completion of the exercise each subject was provided with towels to dry excess perspiration prior to re-weighing. A third saliva sample was collected and used for the

determination of post-exercise deuterium abundance. The subjects then received their post-exercise deuterium dose before commencing a two hour equilibration period. Urine voided during this period was collected and analysed to correct for isotope loss. A post-exercise blood sample was collected after 45 minutes rest following completion of the march. This period ensured that metabolic rates were closer to that of the pre-exercise level and allows for the majority of fluid loss through post-exercise sweating to have ceased. This period also allowed for the return of exercise-induced plasma volume shifts to pre-exercise levels. No food or fluids were allowed during this 2 hour period. Samples were drawn from subjects while seated as for the pre-exercise sample. A final saliva sample was collected and body mass re-measured in order to calculate post-exercise TBW. Total body water (TBW), which consists of extracellular fluid (ECF) and intracellular fluid (ICF), averages approximately 60% of body mass. However due to the influence of body composition, specifically individual variances in fat free mass, the range has been reported from approximately 45 to 75 percent (35) of TBW.

Total body water (kg) was calculated using the preferred method of Halliday and Miller (14) according to the following equation:

$$TBW (Kg) = (((T*A)/a)*((Ea-Et)/(Es-Ep)))/1000) / 1.04, \text{ in which}$$

A = amount of dose solution drunk (grams)

a = amount of dose solution diluted in T (grams)

T = amount of tap water into which 'a' was diluted

Ea = enrichment of diluted dose

Et = enrichment of tap water used to dilute the dose

Ep = enrichment of baseline sample.

Es = enrichment of post dose sample

1.04 = correction factor for over estimation due to exchange with non-aqueous hydrogen

Corrections were made for the ingestion of the isotope dose, metabolic water production and water added to the TBW pool through exchange with atmospheric moisture according to the methods of Schoeller et al. (35). Since most of the correction factors are depended on metabolic rate, additional corrections were made for the post-exercise equilibration period during which an increased metabolic rate (excess post exercise oxygen consumption, EPOC), although marginal (13,3,21) would increase the over-estimation through increased metabolic water production.

Statistical analyses

In order to determine which statistical test would be most suited for the comparisons of pre- and post-exercise values, the differences (paired differences) between the pre- and post-exercise results were calculated. The distributions (in the form of histograms) of paired differences of all the results were plotted with the number of classes as calculated according to the Rule of Sturge. The normality of this distribution was tested by means of the Shapiro-Wilks' W test. The statistical Rule of Sturge states that the number of classes equals N x 1.4 + 1 (where: N = sample size). Student's T-tests were used to compare results where the

distribution of the paired differences was normal. Where the distribution of the paired differences was not normal, the non-parametric alternative to the student's T-test, the Wilcoxon Rank Sum Test was used to compare results. A Pearson's product moment correlation coefficient was used to determine relationships between appropriate variables. Statistical significant differences were indicated by a p-value of less than 0.05. The statistical analyses were completed using the STATISTICA© software package.

4.3   Results

Fifteen subjects volunteered for the study. Thirteen of these subjects were male and two were female. On average the subjects carried a pay load mass of 20.7 kg each. The mean WBGT during the route march was 24.5 °C (21.8-29.3°C). The mean relative humidity was 57.1% (51-65%) while the mean wind speed was 0.99 m/s^{-1} (0-2.2 m/s^{-1}).

Body mass loss, fluid intake and sweat rates

On average the group lost 1.0 kg during the exercise with a range of 0.0 kg to 1.8 kg (Table 1). The group consumed on average 383 ml/hr during the exercise. The subject with the largest intake consumed 665 ml/hr while the subject with the smallest intake consumed only 153 ml/hr (Table 1). The mean sweat rate was 626 ml/hr during the march (Table 1).

Plasma osmolality (POsm) and serum [Na$^+$]

Changes in POsm and serum [Na$^+$] values pre- and post-exercise are also listed in Table 1. Neither POsm nor serum [Na$^+$] changed during exercise.

Core temperature measurements

The mean core temperature of the subjects during the exercise was 37.6°C; the highest individual core temperature was 39.4°C. There was no relationship between the peak body core temperature reached during exercise and the change in body mass ($p > 0.05$; $r = 0.10$).

Table 1: Body mass changes, water intake, sweat rates, plasma osmolality and serum [Na$^+$] changes during exercise

|	Minimum	Mean (±SD)	Maximum
Body mass (pre-exercise) [kg]	59.1	75.5 (12.3)	105.7
Body mass (post-exercise) [kg]	58.5	74.5 (12.3)	104.9
Body mass loss [kg]	0.0	-1.0 (0.5)	-1.8
Body mass loss [%]	-2.6	-1.4 (0.7)	0.0
Total water intake [ml]	610	1530 (601)	2659
Water intake [ml/hr]	153	383 (150)	665
Sweat rate [ml/hr]	420	626 (122)	815
POsm (pre-exercise) [mosm/kg]	280.0	286.8 (4.2)	294.0
POsm (post-exercise) [mosm/kg]	280.0	286.9 (4.5)	295.0
POsm [% change]	-3.1	0.3 (2.1)	2.4
[Na$^+$] (pre-exercise) [mmol/kg]	136.0	139.7 (1.5)	141.0
[Na$^+$] (post-exercise) [mmol/kg]	136.0	139.3 (1.8)	141.0
[Na$^+$] [% change]	-2.9	-0.3 (2.1)	1.4

Total body water measurements

Table 2 presents the pre- and post-exercise TBW results of the subjects. TBW fell insignificantly (~500 ml) (0.6% body mass) during exercise. Figure 1 shows that the change in body mass was unrelated to the change in TBW (r = -0.49). Note that a zero change in body mass was associated with a ~ 400 g increase in TBW. There was a non-significant relationship (r = -0.42) between post-exercise serum [Na$^+$] and total % body mass change during exercise (Figure 2) and a significant relationship (r =-0.59) between post-exercise serum [Na$^+$] and post-exercise TBW expressed as a % of body mass (BM) (Figure 3).

Table 2: TBW changes during exercise

|	Minimum	Mean (±SD)	Maximum
Pre-exercise TBW [kg]	25.85	40.92 (6.7)	48.29
Post-exercise TBW [kg]	25.46	40.39 (6.4)	47.72
Pre-exercise TBW [% body mass]	42.95	56.36 (7.5)	66.08
Post-exercise TBW [% body mass]	43.58	56.42 (7.4)	66.97
TBW change during exercise [kg]	-1.74	-0.52 (0.83)	1.04
TBW change during exercise [% body mass]	-1.55	0.6 (1.07)	2.18
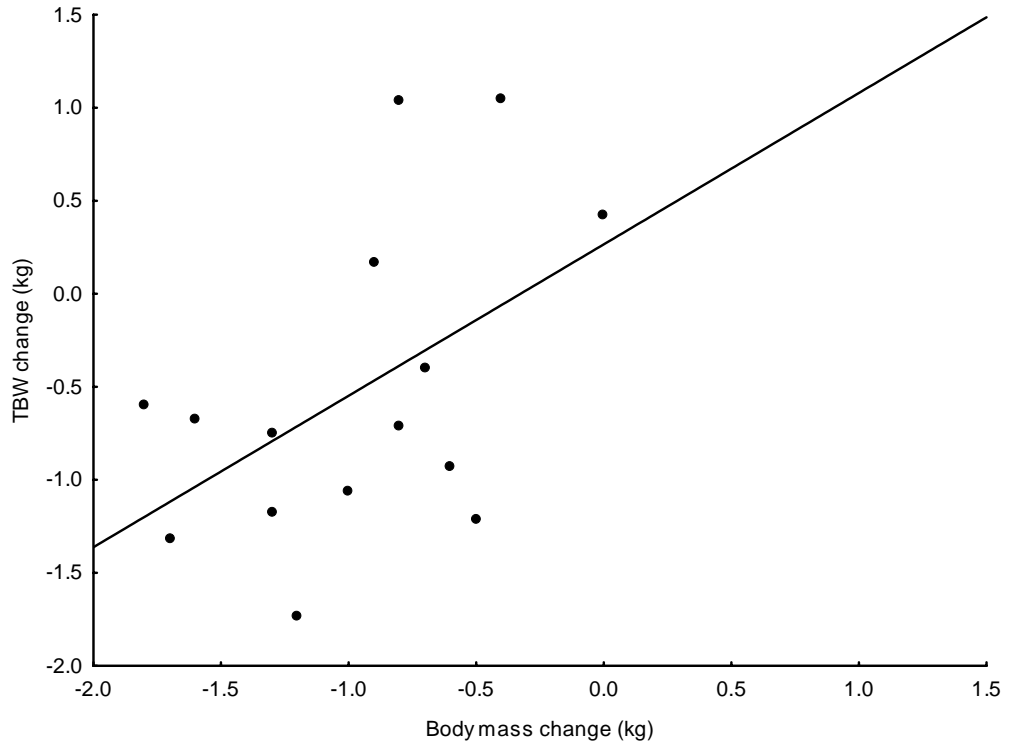
Figure 1: The relationship between changes in body mass and TBW

(p>0.05, r = -0.49)
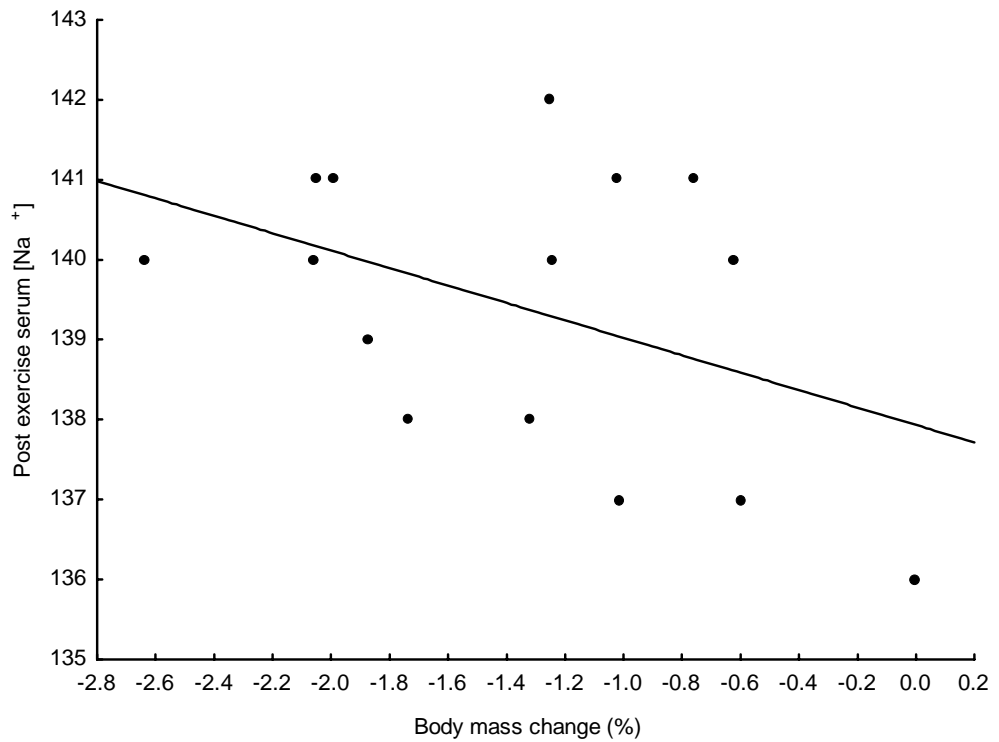
Figure 2: Relationship between post-exercise serum [Na$^+$] and body

mass change during exercise (p>0.05, r = -0.42)
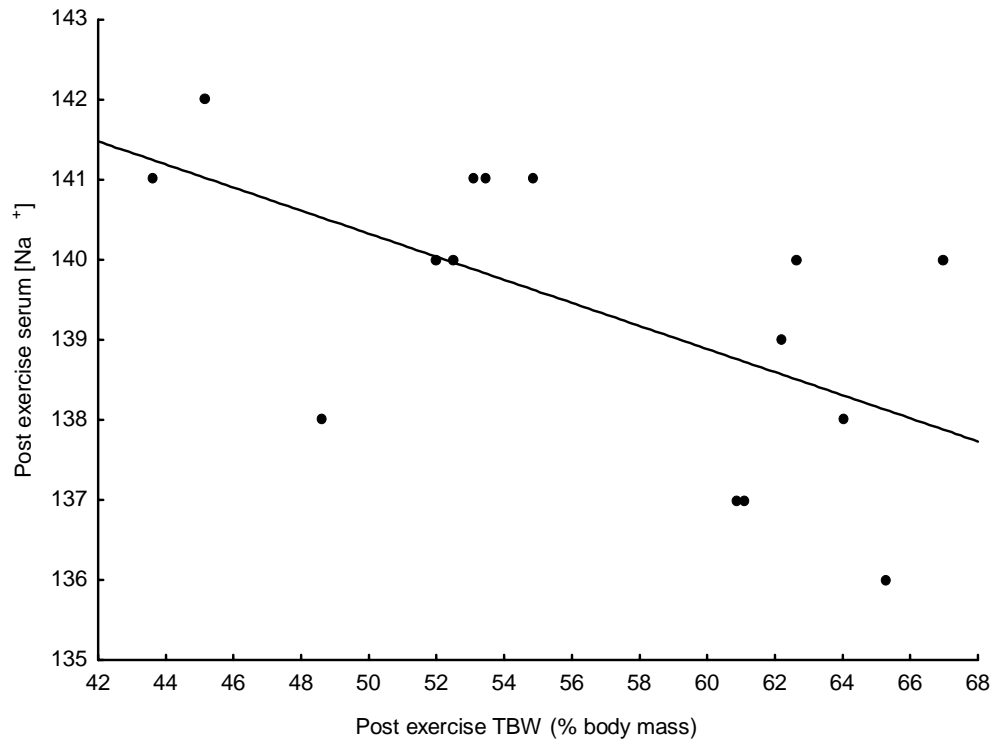
Figure 3: Relationship between post-exercise serum [Na$^+$] and post exercise % TBW ($p<0.05$, $r = -0.59$)

4.4   Discussion

The first finding of the study was that soldiers participating in a 16 km route march while carrying packs of 20.7 kg and wearing standard issue battle dress maintained safe body temperatures (less than 39.5°C) and regulated their serum [Na$^+$] and plasma osmolality within the normal range while drinking "*ad libitum*" at a mean rate of 383 ml/hr even though this drinking rate replaced only 61% of their measured hourly body mass loss (626 ml/hr). As a result these soldiers showed a mean body mass loss of 1.01 kg (1.4% BM) during the exercise. TBW fell insignificantly by a mean of 526 g during exercise. These findings suggest a number of important conclusions.

The first conclusion is that changes in body mass did not accurately predict changes in TBW in these soldiers (Figure 1). Similar findings were reported in soldiers performing a 194 km unsupported desert march during which their mean hourly fluid intakes of 458 ml were adequate to maintain thermoregulation (mean core temperature of 38.1°C) while producing a 300 g increase in TBW despite a body mass loss of 3.3 kg at the end of exercise (28).

In contrast, Baker et al. (1) have recently reported that body mass loss accurately predicts TBW changes during 2 hrs of exercise. However their methodologies differed significantly from those reported here and there are other uncertainties regarding the manner in which the data were analysed. Thus the authors designed their experiment so that 4 different levels of body mass loss would be produced. They then analysed the total data as if all came from a single experiment and not from four different experiments. The differences in results are most probably further confounded by the unusual manner in which they used different biological samples (urine or serum) to measure changes in TBW in the same individuals and which suggests that this conclusion may require revision.

The debate of whether the change in body mass (in kg) during exercise can be used as a 1:1 predictor of the change (in litres) in TBW is crucially important since it raises the question of whether or not there is a body fluid reserve of perhaps up to 2 litres that may not require replacement in order to insure that whole body fluid homeostasis is maintained during exercise. Indeed this finding is compatible with the theory first proposed

by Ladell during and after the Second World War (18,19,20). In particular Ladell (18,19,20) observed, as did we, that body mass losses of 2 kg are possible before any urine effects become visible. If this fluid volume exists in the gut it would explain why some believe that body mass losses of up to at least 3% may not carry any physiological penalty during prolonged exercise (19,24,27,37). Indeed the new American College of Sports Medicine Position Stand (34) appear to support this interpretation.

Our second conclusion was that the serum [Na$^+$] was maintained by *ad libitum* drinking. This is to be expected since drinking behaviour is determined by changes in plasma osmolality so that drinking according to the dictates of thirst would be expected to produce minimal changes in plasma osmolality and the serum [Na$^+$] (15). In contrast drinking to stay "ahead of thirst" by "drinking as much as tolerable", (8,2) must cause serum [Na$^+$] to fall (30,32) if renal free water clearance is insufficient to prevent an increase in TBW. This occurs when arginine vasopressin (ADH) secretion is not appropriately suppressed by an increasing plasma osmolality (31).

In contrast our data show that an increase in TBW produced a fall in serum [Na$^+$] whereas a fall in TBW produced a rise in serum [Na$^+$]. These data are compatible with our findings that an increase in body mass (and hence TBW) is the major determinant of exercise-associated hyponatremia (EAH) (30,31,39).

Thus we conclude that the *ad libitum* intake of water during a 16 km route march was sufficient to maintain serum $[Na^+]$. This is compatible with the finding that sodium ingestion is not required to maintain serum $[Na^+]$ during exercise (16). Rather it is the inappropriate regulation of the TBW that determines the extent to which the serum $[Na^+]$ falls during prolonged exercise (30).

Finally we show that although the core body temperature of all subjects rose steadily from the start of the exercise, none exceeded 39.5°C at the end of exercise. These values are substantially lower than those measured in athletes competing in a 21 km race under comparable environmental conditions (mean WBGT of 26.5 °C), some of whom reached values > 40.5°C without developing symptoms (5). Similar high values have recently been reported in athletes running 8 km without any limit to their performance in slightly warmer environmental temperatures (mean WBGT of 27 °C) (10).

Thus the relatively low core body temperatures measured in our subjects indicate that none was under extreme physiological stress or suffering from excessive thermal strain. Accordingly we conclude that their *ad libitum* fluid intake was adequate to maintain a safe thermoregulation during the march for the particular environmental conditions. A number of previous studies indicate that *ad libitum* fluid replacement strategies in which between 60-70% of sweat losses are replaced, are effective in maintaining thermoregulation in athletes despite body mass losses of up to 3% (7,22).

In summary the results of this study indicate that an *ad libitum* fluid replacement strategy, which replaced approximately 61% of sweat losses (383 ml/h) maintained core temperature, plasma osmolality and serum [Na$^+$] values despite a 1.4% body mass loss. This is compatible with the current ACSM Position Stand which promotes *ad libitum* drinking provided that the body mass loss during exercise does not exceed 2% (34).

However it does not exclude the possibility that greater levels of body mass loss may not be detrimental to either health or performance in those who drink to prevent the development of thirst during exercise (33). Data to address that question need to be collected.

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Chapter 5

Protection of total body water content and absence of hyperthermia despite 2% body mass loss ("voluntary dehydration") in soldiers drinking *ad libitum* during prolonged exercise in cool environmental conditions

5.1   Introduction

The extent to which humans need to replace their fluid losses during exercise remains contentious despite more than 60 years of focused research.[2,22,32,33,34,42,48,58,63]   Prior to the 1970's athletes were encouraged to avoid drinking during exercise since it was believed that fluid ingestion impaired exercise performance.[47]   This despite the evidence collected in the classic Nevada desert studies [2] which showed that soldiers benefited from ingesting fluid during 8 hour marches in the desert.   At that time drinking guidelines for the US military prescribed only the need to drink within a daily range of fluid intakes, for example from 4-12 litres/day in hot environments.[37,41]

However after the mid-1990's guidelines were introduced that encouraged soldiers to drink up to 1.8 L of fluid for each hour that they were on active duty in hot environments (WBGT>30°C).[5,30,41]   These guidelines which mirrored those of the American College of Sports Medicine (ACSM) [15] invoked the theory that only by maintaining the pre-exercise body mass could exercisers ensure that they would not suffer an impaired exercise performance or risk the development of heat illness.[5,30]

The adoption of these guidelines had two consequences.   First was an increased prevalence of exercise-associated hyponatremia (EAH) [21,51] sometimes associated with an encephalopathy (EAHE) that proved fatal in a small number of US soldiers [56] and marathon runners.[49] Subsequently those guidelines were revised with the result that the incidence of EAH and EAHE in the US military has fallen substantially

[21] as it has also amongst marathon runners and other endurance athletes (Noakes TD, 2010). Second, these guidelines required soldiers to carry more water which led to an increased load while on active duty.[44,54]

While it is now accepted that exercisers should not be encouraged to drink "as much as tolerable" there is still no consensus of the optimum rate of fluid ingestion during exercise. The recently modified ACSM guidelines advise that exercisers should drink sufficiently to ensure that their body mass loss during exercise is less than 2%.[64] More recently the US Army Research Institute of Environmental Medicine (USARIEM) proposed sweat loss prediction equations ranging from ~575 g/hr to ~1092 g/hr (based on an individual with a body surface are of 1.9 m^2) for various workloads, environmental and clothing configurations in order to more accurately predict fluid replacement volumes during work and exercise.[22] Body mass losses of up to 2% are often regarded as "voluntary dehydration". This term refers to the observation that humans does not voluntarily drink as much water as believed to have been lost (using body mass losses as a measure) even when water is readily available. Some believe that voluntary dehydration occurs since the thirst mechanism is an inadequate stimulus to drinking.[2,34,58] Others [48,52,54,73] argue that drinking to the dictates of thirst is the biologically appropriate behaviour that optimizes performance and prevents heat illness regardless of the exact level of dehydration that develops during exercise.

This debate has special relevance for the military since soldiers ingesting fluid *ad libitum* will always drink less that those who are forced to drink in order to lose less than 2 % of their body mass during exercise. Many soldiers drinking *ad libitum* according to the dictates of their thirst will drink less, develop "voluntary dehydration" and will therefore need to carry less water during military operations. To distinguish between these possibilities, there is a need for field studies to establish the optimum rates at which soldiers should ingest fluid during exercise.

Accordingly the primary objective of this study was to evaluate the effect of an *ad libitum* fluid replacement strategy on selected hydration status markers to determine whether this approach could prevent "dehydration". In addition we wished to determine whether body mass loss can be used as an accurate surrogate measure of the changes in total body water during exercise. For example body mass loss during exercise that may at least theoretically not contribute to body water losses include substrate oxidation and the release of water associated with the storage of glycogen (if that water does not constitute part of the total body water measured with stable isotope tracers). Thus many scientists [39,44,59,61,71] conclude that some degree of body mass loss can be explained by losses other than water so that some degree of body mass loss (not replacing all body mass losses through fluid intake) is essential to maintain normal plasma osmolality especially during prolonged exercise. This is because the POsm and not the body mass is the homeostatically regulated variable during exercise.[26] Others have argued since the Second World War, that the body contains a 2L fluid

excess that can be lost before there are any effects of "dehydration".[32,33,34]

Thus the scientific hypothesis that we tested was that *ad libitum* fluid replacement is effective in protecting TBW despite body mass loss during prolonged exercise in cool environmental conditions since this method of drinking is driven to maintain plasma and tissue osmolality.

5.2   Materials and methods

Subject Selection

Ethical clearance for this study was obtained from the Research Ethics Committee from the South African Military Health Services (SAMHS) of the South African National Defence Force (SANDF). All soldiers taking part in an official military exercise (N = ~ 250) were eligible for the study; twenty Operational Emergency Care Practitioners (OECPs) were identified and invited to volunteer for this study. All the subjects were experienced and conditioned to route marches with payloads of up to 35 kg; they were medically fit to participate in the study and were without any musculoskeletal injuries. Subjects were told that they could terminate their participation at any stage without any consequences to their military careers. All subjects were required voluntarily to read and sign an informed consent. The subjects were asked to provide basic demographic information for record purposes. Three days prior to the route march, the sub-maximal oxygen consumption of each subject was directly measured using a MetaMax™ portable gas analyser (Cortex Biophysik, Germany) during the Harvard graded step-up test. Their predicted aerobic capacity was calculated from the sub-maximal exercise

test results and the resulting individual regression equation for heart rate versus oxygen consumption at different workloads according to the method described in ISO 8996.[28]

Exercise intervention

The exercise intervention took the form of a competitive route march of 14.6 km. Individuals competed against each other to complete the route march in the fastest time possible without running. Each individual had to carry a mass of 26.5 kg including 4 litres of water, rifle and bush hat or cap. Participants were dressed in standard issue SANDF combat dress. Water was available for additional replenishment if required at two points along the route. The subjects drank according to the dictates of their thirst (*ad libitum*) during the march. The core body temperature of the subjects was recorded at one minute intervals with a CorTemp ^{TM}2000 (HQ Inc, USA) ambulatory remote sensing system. Core temperature data were evaluated for the potential confounding effect of fluid ingestion invalidating the ingestible sensor.[37] The ambient temperature, wind speed, relative humidity, solar radiation were recorded for the duration of the experiment (WBGT Temperature Measurement System, Questtemp, Quest Technologies, South Africa).

Hydration markers

Prior to the exercise intervention each subject emptied his/her bladder and provided a urine and saliva sample for analysis for urine specific gravity (USG), urine osmolality (UOsm) and deuterium abundance (saliva). Saliva was chosen due to the ease and non-invasive nature of its collection, as well as the fact that saliva has been proven as a valid

and convenient sampling medium for determining TBW through the diluted isotope technique.[25,65,74] Furthermore it has been documented that enrichments of deuterium oxide in saliva and plasma samples were identical and reached a 2 hour plateau after administration of an oral dose of the tracer. Determining TBW through the diluted isotope technique remains the most reliable method currently available, producing lower coefficient of variation values than methods such a bioelectrical impedance.[65] Nude body mass were obtained on a scale accurate to 0.1 kg (Scale CPW 150, Adam equipment). Deuterium oxide (99%) was used to prepare a 4% (weight to weight) solution with water. This solution was then used to prepare the individual deuterium oxide doses according to individual body mass (± 0.05 g/kg body mass). Appropriate weighing of the dose bottle (to the nearest 0.1g) was performed in order to determine the exact dose consumed by each participant. After a two hour equilibration period [9,13,17,18,19,27,31,38,66,69,70,73,74], a second saliva sample was collected in order to determine the pre-exercise total body water (TBW). At the completion of the exercise each participant was provided with a towel to dry excess perspiration prior to re-weighing. A second urine sample and third saliva sample were collected and used for the determination of post-exercise USG, UOsm and deuterium abundance (saliva). The participants then received their post-exercise deuterium oxide dose followed by a two hour equilibration period. Urine voided during this period was recorded for correction of isotope loss. A final saliva sample was collected and body mass measurement performed in order to calculate post-exercise TBW.

Total body water (kg) was calculated using the preferred method of Halliday and Miller [25] according to the following equation:

TBW (Kg) = ((((T*A)/a)*((Ea-Et)/(Es-Ep)))/1000) / 1.04, in which

A = amount of dose solution drunk (grams)

a = amount of dose solution diluted in T (grams)

T = amount of tap water 'a' was diluted in

Ea = enrichment of diluted dose

Et = enrichment of tap water used to dilute the dose

Ep = enrichment of baseline sample.

Es = enrichment of post dose sample

1.04 = correction factor for over estimation due to exchange with non-aqueous hydrogen

Diluted isotope methods designed to measure TBW at the time of isotope administration are subject to systematic errors from water entering the body between the time of dosing and the sample collection.[65] Corrections were made for the ingestion of the isotope dose, metabolic water production and water added to the TBW pool through exchange with atmospheric moisture according to the methods of Schoeller et al.[65] Since most of the correction factors depend on metabolic rate, additional corrections were made for the post-exercise equilibration period during which an increased metabolic rate (excess post exercise oxygen consumption (EPOC), although marginal [8,23,35] would augment the over-estimation through increased metabolic water

production. The sweat losses of the participating soldiers were calculated according to method previously described by Rogers et al.[61] Respiratory water loss was calculated by the methods of Mitchell et al.[40] For all calculations and estimations involving respiratory exchange ratio (RER) and oxygen consumption (VO_2), we assumed that the RER averaged 0.85 and that the oxygen consumption averaged 65% of VO_{2max} throughout the exercise.[16,45,61] Even if the actual RER and VO_2 of the participants differed slightly from these assumptions, the outcomes would not have been greatly different.

Statistical analyses

In order to determine which statistical test would be most suited for the comparisons of pre- and post-exercise values, the differences (paired differences) between the pre- and post-exercise results were calculated. The distributions (in the form of histograms) of paired differences of all the results were plotted with the number of classes as calculated according to the Rule of Sturge. The normality of this distribution was tested by means of the Shapiro-Wilks' W test. The statistical Rule of Sturge states that the number of classes equals N x 1.4 + 1 (where: N = sample size). Student's T-tests were used to compare results where the distribution of the paired differences was normal. Where the distribution of the paired differences was not normal, the non-parametric alternative to the student's T-test, the Wilcoxon Rank Sum Test was used to compare results. A Pearson's product moment correlation coefficient was used to determine relationships between appropriate variables. Statistical significant differences were indicated by a p-value of less than

0.05. The statistical analyses were completed using the STATISTICA©
software package.[72]

5.3   Results

Twenty participants volunteered for the study. Sixteen of these subjects
were male, four were female. The mean stature of the male and female
participants was 1.72 ± 0.05 m and 1.58 ± 0.04 m respectively. The
mean predicted VO_{2max} of the male and female participants was 45 ±
10.3 ml/kg/min and 37.8 ± 2.3 ml/kg/min respectively. Two men failed to
provide sufficiently large saliva samples required for deuterium
abundance analysis and their data were therefore excluded from all
analyses. On average the subjects carried a pay load mass of 26.5 kg
each. The mean Wet Bulb Globe Temperature (WBGT) index value for
the duration of the exercise period was 14.1°C.

Body mass loss, fluid intake and sweat rates

Body mass was reduced significantly ($p < 0.05$) during exercise, on
average the group lost 1.3 ± 0.5 kg (Table 1). The group consumed on
average 850 ± 594 ml/hr during the march (Table 1). The mean sweat
rate was 1289 ± 530 ml/hr (Table 1). There was no significant
relationship ($p > 0.05$) between exercise time and rates of fluid intake ($r = -0.13$), changes in % body mass ($r = 0.18$) and sweat rates ($r = -0.25$).
Figure 1 indicates the total fluid intake, hourly fluid intake as well as
predicted hourly sweat rates.

Table 1: Summary of change in body mass, TBW, USG and UOsm during

exercise

	Pre-exercise mean (± SD)	Post-exercise mean (± SD)	% Change mean (± SD)
Body mass [kg]	63.7 (6.7)	62.4 (6.5)	-1.98 (0.6)
TBW [kg]	37.07 (5.8)	37.26 (6.0)	0.53 (4.0)
USG	1.023 (0.002)	1.021 (0.007)	-0.15 (0.0)
UOsm [mmol/kg]	977.6 (187.3)	924.5 (164.3)	-4.10 (14.7)
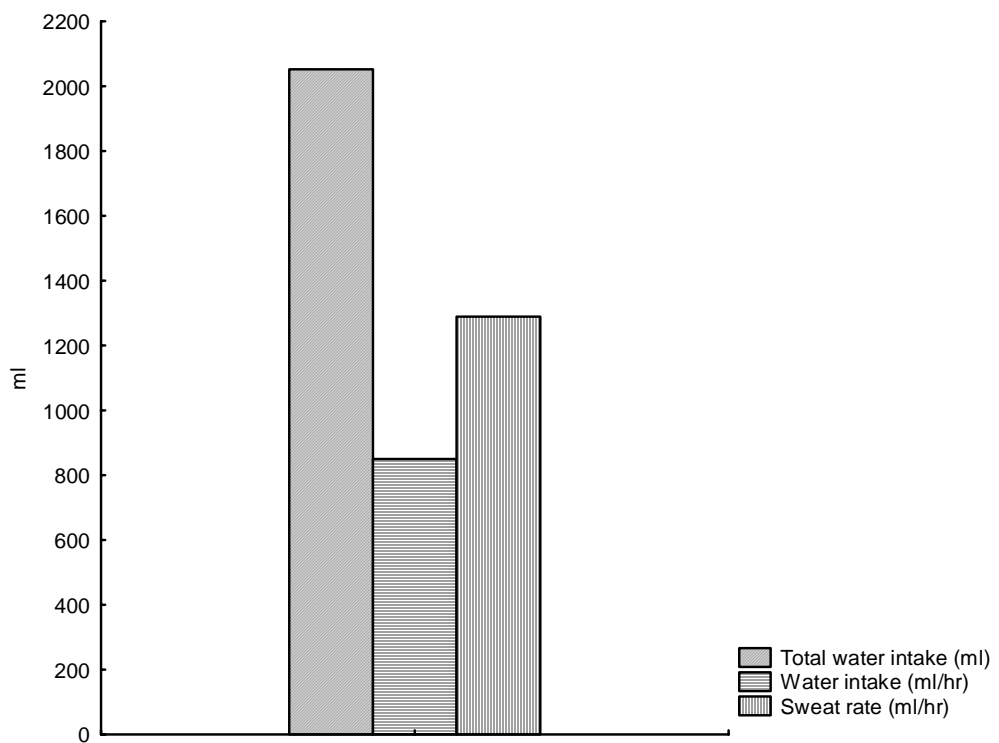
Figure 1: Mean total fluid intake, hourly fluid intake and predicted hourly

sweat losses

Urine specific gravity (USG) and urine osmolality

Neither the USG nor the UOsm changed significantly during the exercise period (Table 1). Figure 2a and 2b show that there was no significant relationship between either USG or UOsm and TBW at the end of exercise or between changes in these variables during exercise.
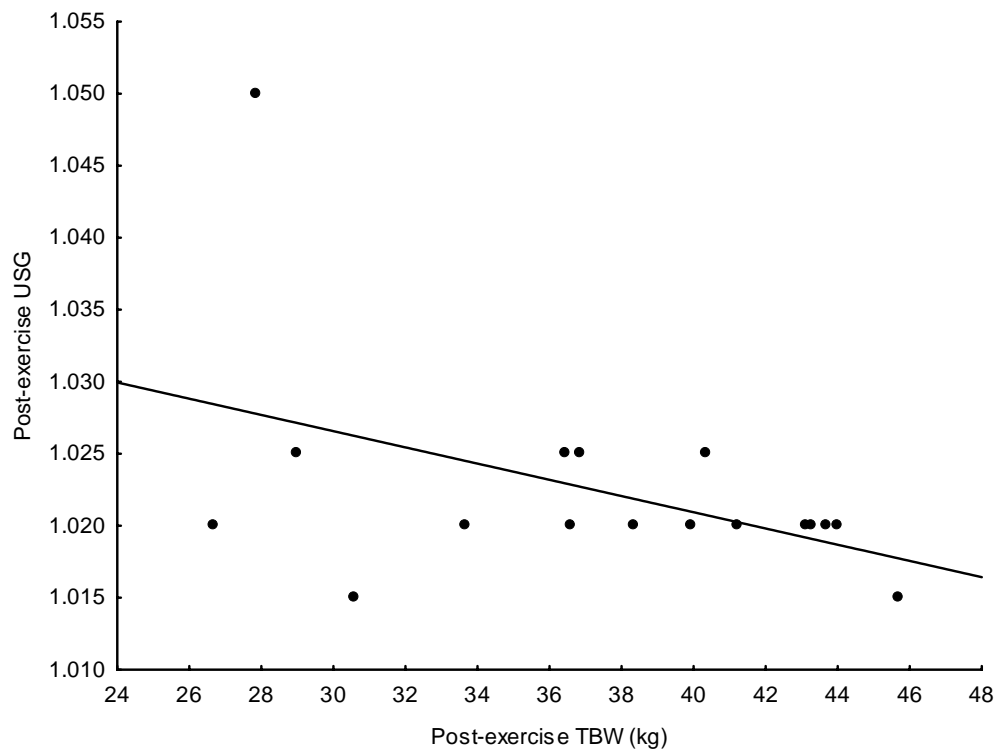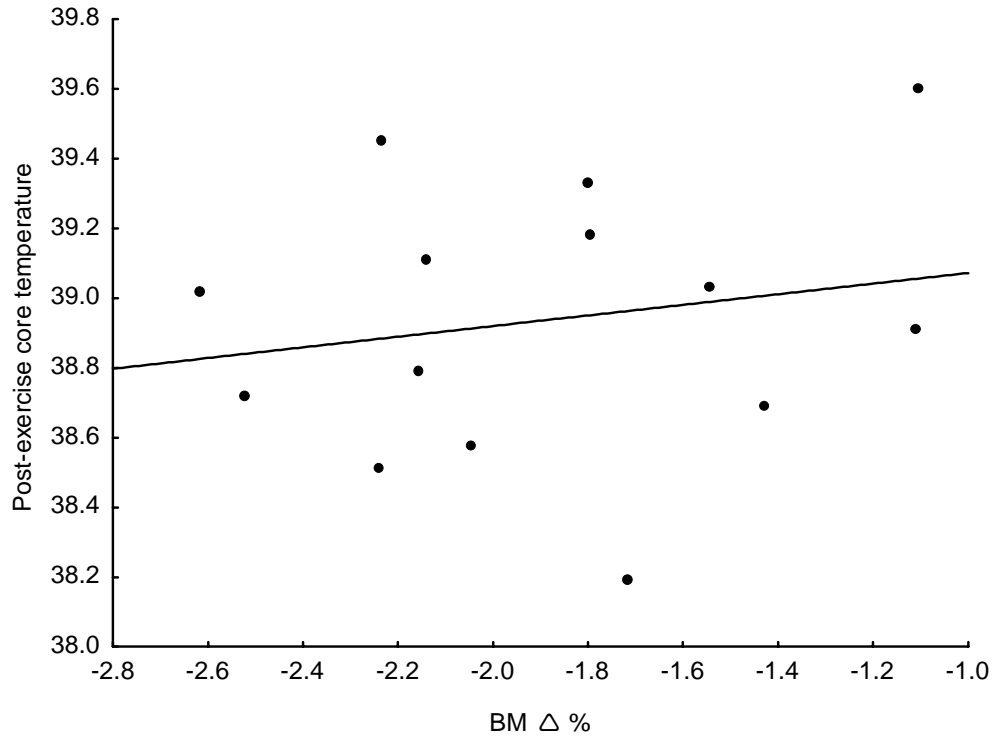

**Figure 2a:** The relationship between post-exercise TBW and USG

(p>0.05; r = 0.53)
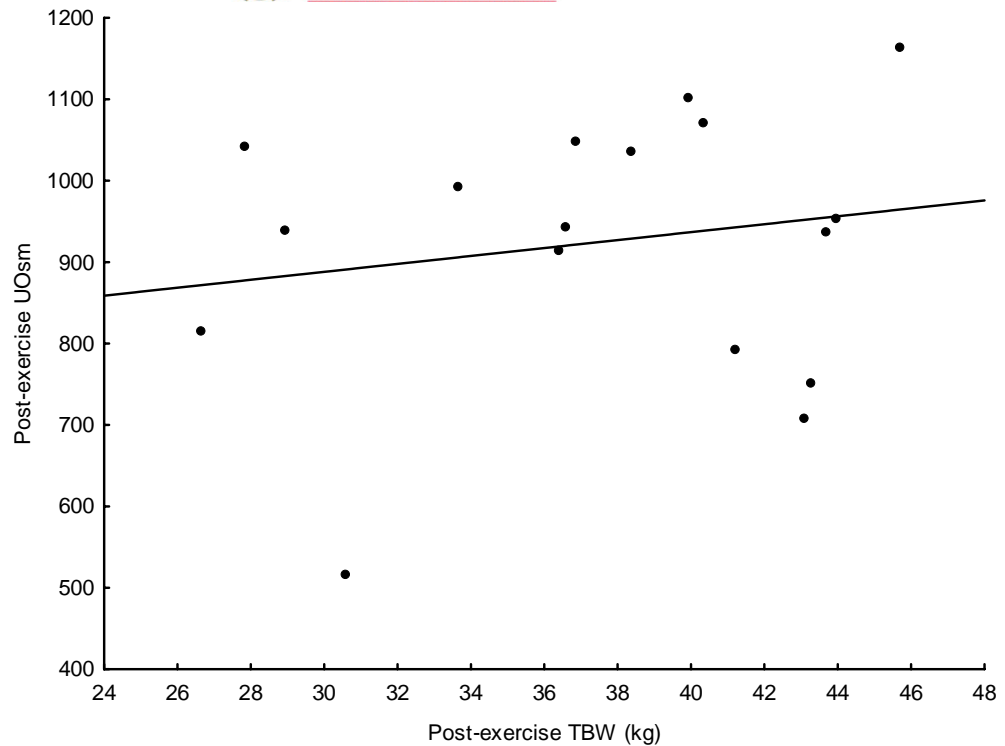
Figure 2b: The relationship between post-exercise TBW and UOsm (p>0.05; r = 0.15)

Core temperature measurements

The average peak core temperature of the soldiers during the exercise was 38.9 ± 0.3°C while the highest individual peak core temperature was 39.6°C. Figure 3a shows that there was no relationship between core temperature and the change in body mass during exercise. However there was a significant positive linear relationship between total fluid intake and core temperature during exercise (Figure 3b). This could be explained by significant relationships between finishing time (marching speed) and core temperature (Figure 3c) as well as sweat rate (Figure 3d).
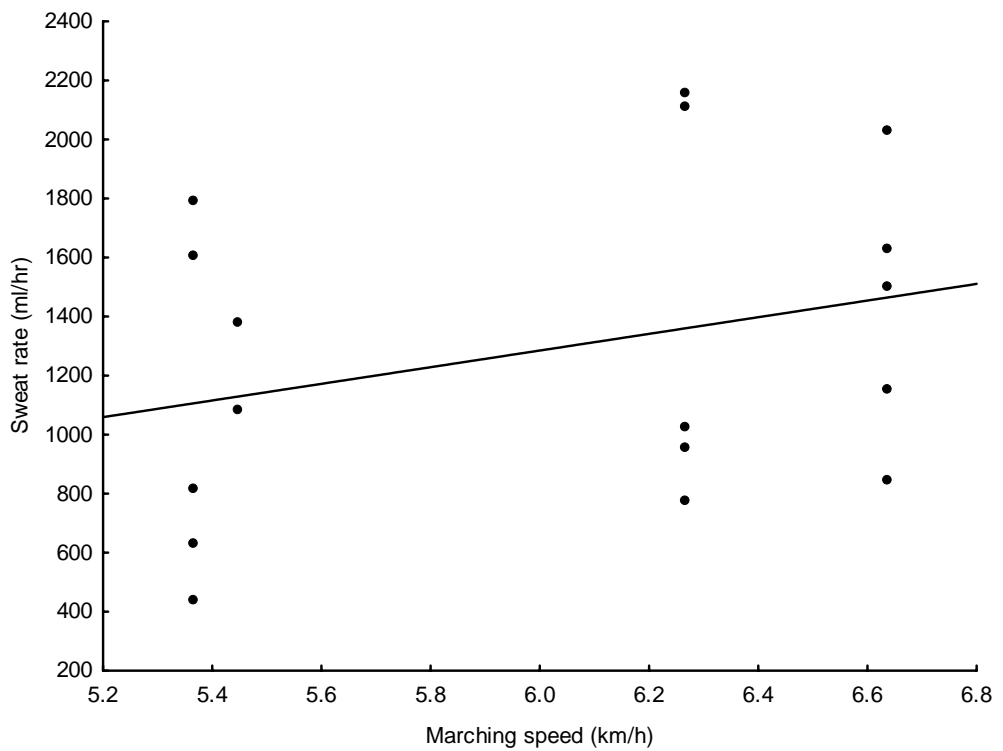
Figure 3a: The relationship between core temperature and changes in body mass (p>0.05; r = 0.18)
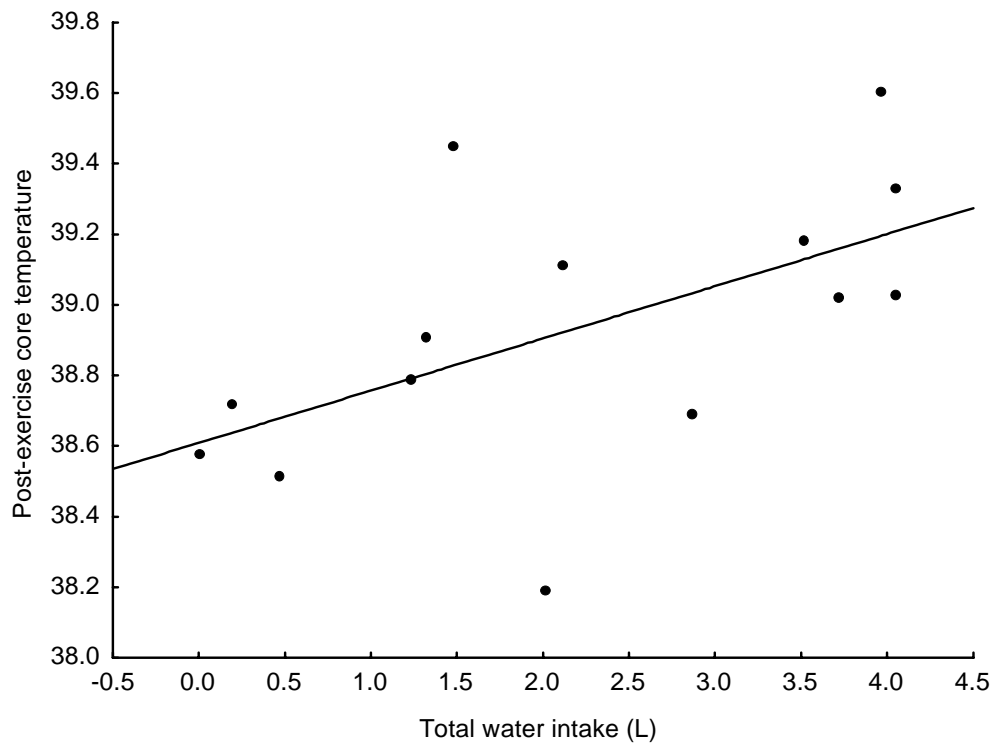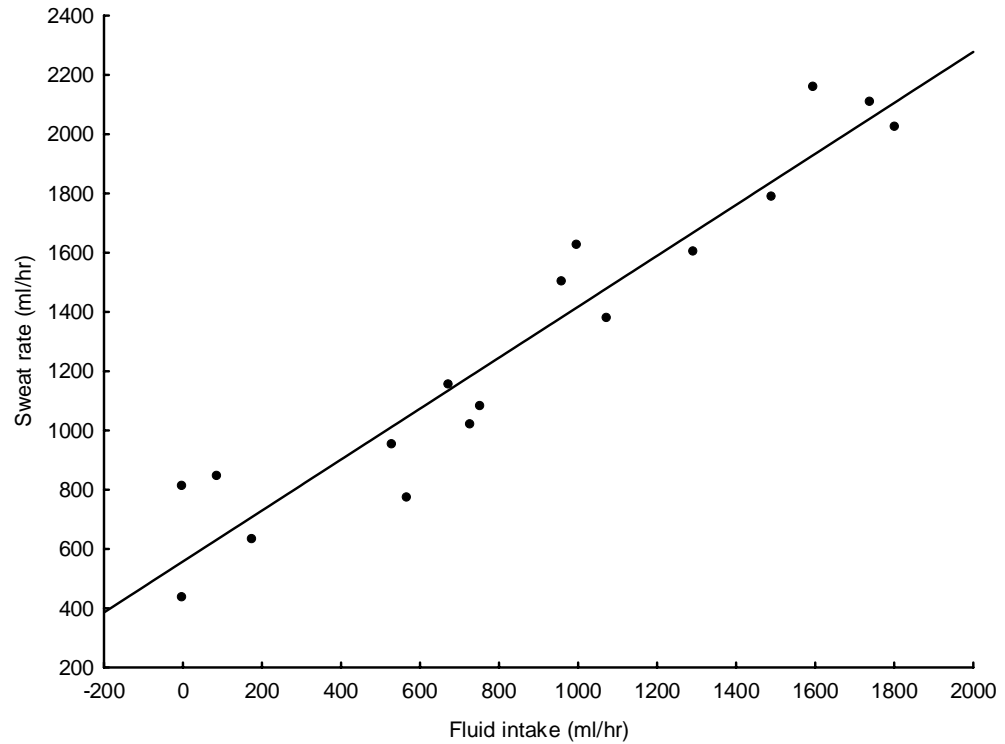

Figure 3b: The relationship between core temperature and total water intake (p<0.05; r = 0.56)
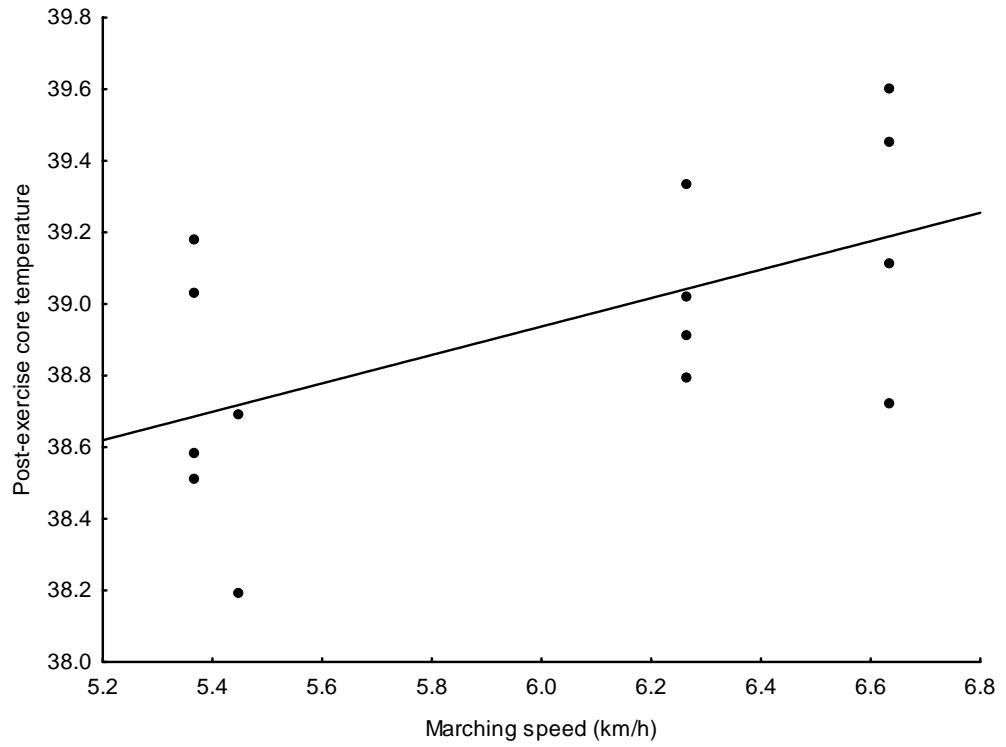
**Figure 3c:** The relationship between core temperature and marching speed ($p < 0.05$; $r = 0.58$)

Figure 3d: The relationship between marching speed and sweat rate ($p > 0.05$; $r = 0.29$)

Figure 3e: The relationship between fluid intake and sweat rate

(p<0.05; r = 0.96)

Total body water measurements

Table 1 presents the pre- and post-exercise TBW results. Mean TBW did not change despite 1.3 ± 0.5 kg body mass loss. Figures 4 shows that changes in these variables were unrelated. Of the 18 subjects that were tested, all lost between 0.7 and 2.4 kg body mass. Yet total body water increased in 9, stayed the same in 2 and was reduced in 6 subjects.
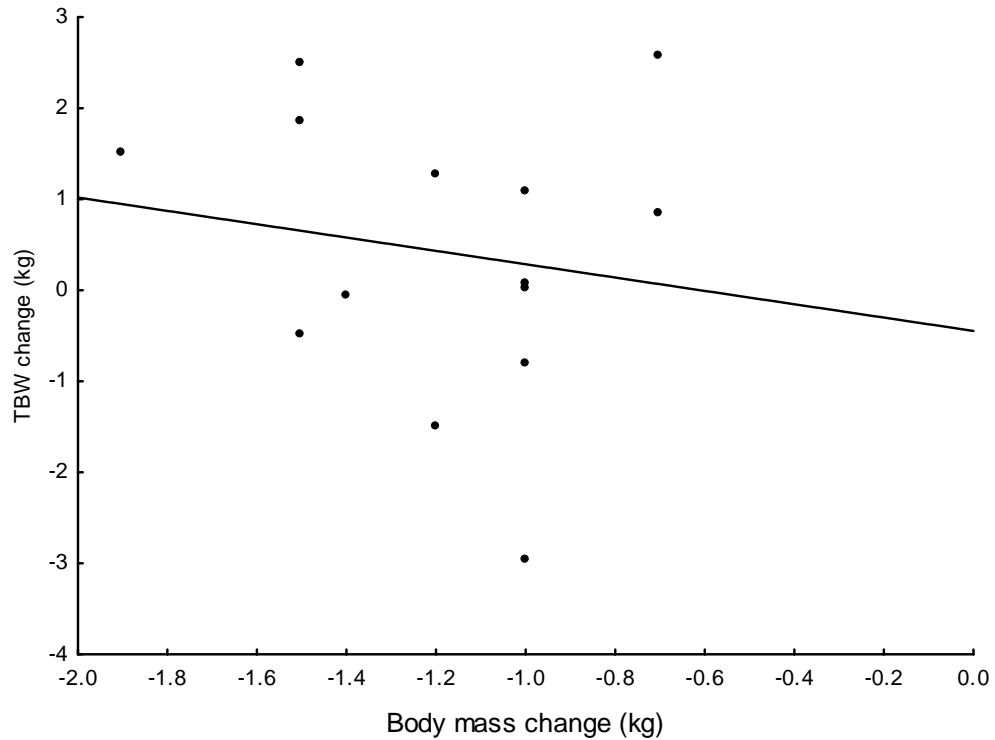
Figure 4: The relationship between body mass change and TBW change ($p > 0.05$; $r = -0.16$)

5.4 Discussion

The first important finding of this study was that there were no significant changes to TBW, USG or UOsm in any of the subjects despite a significant ($p < 0.05$) average body mass loss of 1.3 kg (1.98%). Thus although the subjects developed "voluntary dehydration" as classically described [2] they did not show a decrease in TBW and so were not "dehydrated". Instead TBW increased marginally by about 197 g during the route march. This increase in the TBW occurred even though the mean *ad libitum* fluid intake was only 850 ± 594 ml/h, substantially less than values of 1.2 L/hr originally prescribed by the 1996 ACSM guidelines. An important finding considering that this rate of intake was less than their hourly fluid loss. These findings suggest

that changes in body mass may not accurately predict changes in TBW and raise doubts about the accuracy of using body mass losses during exercise as a surrogate marker for changes in TBW at least in the range of body mass losses that we measured.[7]

Maughan et al. [39] have recently reviewed the possible reasons why water loss alone may not explain all the mass loss during exercise. First is the production of metabolic water during fuel consumption. This endogenous source of water gain involves the transformation of one form of body mass (fat and carbohydrates) to another form (H_2O) with the exhaled loss of CO_2.

A second source of water gain during exercise is the intake of exogenous water in the form of either water or the water present in food eaten during exercise. A third theoretical source is the release of water with the breakdown of muscle and liver glycogen. It has been estimated that 3-4 grams of water may be complexed with each gram of glycogen stored in the liver or muscles.[57] Since humans can store at least 450 g of glycogen [1,20], in theory at least, 1350g of water could be stored in this way.[57] This water would become available to the TBW pool even when there is a body mass loss resulting from irreversible glycogenolysis.[61] It has been calculated that an athlete who loses 2 kg of mass during a marathon race could, in fact, be dehydrated (fluid loss) by only ~ 200 g when allowance is made for the body mass loss and alternatively body water gain from these three sources.[39,50,59] Calculations based on the method of Rogers et al.[61] predict the mean rate of carbohydrate oxidation during this study to be approximately 60 ± 15 g/hr. Considering

the assumptions regarding water associated with glycogen storage and the mean duration of the route march a mean of 436 ± 113 g of water could have become available to our subjects during this march, this excludes exogenous water as well as metabolic water production.

While the presence of this fluid store remains hotly debated [7,39,53], the findings of this study indicate that the TBW was unchanged in subjects who lost an average of 1.3 kg during exercise. This was also found in our earlier study.[54] This finding is therefore compatible with the presence of an endogenous body water source that is released during exercise and which therefore "protects" the TBW despite a body mass loss during exercise.   While the source of this fluid may be uncertain [42] this does not negate the importance of our finding that up to 1.3 kg of this mass lost during exercise may not be due to this loss of water from the TBW as measured with the diluted isotope (deuterium oxide) method.   Indeed this finding is compatible with the theory first proposed by Ladell during and after the Second World War.[32,33,34] In particular Ladell [32,33,34] observed, as did we, the loss of 2kg body mass prior to any urine effects becoming noticeable.

There are a number of other findings in the literature which are compatible with this interpretation. Thus Astrand and Saltin [6] found that another indicator of hydration status, plasma volume, increased during an 85 km ski race despite an average 5.5% decrease in body mass. Colt et al. [14] found that TBW increased by 2.4% during a 16 km foot race in subjects who lost 2.3% of body mass. Similarly Pastene et al. [59] also reported that plasma volume did not fall in 42 km

marathon runners despite a significant decrease in body mass (2.0 kg). The author suggests that the production of 402 g of metabolic water and the release of 1 280 g of water stored in glycogen complexes in muscle and liver prevented a change in TBW despite this body mass loss of ~2 kg.[59] Similar findings were reported in soldiers performing a 194 km unsupported desert march during which their mean hourly fluid intakes of 458 ml maintained thermoregulation (mean core temperature of 38.1°C) while producing a 300 g increase in TBW despite a body mass loss of 3.3 kg at the end of exercise.[44]

In addition, Speedy et al. [71] showed that serum [Na$^+$] was maintained despite an average body mass loss of 2.5 kg during a 226 km Ironman triathlon. Significantly subjects in that study did not regain body mass losses until 24 hours after the race compatible with the theory that the pre-race body mass is not regained until the glycogen stores with (a theoretical) associated mass of water are fully replenished. Laursen et al. [36] also found that changes in body mass were unrelated to core temperature, plasma [Na$^+$] or urine specific gravity at completion of another 226 km Ironman triathlon. The authors concluded that body mass losses of up to 3% are well tolerated by properly trained athletes exercising in warm conditions and who show no evidence for thermoregulatory failure.

Thus all these studies show that significant loss of body mass, perhaps between 1-3%, may be incurred without the development of significant hypohydration.[39]   Indeed the most recent ACSM Position Stand acknowledges that a body mass loss of up to 2% may not incur any

physiological cost. Our data are compatible with that interpretation as are the historical studies of Ladell.[32,33,34]

However, we and others have also shown that much greater mass losses occur in successful athletes who drink *ad libitum* during prolonged exercise.[67,68,71] Indeed the study of Kao et al. [29] found that the athletes who lost the most body mass (%) during a 24 hour race ran the furthest. In that study there was a linear relationship with a negative slope between the distance run in 24 hours and the extent of body mass loss. Data on marathon runners reviewed by Cheuvront et al. [12] also found that those who lost the most mass during the race also ran the fastest.

Thus we do not exclude the possibility that body mass losses greater than 2% may also not carry adverse physiological cost in those who drink according to the dictates of their thirst during prolonged exercise.[63]

Instead we conclude that these data suggests that the validity of the term "voluntary dehydration" needs to be re-considered since it now seems clear that some body mass loss can occur in athletes drinking *ad libitum* during exercise without the decrease in TBW that is necessary for the use of the term "dehydration".

Our second important finding was that there was no relationship between % body mass loss and the peak exercise core temperature as now frequently reported.[10,46,68] The core body temperature of all

the participants rose steadily from the start of the exercise, but did not exceed 40°C let alone 42°C; which is considered the danger level for core body temperature resulting in serious health consequences.[55] Instead core temperatures were homeostatically regulated within a normal range unrelated to the degree of mass loss during exercise.

Cheuvront and Haymes [11] and Byrne et al. [10] have reported similar findings in athletes drinking *ad libitum* during exercise. Paradoxically the subject who developed the highest core body temperature (39.6°C) in this study was also the subject who consumed fluid at the highest rate (1800 ml/h), thus presenting with the lowest level of dehydration. This subject lost 0.7 kg body mass while replacing 89% of his high sweat losses (2026 ml/hr). He was also amongst the first group of finishers (Figure 3c). The finding that race winners in endurance events are usually both the hottest and the most dehydrated is frequently observed [43,60,75] yet infrequently acknowledged.

Thirdly we found no relationship between changes in TBW and urinary markers of "dehydration". Thus there were no significant correlations between either UOsm or USG and TBW changes during exercise as also reported by others.[24,62] This suggests that these markers should not be used as a measure of hydration status in athletes. This conflicts with some popular guidelines [3,4] but reflects the historical evidence.[32,33,34]

Finally, we found a relationship between the rates of fluid intake and of sweating (Figure 4e). We are unaware of other studies that have evaluated this relationship. Nor do we know whether these variables are causally related or explained by their co-dependence on a third variable, for example, the exercising metabolic rate.

Conclusion

In summary, there were a number of important findings of this study which add to the debate on the extent to which fluid and weight losses incurred during exercise need to be replaced. Until recently it was advised that full replacement of body mass losses should be achieved during exercise.[15] Currently this position has been revised so that the newest ACSM Position Stand proposes that the mass loss during exercise should not exceed 2% of the starting body mass.[64] Thus our findings could be interpreted as supportive of that proposal since we showed that subjects maintained their pre-exercise TBW despite an average body mass loss of 1.3 kg (equivalent to a 2% body mass loss) and showed no evidence for any homeostatic failure since serum [Na^+], urinary osmolality and core body temperatures did not change substantially. However this conclusion could also be an artefact of the study design in which subjects lost only ~2% body mass during the exercise intervention.

Left unanswered by our study is whether higher levels of body mass loss during more prolonged exercise, for example losses of 4-8% as reported recently in athletes competing in 160 km/ 24 hour races [29] and which losses appear to be homeostatically regulated since they

remained constant and did not increase after ~8 hours, are also associated with an unchanged TBW. Alternatively, whether large changes in body mass cause changes in TBW that is associated with deleterious physiological changes such as significant reductions in plasma volume and/or electrolyte imbalances.

Regardless, our study raises questions about the validity of the term "voluntary dehydration" that was first coined more than 60 years ago. Additional studies during more prolonged exercise in which athletes undergo greater changes in body mass are required to determine whether "voluntary dehydration" does indeed occur in those who ingest fluids *ad libitum* during more prolonged exercise.

Indeed this study invites a more thorough interrogation of the use of the term "dehydration" which should be used only when there is a proven reduction in TBW and not, as this study shows, merely a reduction in body mass during exercise.

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Chapter 6

Appropriately trained humans can safely perform vigorous, competitive self-paced exercise in extreme heat (44°C) when drinking water *ad libitum*

Article:

6.1 Introduction

The goal of the American College of Sports Medicine (ACSM) Position Stands on fluids and exercise [2,7] is to insure that humans drink adequately and do not exercise in inappropriately hot conditions. The guidelines to establish safe environmental conditions for exercise are based in part on the concept of a thermal prescriptive zone [27,40,44,10,20] in which humans exercising at an externally-regulated (fixed) work rate (exercise intensity) are only able to reach thermal equilibrium at an increasingly higher level of core temperature [27] or unable to reach such equilibrium at all. Instead, continuing to exercise at that fixed work rate produces a progressive heat accumulation leading to an elevated brain temperature causing central fatigue [52] or "heat illness" including heat stroke.

Thus Eichna et al. [10] concluded that: "At wet bulb temperatures exceeding 94°F (34.4°C), most men are incapable of sustained effort; those who work do so inefficiently and ineffectively. A high incidence of heat casualties (often severe) is to be expected…clothing probably lowers the environmental upper limits" (p.82).

Those studies have encouraged the concept that humans have a limited capacity to prevent any of these outcomes if they exercise in extremely hot conditions, particularly if they fail to ingest fluid at rates to prevent either no [1] or a minimal body mass (BM) loss (<2% BM) during exercise. Thus human athletes exercising in the heat are sometimes considered to live on the edge of thermoregulatory catastrophe.[19,20]

Yet there are isolated reports of unusual human athletic performances in extreme heat. A feature of all these examples is that the participating athletes were able freely to modify their exercising work rate intensities in response to the prevailing environmental conditions. For example Karoha Langwane, the !xo San Bushman hunter was followed as he hunted for 4-6 hours in the Kalahari Desert, South Africa in an air temperature of 40-46°C covering in excess of 30km in loose sand while he drank only about 1L of water.[12] Similarly the woman's 42km marathon footrace at the 2000 Olympic Games was won by a 40kg Japanese athlete who ran 2:23:14 in conditions reported to be 35°C with relative humidity of 55%. This performance was only 3 minutes (~2.4%) slower than her personal best time run in much cooler conditions.

As part of a series of experiments to determine the minimal fluid requirements of soldiers during route marches [37,39], we were granted the opportunity to study a group of 18 exceptionally well conditioned and heat-adapted members of the South African Special Forces. The soldiers participated in an individually-timed, competitive 25km route march in a dry bulb temperature that reached 44.3ºC (mean WBGT index of 30ºC; maximum value of 31.3ºC). The soldiers were inappropriately dressed for the activity since they wore full combat dress which covered their arms and legs. Furthermore each carried a rifle and a combat backpack (weighing 26 kg). During the march soldiers had free access to all the water they required.

While the data collection in this study is descriptive and do not address any specific hypothesis, we believe they may represent a unique testimony to the remarkable physiological capacity of some appropriately-trained humans to safely sustain high rates of energy expenditure for prolonged periods in extreme heat even when heavily burdened and inappropriately attired.

Furthermore this data is compatible with the theory that a critical determinant of the direction of human evolution was the development of a superior thermoregulatory capacity that allowed hominids to successfully perform persistence hunts of non-sweating mammals in extreme dry heat on the African savannah beginning about 2 million years ago.[26,15] They also indicate that the biological controls that likely evolved in that process appear to be sufficient to maintain whole body homeostasis in subjects who drink only according to the dictates of thirst (*ad libitum*) and who are able to modify their exercise intensities according to the prevailing environmental conditions.

6.2   Methods

Subject Selection

Ethical clearance for this study was obtained from 1 Military Hospital Research Ethics Committee within the South African Military Health Services (SAMHS) of the South African National Defence Force (SANDF). Eighteen subjects volunteered for this study. All were experienced and conditioned to route marches with payloads of up to 35 kg, medically fit to participate in the study and without any musculoskeletal injuries. Subjects were told that they could terminate

their participation at any stage without any consequences to their careers. All were required voluntarily to sign an informed consent form before they were accepted for participation in the study. The subjects were asked to provide basic demographic information for record purposes. Two days prior to the route march, the sub-maximal oxygen consumption of each subject was directly measured using a MetaMax™ portable gas analyser (Cortex Biophysik, Germany) during the Harvard graded step-up test. Their predicted aerobic capacity was calculated from the sub-maximal exercise test results and the resulting individual regression equation for heart rate versus oxygen consumption at different workloads according to the method described in ISO 8996.

Exercise intervention

During the route march of 25 km each participant carried a mass of 26 kg which included their backpack, rifle, and water supply during the march. All backpacks were packed in a similar configuration and weighed prior to the start of the exercise. Water was the only fluid allowed during the march and was available for replenishment if required at frequent intervals during the exercise. Soldiers were instructed to drink *ad libitum* during the march. Their core body temperatures were continuously measured at one minute intervals with a CorTemp ™2000 ambulatory remote sensing system (HQ Inc, USA). Core temperature data were evaluated for the potential confounding effect of fluid ingestion invalidating the ingestible sensor.[25,50] The wet bulb globe temperature (WBGT) index, temperature and relative humidity were monitored for the duration of the exercise (Davis Health

Environmental Monitor and Questemp, Quest Technologies, South Africa).

Hydration markers

Prior to the exercise intervention each subject emptied his bladder and provided a saliva sample for analysis of deuterium abundance. Saliva was chosen due to the ease and non-invasive nature of its collection, as well as the fact that saliva has been proven as a valid and convenient sampling medium for determining TBW through the diluted isotope technique.[14,45,51] Furthermore it has been documented that enrichments of deuterium oxide in saliva and plasma samples were identical and reached a 2 hour plateau after administration of an oral dose of the tracer.[74] Determining TBW through the diluted isotope technique remains the most reliable method currently available, producing lower coefficient of variation values than methods such a bioelectrical impedance.[45]

A pre-exercise blood sample (5 ml) was collected from the antecubital vein to determine serum sodium concentration [Na^+] and plasma osmolality. Samples were collected after the subjects had been seated for 45 minutes.

Subjects were weighed wearing only their underclothing (to the nearest 0.02 kg). As previously described (38), individual deuterium oxide doses (± 0.05 g/kg body mass) were pre-mixed from 99% deuterium oxide (made up as a 4% weight-to-weight solution with water). Appropriate weighing of the dose bottle (to the nearest 0.1g) was

performed in order to determine the exact dose consumed by each participant. After a two hour equilibration period [9,48] a second saliva sample was collected in order to determine the pre-exercise total body water (TBW).

The exercise intervention followed, at the completion of which each participant was provided with a towel to dry excess perspiration prior to re-weighing. A third saliva sample were collected and used for the determination of post-exercise deuterium abundance. This value was used as the new deuterium abundance baseline post-exercise. The participants then received their post-exercise deuterium oxide dose followed by a two hour equilibration period.

Participants also received a post-exercise dose of oxygen-18 in order to perform a concurrent measure of TBW with an additional tracer to ensure that the second (post-exercise) deuterium oxide dose did not overestimate the TBW.

No food or fluids were allowed during any of the two 2-hour equilibration periods. To avoid contamination of the saliva, care was also taken to ensure that no food or fluids were ingested for at least 45 minutes prior to any of the saliva sampling. Urine voided during this period was recorded for correction of isotope loss.

A post-exercise blood sample was also collected 45 minutes after completion of the march. This period ensured that metabolic rates were closer to that of the pre-exercise level and allows for the majority of fluid

loss through post-exercise sweating to have ceased. This period also allowed for the return of exercise-induced plasma volume shifts to pre-exercise levels. Samples were drawn from subjects while seated as for the pre-exercise sample.

A final saliva sample was collected and body mass measurement performed in order to calculate post-exercise TBW. The samples were analysed by continuous flow isotope ratio mass spectrometry using a Europa Scientific ANCA-GSL and Geo 20-20 isotope ratio mass spectrometer. TBW, which is comprised of extracellular fluid (ECF) and intracellular fluid (ICF), averages approximately 60% of body mass. However due to the influence of body composition, specifically individual variances in fat free mass, the range has been reported from approximately 45 to 75 percent [45] of TBW. Total body water (kg) was calculated using the preferred method of Halliday and Miller [14] according to the following equation:

TBW (Kg) = (((($T*A$)/a)*((E_a-E_t)/(E_s-E_p)))/1000) / 1.04, in which

A = amount of dose solution drunk (grams)

a = amount of dose solution diluted in T (grams)

T = amount of tap water 'a' was diluted in

E_a = enrichment of diluted dose

E_t = enrichment of tap water used to dilute the dose

E_p = enrichment of baseline sample.

E_s = enrichment of post dose sample

1.04 = correction factor for over estimation due to exchange with non-aqueous hydrogen.

Diluted isotope methods designed to measure TBW at the time of isotope administration are subject to systematic errors from water entering the body between the time of dosing and the sample collection.[45] Corrections were made for the ingestion of the isotope dose, metabolic water production and water added to the TBW pool through exchange with atmospheric moisture according to the methods of Schoeller et al.[45] Since most of the correction factors are depended on metabolic rate, additional corrections were made for the post-exercise equilibration period during which an increased metabolic rate (excess post exercise oxygen consumption (EPOC), although marginal [13,3] would augment the over-estimation through increased metabolic water production.

The sweat losses during the march were calculated according to methods previously described by Rogers et al.[42] Respiratory water loss was calculated by the methods of Mitchell et al.[29] For all calculations and estimations involving respiratory exchange ratio (RER) and oxygen consumption (VO_2), we assumed that the RER averaged 0.85 and that the oxygen consumption approximated 65% of the VO_{2max} throughout the exercise [8,34,42]. Even if the actual RER and VO_2 of the participants differed slightly from these assumptions, the outcomes would not have been greatly different.

Statistical analyses

In order to determine which statistical test would be most suited for the comparisons of pre- and post-exercise values, the differences (paired differences) between the pre- and post-exercise results were calculated. The distributions (in the form of histograms) of paired differences of all the results were plotted with the number of classes as calculated according to the Rule of Sturge. The normality of this distribution was tested by means of the Shapiro-Wilks' W test. The statistical Rule of Sturge states that the number of classes equals N x 1.4 + 1 (where: N = sample size). Student's T-tests were used to compare results where the distribution of the paired differences was normal. Where the distribution of the paired differences was not normal, the non-parametric alternative to the student's T-test, the Wilcoxon Rank Sum Test was used to compare results. A Pearson's product moment correlation coefficient was used to determine relationships between appropriate variables. Statistical significant differences were indicated by a p-value of less than 0.05. The statistical analyses were completed using the STATISTICA© software package.

6.3   Results

Eighteen male subjects with an average age of 26 years (range of 21-38 years) volunteered for the study. The subjects had an average stature of 175 cm (165-190 cm). Their mean predicted VO_{2max} was 55 ml/kg/min (40-65 ml/kg/min). On average the subjects carried a pay load mass of 26 kg each. The mean WBGT during the route march was 28.8 °C (26.9-31.3°C). The mean relative humidity was 16.8% (15-

28%) while the mean dry bulb temperature was 40.2 °C (34.9-44.3°C). The mean time to complete the route march was 4h17min with a range of 3h57min to 4h47min.

Body mass loss, fluid intake and sweat rates

On average the group lost 2.73 kg (3.8%) ($p<0.05$) during the exercise with a range of 1.5 kg to 4.7 kg (2-6%) (Table 1). The group consumed on average 1264 ml/hr during the exercise. The subject with the largest intake consumed 1782 ml/hr while the subject with the smallest intake consumed 999 ml/hr (Table 1). There was no significant relationship ($p>0.05$) between body mass loss (%) ($r = 0.57$) or amount of fluid consumed ($r = 0.10$) and peak exercise core temperature during exercise (Figures 1 and 2).

Table 1: Body mass change, water intake, sweat rates, plasma osmolality and serum [Na$^+$] during a 25km march.

	Minimum	Mean (±SD)	Maximum
Body mass (pre-exercise) [kg]	59.7	72.9 (8.0)	89.2
Body mass (post-exercise) [kg]	57.9	70.1 (8.0)	87.0
Body mass loss [kg]	-1.52	-2.73 (0.98)	-4.74
Body mass loss [%]	-2.0	-3.8 (1.4)	-6.2
Total water intake [ml]	4199	5410 (831)	7101
Water intake [ml/hr]	999	1264 (229)	1782
Sweat rates [ml/hr]	1449	1789 (267)	2191
POsm (pre-exercise) [mosm/kg]	294.0	300.6 (4.5)	312.0
POsm (post-exercise) [mosm/kg]	291.0	303.6 (5.8)	311.0
[Na$^+$] (pre-exercise) [mmol/kg]	140.0	143.3 (2.0)	147.0
[Na$^+$] (post-exercise) [mmol/kg]	140.0	144.0 (2.5)	148.0
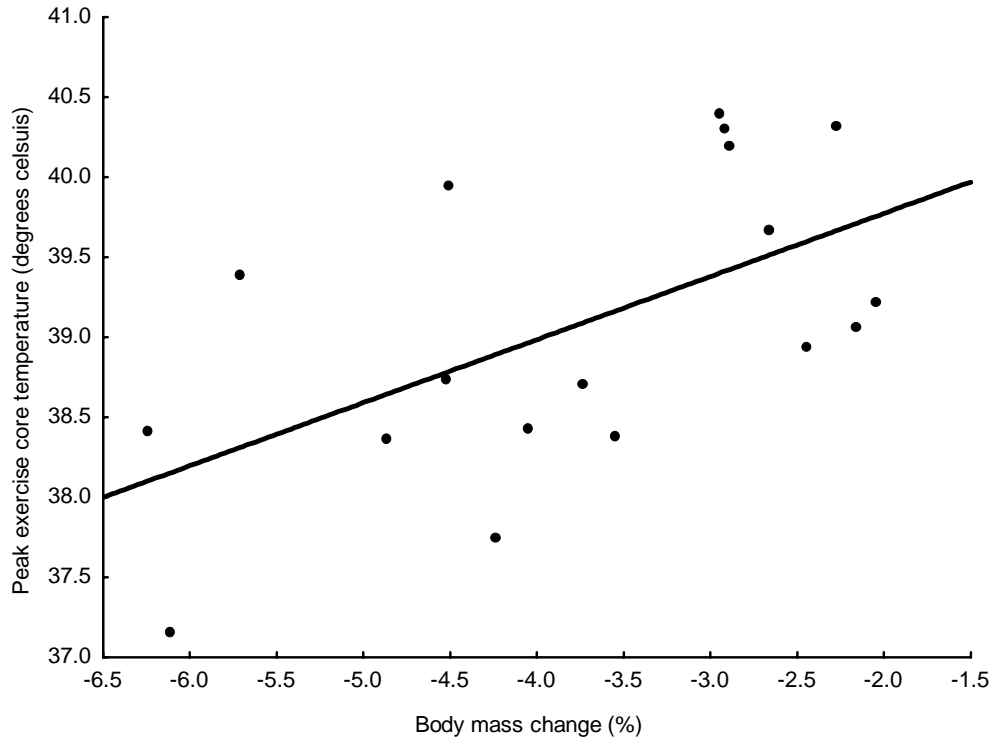
**Figure 1:** The relationship between changes in body mass and peak exercise core temperature (p>0.05, r = 0.57).
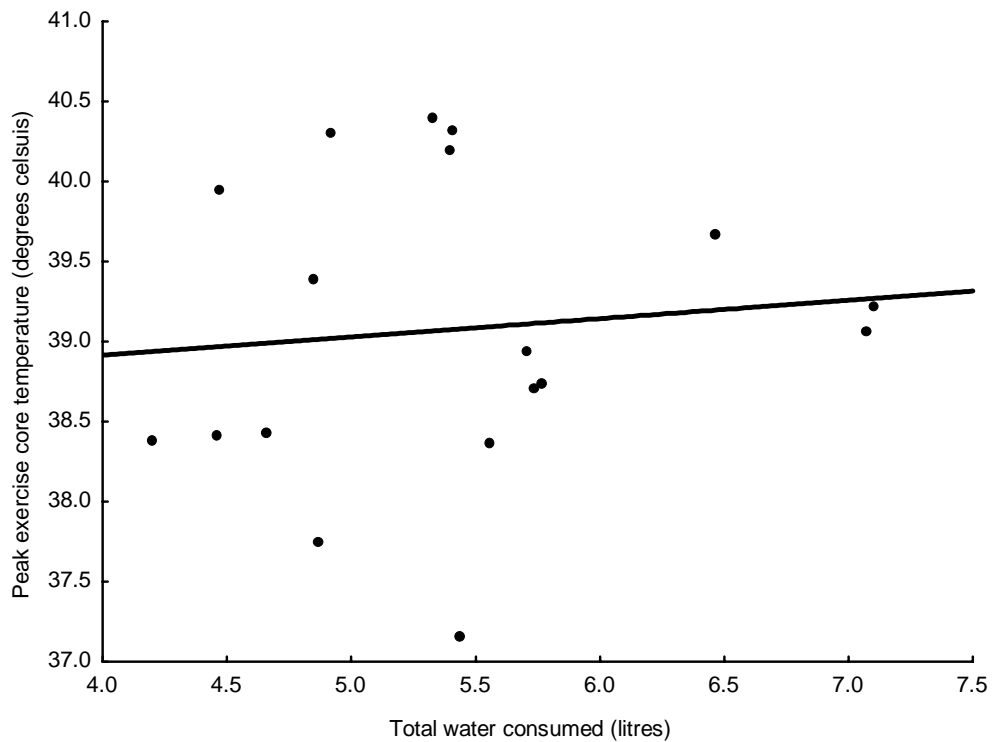
Figure 2: The relationship between fluid consumption and peak exercise core temperature (p>0.05, r = 0.10).

Plasma osmolality (POsm) and serum [Na⁺]

POsm and serum [Na$^+$] values pre- and post-exercise are also listed in Table 1. Neither POsm nor serum [Na$^+$] changed significantly during exercise. There was a significant relationship (r = -0.76) between post-exercise serum [Na$^+$] and body mass change (kg) during exercise (Figure 3) and a significant relationship (r =-0.73) between post-exercise serum [Na$^+$] and change in TBW (kg) (Figure 4).
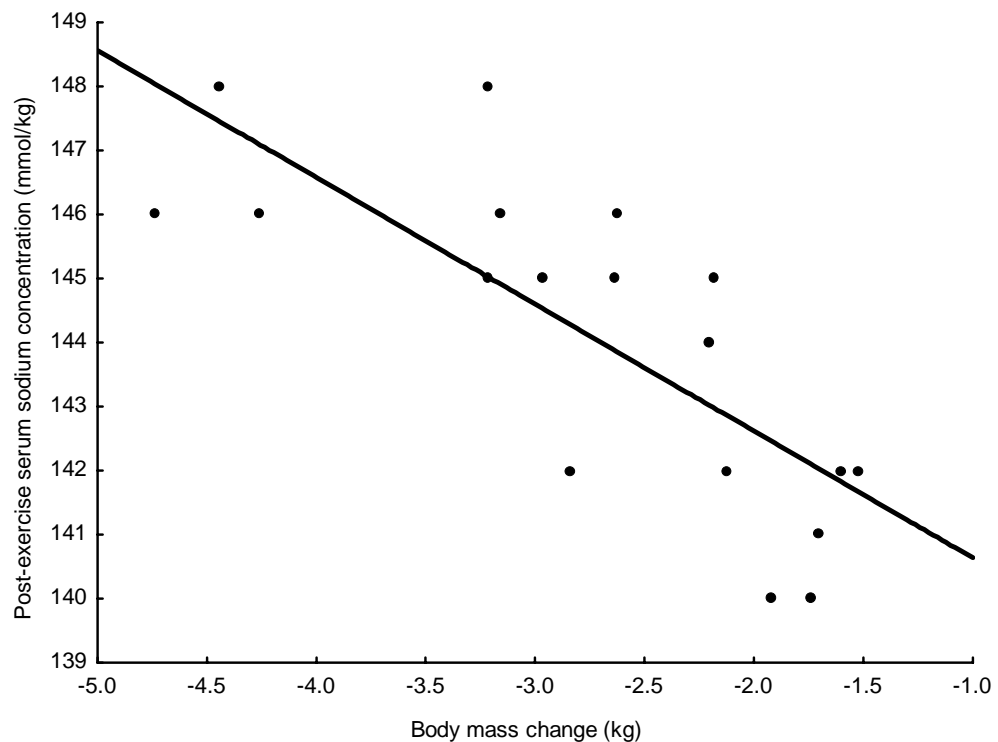

Figure 3: Relationship between post exercise serum [Na$^+$] and body mass change during exercise ($p < 0.05$, r = -0.76)
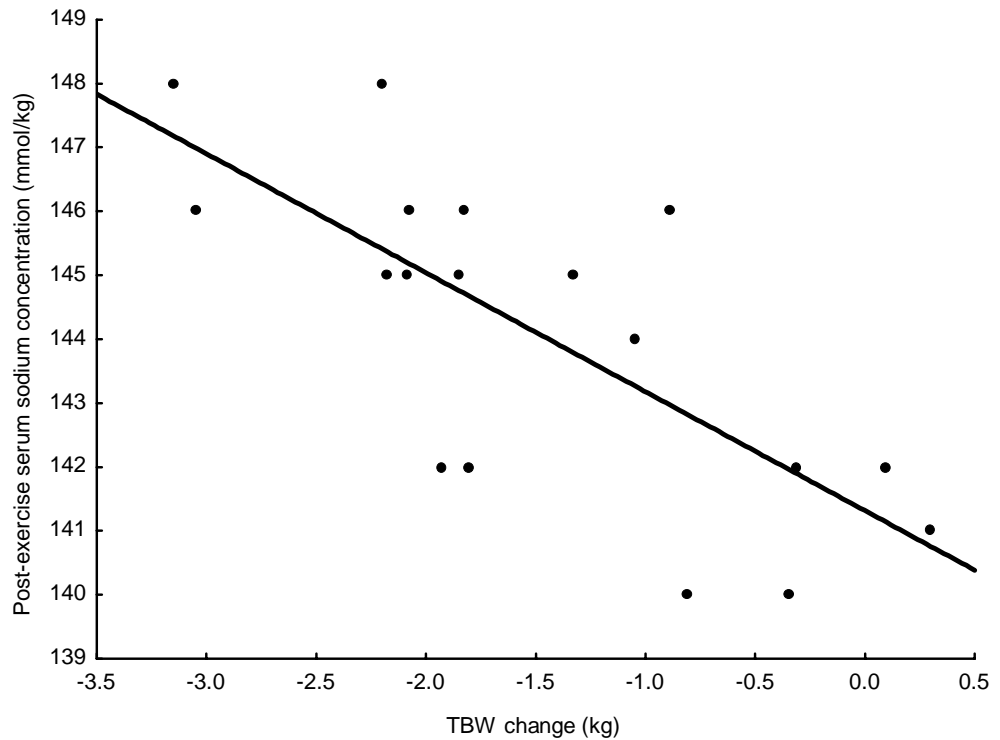
Figure 4: Relationship between post exercise serum [Na$^+$] and change in TBW (kg) during exercise (p<0.05, r = -0.73).

Peak core temperature measurements

The mean peak core temperature of the subjects during the exercise was 39.0°C; the highest individual core temperature was 40.3°C. Figure 5 (a, b, c) plots the individual core temperature responses as a function of magnitude during the march. The oscillatory pattern in most subjects indicates the presence of a pacing strategy in which subjects included frequent (up to 4 during the march) rest periods in the shade. The decision to rest seems not to have been determined purely by the intestinal temperature since of approximately 4 separate bouts of rest per subject, the vast majority occurred when the intestinal temperature was less than 39°C (all but two cases). Indeed the highest temperature reached before a rest period was ~40°C.
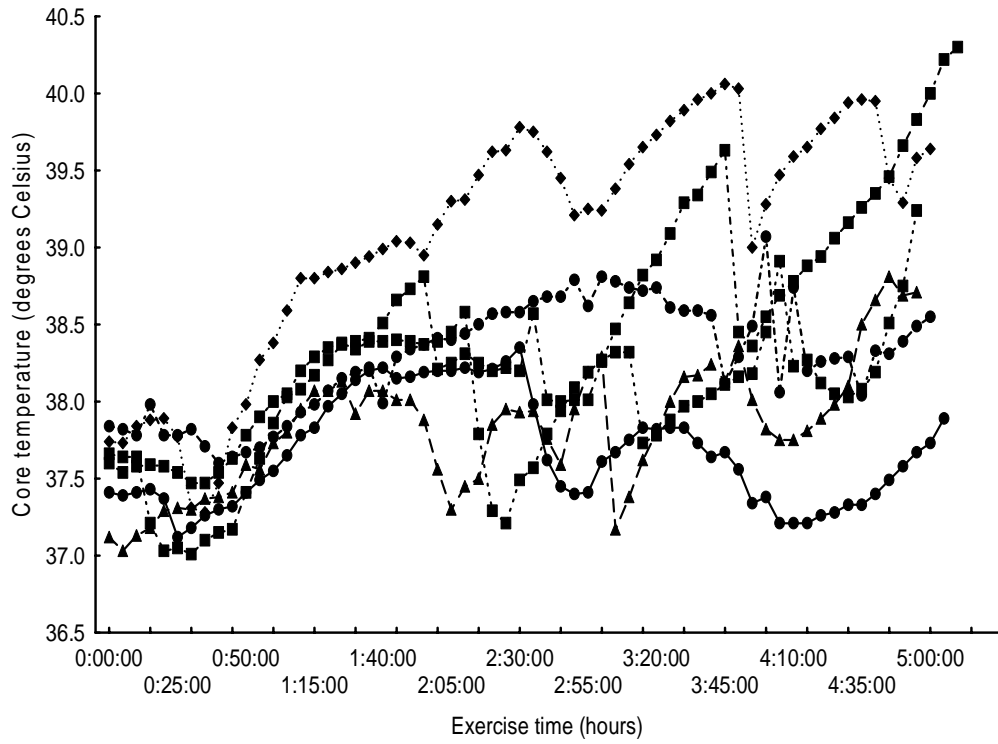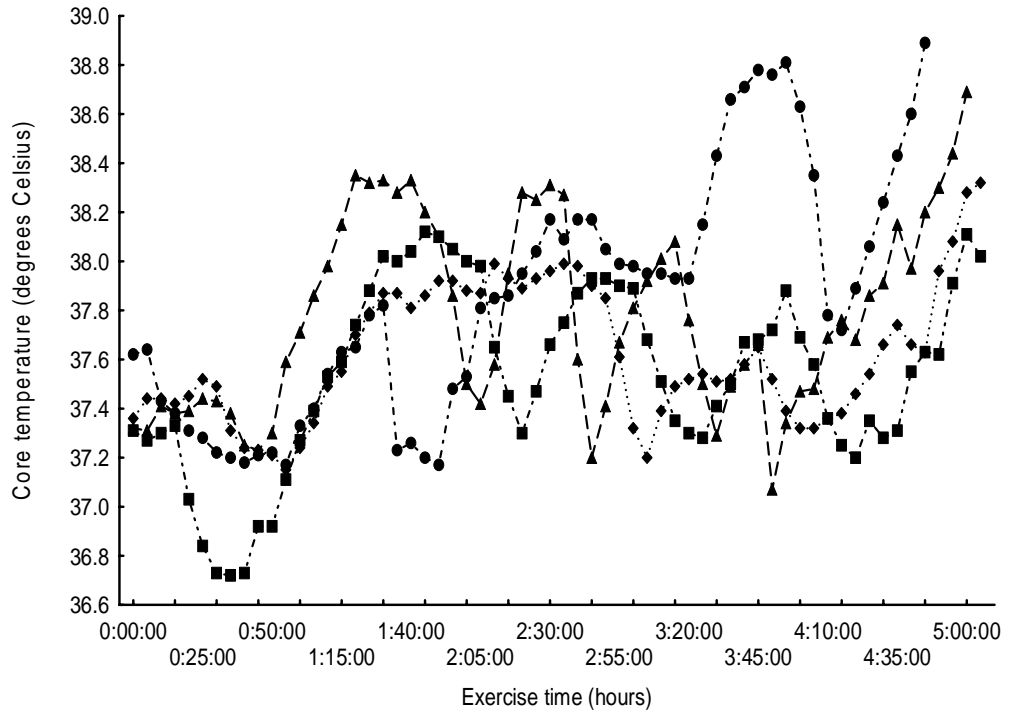
Figure 5a: Core temperature changes during the route march from the

6 subjects with the highest core temperatures
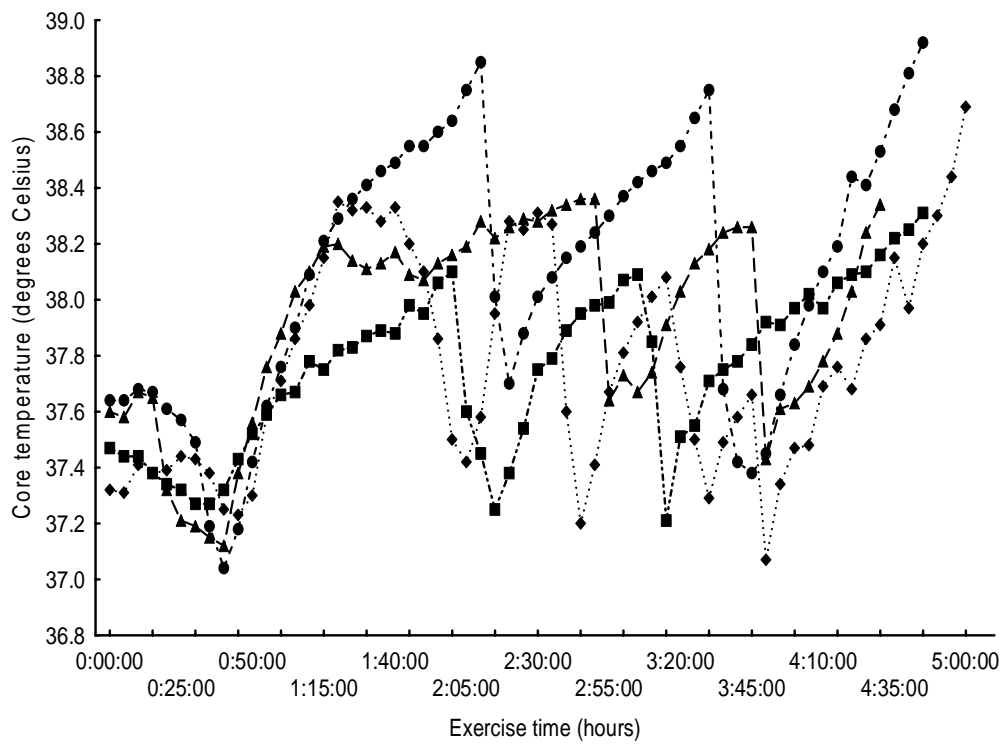
Figure 5b: Core temperature changes during the route march from the

6 subjects with moderate core temperatures

Figure 5c: Core temperature changes during the route march from the

6 subjects with the lowest core temperatures

Total body water measurements

Table 2 presents the pre- and post-exercise TBW results as determined

by deuterium oxide dilution and the post-exercise TBW results as

determined by oxygen-18 dilution for all the subjects. TBW fell

significantly ($p < 0.05$) (1.47 kg) (2.0% body mass) during exercise.

Table 2: TBW changes during a 25km march at 40°C

|	Minimum	Mean (±SD)	Maximum
Pre-exercise TBW [kg]	33.36	44.87 (6.0)	54.67
Post-exercise TBW [kg] [deuterium oxide]	31.55	43.39 (6.0)	53.34
Post-exercise TBW [kg] [oxygen-18]	32.86	44.75 (5.3)	54.92
Pre-exercise TBW [% body mass]	52.91	61.50 (3.9)	66.74
Post-exercise TBW [% body mass]	52.42	61.81 (4.3)	68.57
TBW change during exercise [kg]	-3.15	-1.47 (0.99)	0.302
TBW change during exercise [% body mass]	-1.3	0.3 (0.9)	1.8

Figure 6 shows that the change in body mass was significantly related to the change in TBW ($r = 0.77$). However the change in body mass did not accurately predict the changes in TBW as a 1:1 ratio. Rather a 1000g loss in body mass was associated with only a 200g loss in TBW.
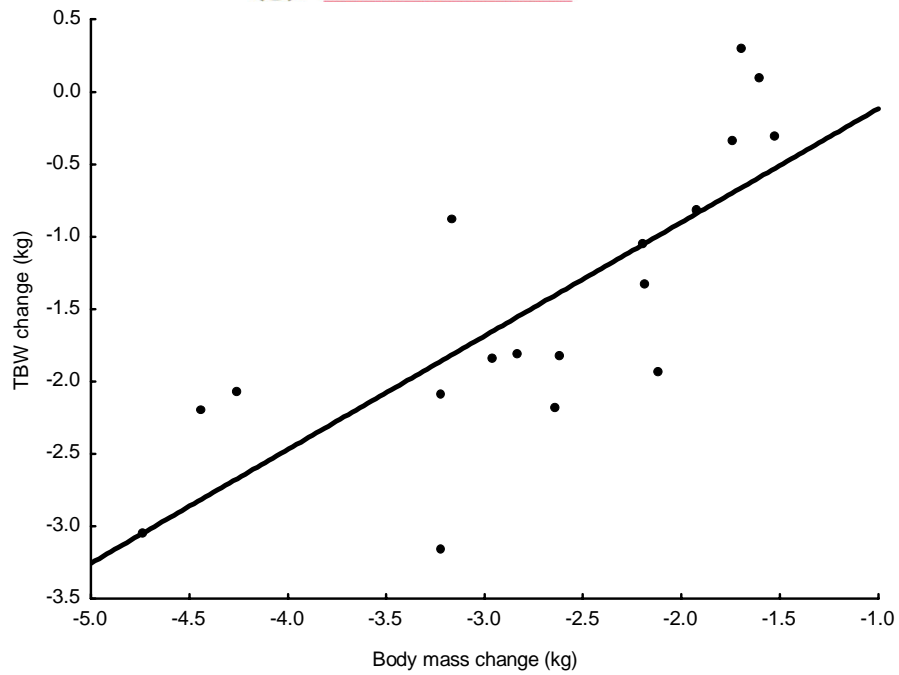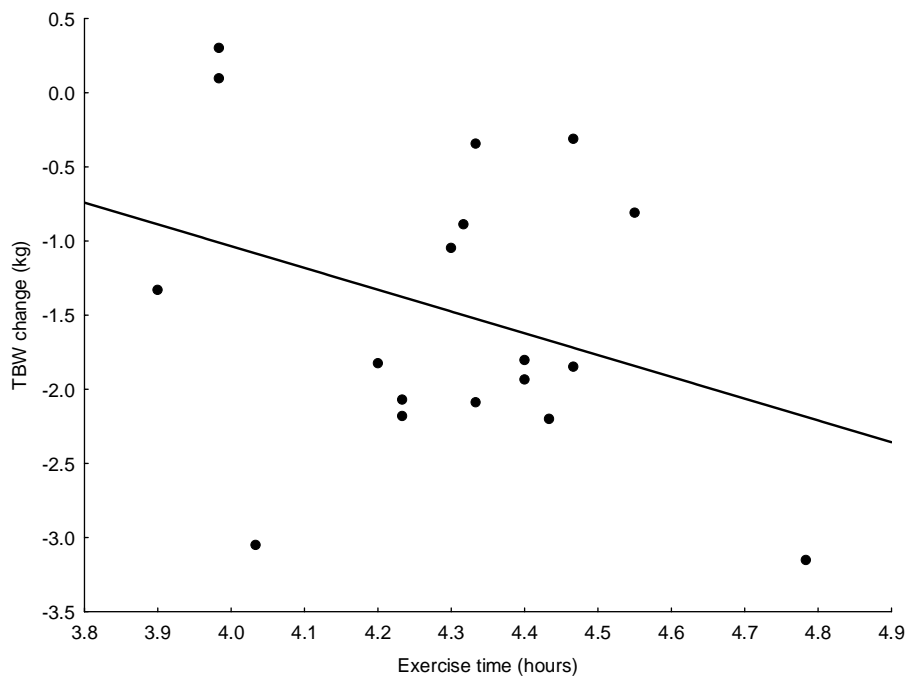
Figure 6: The relationship between changes in body mass and TBW

(p<0.05, r = 0.77)

Weight loss and performance

Figure 7 shows that there was no relationship between the change in body mass and exercise time (p>0.05). Nor was there any relationship (p>0.05) between change in TBW and exercise performance (Figure 8).
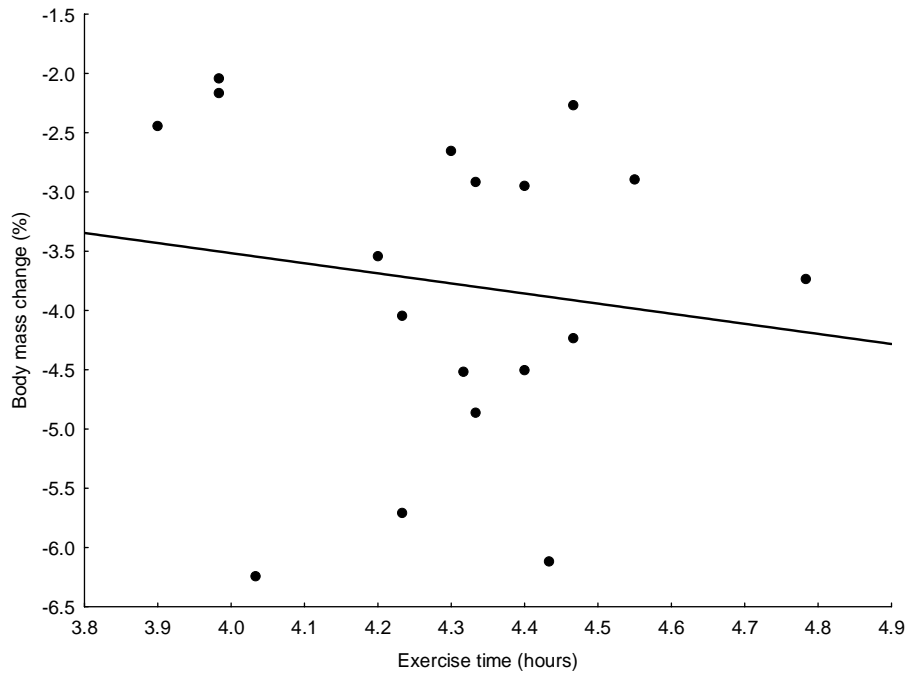
Figure 7: The relationship between exercise time and changes in body mass and ($p > 0.05$, $r = -0.14$).

Figure 8: The relationship between exercise time and changes in TBW and ($p > 0.05$, $r = -0.33$).

6.4   Discussion

To our knowledge this may be the first reported study of the physiological responses of a relatively large number of fully-burden subjects to competitive exercise in conditions of extreme dry heat while they wore clothing that was somewhat inappropriate for both the activity and the prevailing environmental conditions.

Accordingly, our first important finding was that despite carrying packs of 26 kg and wearing standard issue battle dress, soldiers participating in a competitive 25 km route march maintained safe body temperatures of less than 40.3°C while exercising in environmental conditions that approach those considered to be unsafe for practice and competition by the American College of Sports Medicine.[2]   Furthermore, all completed the study successfully and none presented with either the signs or symptoms of "heat illness".   Instead the relatively low core body temperatures measured in our subjects indicate that none was under extreme physiological stress or suffering from excessive thermal strain.

Indeed the core body temperatures measured in our subjects were lower than those measured in athletes competing in a 21 km race under significantly less strenuous environmental conditions (mean WBGT of 26.5 °C), some of the subjects in that race reached intestinal temperatures > 40.5°C without developing symptoms.[6,25]   Similar high values have been reported in athletes running 8 km in slightly warmer environmental temperatures (mean WBGT of 27 °C).[11]   Yet higher values (>41°C) were measured by Robinson [41] in a pair of

runners completing 5km runs in the heat and in a single marathon runner.[31]

The lower temperatures in these subjects would be explained by the relatively lower exercise intensities and hence metabolic rates that can be sustained for exercise lasting ~ 4 hours. In addition an anticipatory pacing strategy [49] exists to ensure that self-paced exercise performance in the heat is impaired before there is a dangerous elevation of body temperature. Figure 5 shows the pattern of individual core temperature changes during exercise. It is clear that the core temperatures fluctuate according to the pacing of each individual. The observed behaviour during the march was that of individuals performing periods of exercise followed by periods of rest, typically stopping, sitting down (in shade when available) and drinking water. The patterns of the core temperatures follow these periods of increased or decreased exertion.

Although the subjects drank profusely during exercise, maintaining rates of fluid intake that exceed by far rates of 400-800 ml/hr achieved by athletes in competition [35], yet their high rates of fluid intake did not explain their low body temperatures. Thus there was no relationship between % body mass loss or the volume of fluid consumed and the peak exercise core temperature (Figures 1 and 2) as now frequently reported.[6,33,46,38]

Accordingly we conclude that the *ad libitum* fluid intake was sufficient to ensure safe thermoregulation during the march in these subjects who were able to self-regulate their pacing strategies to suit the particular environmental conditions. A number of previous studies indicate that *ad libitum* fluid replacement strategies in which between 60-70% of sweat losses are replaced, are sufficient to maintain thermoregulation in athletes despite body mass losses of up to 3%.[24]

The second important finding was that subjects regulated their serum [Na^+] and POsm within the normal range while drinking only water *ad libitum* at a mean rate of 1264 ml/hr while sweating an average total of 1789 ml/hr. Although we did not measure the sweat [Na^+] in these soldiers, there is no reason to believe they would be lower than values of about 40mmol/L measured in other athletes consuming a typical Western diet. At this sweat [Na^+], average total sweat sodium losses during the march would have been >240mmol. Yet despite such large losses that were unreplaced during exercise, serum [Na^+] was maintained during exercise. This confirms the now well-established finding that the serum [Na^+] can be maintained during exercise without the need for acute sodium replacement during exercise.[17]

Clearly one important contributor to the regulation of the serum [Na^+] was the mean body mass loss of 2.73 kg (3.8%) during the exercise. Figures 3 and 4 shows that an increase in TBW and body mass produced a fall in serum [Na^+] whereas a fall in TBW and body mass produced a rise in serum [Na^+]. This data are compatible with our findings that an increase in body mass (and hence TBW) is the major

determinant of exercise-associated hyponatremia (EAH).[36,43,47,38,18]

Although the magnitude of this body mass loss of nearly 4% is almost double that considered desirable during exercise [44], yet it is clearly the homeostatically-regulated response of these subjects who drank *ad libitum* during exercise. This is because the plasma osmolality and not the body mass is the regulated variable both at rest and during exercise so that drinking according to the dictates of thirst would be expected to produce minimal changes in plasma osmolality and the serum [Na^+].[16] In contrast drinking to stay "ahead of thirst" by "drinking as much as tolerable", [7,4] must cause serum [Na^+] to fall [36,43,47] if renal free water clearance is insufficient to prevent an increase in TBW. This occurs when arginine vasopressin (ADH) secretion is not appropriately suppressed by a decreasing serum osmolality.[43]

Thus we conclude that the *ad libitum* intake of water during a 25 km route march was sufficient to maintain serum [Na^+] despite significant body mass and sweat electrolyte losses. This is compatible with the finding that sodium ingestion is not required to maintain serum [Na^+] during exercise.[17] Rather it is the inappropriate regulation of the TBW that determines the extent to which the serum [Na^+] falls during prolonged exercise.[36]

Our third conclusion is that changes in body mass were not related exactly to changes in TBW according to a 1:1 relationship (Figure 6), so that for each 100g loss of body mass there is a 100ml reduction in

TBW. Furthermore the results of the post-exercise TBW as determined by the deuterium oxide and the oxygen-18 isotopes compared favourably (r=0.98) (Table 2). This is an important finding since it disproves the idea that a second deuterium oxide dose might cause an overestimation of TBW.

This supports our previous findings in soldiers performing prolonged exercise during which their ad libitum water intakes were sufficient to maintain thermoregulation while producing in one group, a 197 g increase in TBW despite a body mass loss of 1.3 kg [38] and in another a 500 g decrease in TBW despite a body mass loss of 1.0 kg.[39]

Similar findings were reported in soldiers performing a 194 km unsupported desert march during which their mean hourly fluid intakes of 458 ml were sufficient to maintain thermoregulation (mean core temperature of 38.1°C) while producing a 300 g increase in TBW despite a body mass loss of 3.3 kg at the end of exercise.[32] Considered in their totality, these findings rekindle the original concept of Ladell [21,22,23] that a body fluid reserve of perhaps up to 2 litres may exist and which may not require replacement in order to insure that whole body fluid homeostasis is maintained during exercise.[37,38] If his fluid volume exists in the gut it would explain why some believe that body mass losses of up to at least 3% may not carry any physiological penalty during prolonged exercise.[22,28,30,46]

Our fourth finding was that performance during the march was unrelated to the extent of the change in either body mass (Figure 7) or TBW (Figure 8). This is an extension of the finding that the amount of fluid ingested during the march also did not predict performance time during the march.

In conclusion, this study extends to much more extreme environmental conditions, than our previous findings [38] that *ad libitum* drinking was sufficient to maintain POsm and serum $[Na^+]$, and prevent an excessive rise in core temperature during a 16 km paced march in a WBGT of 24.5°C. In this study we show that in the most extreme conditions yet studied humans were able to maintain POsm, serum $[Na^+]$ and safe core body temperatures while drinking water *ad libitum*. They achieved this outcome by (i) adopting a pacing strategy which included resting in the shade when available, (ii) by increasing their rates of *ad libitum* fluid intakes to amongst the highest yet recorded in runners/walkers and (iii) by allowing a small reduction in TBW, the latter presumably to insure the maintenance of serum $[Na^+]$ despite a loss of > 200mmol Na^+ in sweat.

Our findings that some selected humans are able to perform competitive exercise in these severe environmental conditions are indeed compatible with the historical interpretation that humans are the mammals with the greatest capacity for exercising in extreme heat [15] and that this adaptation must have evolutionary significance.[5,26]

Acknowledgement

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Chapter 7

Comments on Baker et al.'s "Change in body mass accurately and reliably predicts change in body water after endurance exercise"

<u>**Article:**</u>

Nolte HW and Noakes TD. 2010. Comments on Baker et al.'s "Change in body mass accurately and reliably predicts change in body water after endurance exercise". European Journal of Applied Physiology, 108(5): 1061-1064.*

*Referencing format in the text and list applied as required by the European Journal of Applied Physiology.

7.1 Background to letter

A manuscript by Baker et al. (2009) was published in the European Journal of Applied Physiology stating that changes in body mass accurately and reliably predicts changes in TBW and could therefore by used as an indication of hydration status during prolonged exercise.

Since the results of Baker and associates were in direct conflict with the results of the studies presented in this thesis document, a thorough review of their methods and results were performed. A letter was addressed to the Editor of the European Journal of Applied Physiology in order to raise concerns over the results and the manner in which they were calculated.

7.2 Letter to the editor

To the Editor:

We read with some interest the article reporting research of Baker et al. (2009). The authors concluded that the change in body mass (ΔBM) during exercise is due to a change in total body water (TBW) alone since these two variables are significantly correlated (r=0,77) in subjects who completed exercise with 4 different levels of body mass change (+1.8% BM, -0.2% BM, -2.1% BM, -3.3% BM). As a result, the authors conclude that the change in body mass during exercise is "an accurate and reliable method to assess" exercise-related changes in TBW. This finding supports the argument (Baker et al. 2009; Convertino et al., 1996; Eichner, 2002) that athletes should drink enough to insure that they do not lose any body mass during exercise.

We found the authors' conclusion surprising since many others conclude that water is not the sole constituent of the mass lost during exercise (Maughan et al. 2007). While the authors address these issues in their paper (p965), they nevertheless conclude that their findings finally disprove these contrary arguments. Given the importance of this contrary conclusion, we scrutinized the study to determine whether or not there were any obvious explanations for their unexpected finding.

First we noticed (Experimental procedure, p2) that eight subjects each took part in 12 possible trials for a total of 96 individual experiments. Yet it appears that only 62 of these experiments were included in the final analysis. The authors failed to explain why they excluded 35% of the experimental data from their final analysis. Without an adequate explanation for these exclusions, the validity of their findings cannot be convincingly assessed. We note however in the authors' companion paper that five out of eight subjects were unable to complete the -3.3% BW trial since they could not reach high enough sweating levels during the exercise period (Baker et al., 2008). The inclusion of a table indicating the reasons why data were excluded is important.

The missing data also do not appear to be evenly distributed between the four experimental conditions. Thus it appears from Figure 1 that 18 of 24 (75%) possible trial data were included in the +1.8% BM trial, 15 of 24 (63%) for the -0.2% BM trial, 22 of 24 (92%) in the -2.1% BM trial but only 7 of 24 (29%) for the -3.3% BM trial. Under–representation of data in the trial with the greatest body weight loss could have biased the conclusions.

Second, we find it surprising that carbohydrate (CHO) was included in the tested solution. While we appreciate that this study is funded by a commercial company that produces a carbohydrate-containing "sports" drink, the presence of the CHO in the solution ingested during this experiment confounds the interpretation of the data.

For example the ingestion of glucose will reduce the rate of oxidation of the endogenous CHO stores (Bosch et al., 1994). This will influence the amount of endogenous fuel irreversibly oxidized during prolonged exercise. Although the influence of this effect is probably small, the point remains that the tested solution was not the most appropriate choice for these experiments.

Third the authors claim that they studied *prolonged* exercise whereas in fact they studied 105 minutes of interval running interspersed with 14 minutes of rest between exercise bouts. The extent of irreversible oxidation of endogenous fuels is determined by both the intensity and the duration of exercise and will therefore be greater the longer the duration of exercise. Thus, as already pointed out by Weschler (2008), exercise bouts of at least 4 or more hours would have provided a more appropriate test of the hypothesis that exogenous fuel oxidation does not contribute to the mass loss during exercise. The authors' apparent finding that body mass loss accurately predicts TBW changes may not apply to more prolonged exercise like the 226 km Ironman Triathlon, in which irreversible oxidation of endogenous fuels can theoretically

contribute as much as 0.875 kg to the body mass loss during exercise (Noakes, 2000).

Fourth we have certain concerns with the manner in which body mass and TBW were measured. For example, during each of the rest periods of the interval runs subjects were towelled off before their body mass was measured whereas at the end of exercise, convective cooling was used to promote the evaporation of sweat. What was the reason for this difference?

The text states that: "*Subjects drank fluid or no fluid during recovery to maintain the desired %BM change*" (p3). However the authors do not describe the time sequence for this intervention. If subjects drank water less than 1-2 hours before sampling (blood or urine) then that fluid would not be in equilibrium with the TBW and therefore might not add to the dilution of the tracer. The effect would be to reduce the extent of the body mass loss for the measured change in the TBW pool. This would underestimate the extent of the body mass loss for any change in TBW and would favour the authors' conclusion that irreversible fuel oxidation does not contribute to the mass loss during prolonged exercise.

Next, the authors used the "rinse" technique during the D_2O dosing procedure (p3). This is an acceptable methodology but requires that the amount of rinse water, consumed to ensure complete consumption of the tracer, be subtracted from the total body water pool. Importantly the authors do not clearly state whether they indeed corrected for this 100 ml tracer water by subtracting it from the total body water pool.

Furthermore the authors state that: "*When serum [D$_2$O] measurements were not available (e.g., when there was not enough sample volume or the sample was contaminated during the D$_2$O extraction procedure), the pre-dose urine [D$_2$O] or post-experiment urine [D$_2$O] was used in place of the pre-dose serum [D$_2$O] or post-experiment serum [D$_2$O], respectively, to complete the calculation of Npost*".

We are not aware of any previous publication in which this particular method has been adopted and its accuracy and reliability evaluated. Due to differences in equilibration time required for the different sampling fluids (blood or urine), this untested method could have introduced a methodological error and may therefore explain the wide range of ΔTBW that were measured for each level of dehydration (their Figure 1 and our figures added here). The authors do not present evidence that they validated this method by taking multiple samples in order to insure that the D$_2$O enrichment reached stable levels within the sampling period. For example error could have been introduced if the pre-exercise TBW was determined from blood measurements whereas the post-exercise TBW in the same individual was determined by urine sampling on the assumption that the equilibration period was the same for the both methods.

In addition, another problem with urine sampling is that often the first post-dose urine sample contains un-enriched urine that had been collecting in the bladder prior to dosing. Since the authors did not detail when either urine of serum was used for the TBW calculation, nor if

they sampled the first or second urine voiding, they do not provide sufficient evidence to evaluate the real accuracy and repeatability of the test methods.

We also note that 3 athletes were able to produce a BM loss of ~4% within 105 minutes of exercising requiring a sweat rate of about 1.2L.hr in their average 66kg subjects. We note that the study was undertaken in the heat which produces a greater water loss at a lower overall metabolic rate (and hence a lesser irreversible oxidation of endogenous fuel stores) than would exercising at a higher metabolic rate but in much cooler environmental conditions especially if convective cooling was inappropriate (Saunders et al., 2005) as is usually the case in many exercise laboratories.

Finally and most importantly we have major concerns with the manner in which the data were analysed (their Figure 1, reproduced here). Thus the authors designed the experiment so that 4 different levels of body mass loss would be produced. They then analysed the total data as if all came from a single experiment and not from four different experiments. However if the linear one-to-one relationship they apparently found between the change in TBW and BM is valid for the total experiment, then it must be equally valid for each of the 4 separate experiments that were conducted.
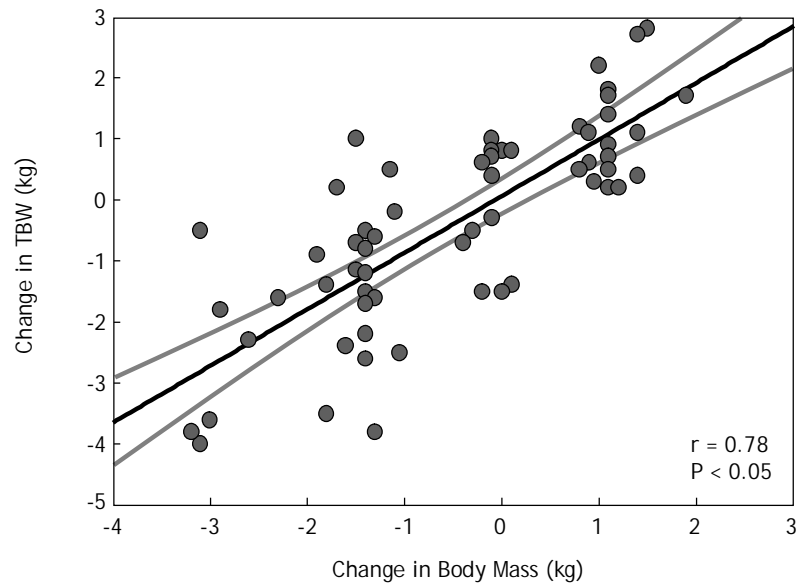
Figure 1: All data points plotted together (p<0.05, r=0.78) (replicated

from Baker et al., 2009) with 95% confidence intervals
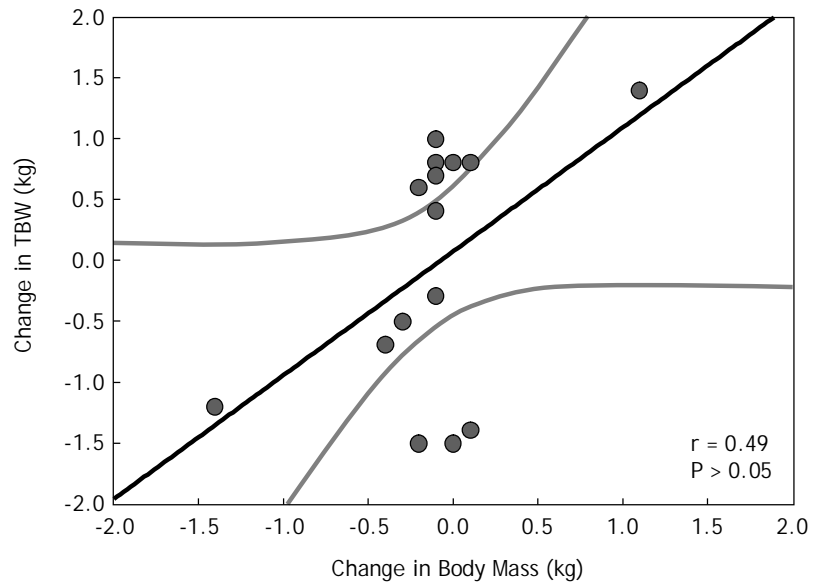
included

Accordingly we analysed the data individually for the four separate

experiments in which data for TBW and BM loss were reported,

according to our analysis, for 18, 15, 22 and 7 subjects in the four

different experiments. We have plotted those data separately below
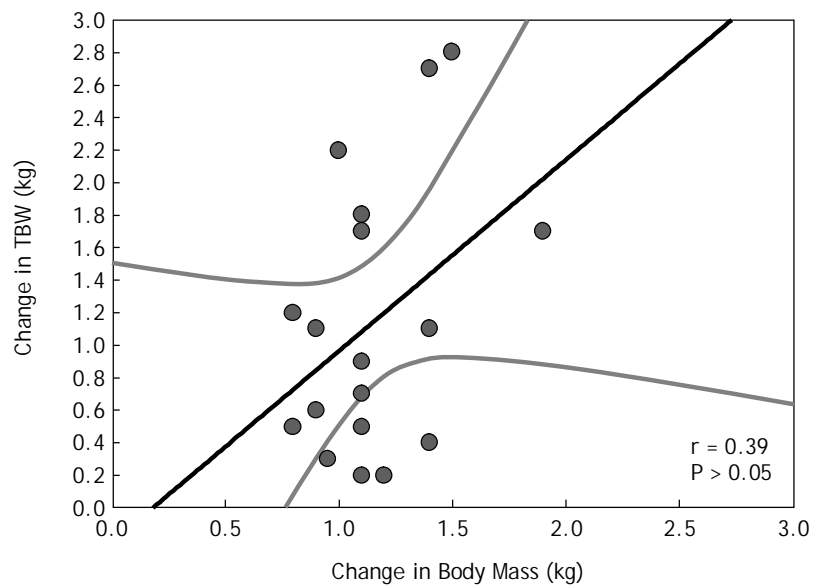
(Figures 2a-2d).
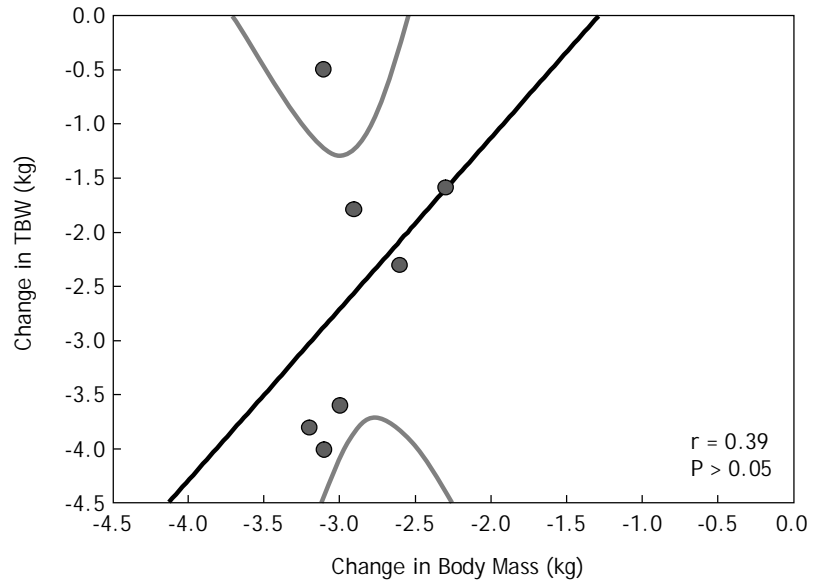
Figure 2a: Data for the -3.3%ΔBM group (p>0.05)
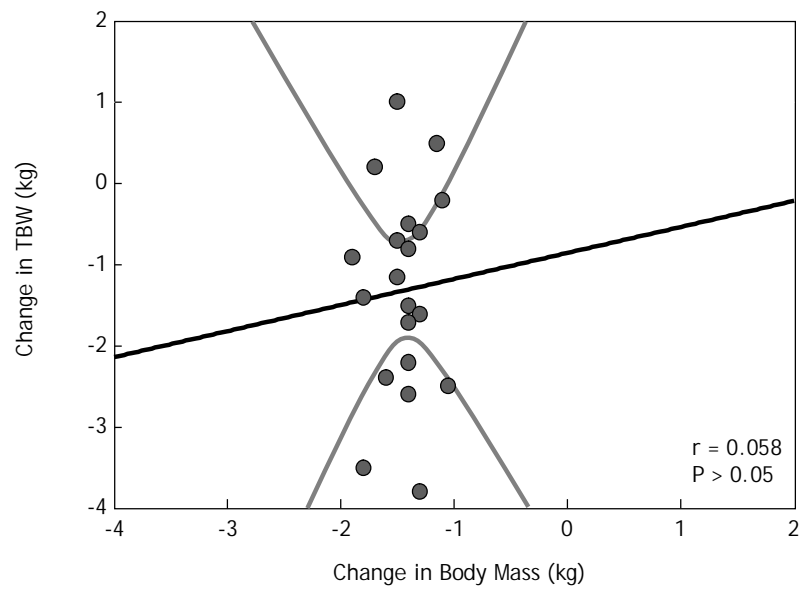
Figure 2b: Data for the -2.1%ΔBM group (p>0.05)

Figure 2c: Data for the -0.2%ΔBM group (p>0.05)

Figure 2d: Data for the +1.8%ΔBM group (p>0.05)

The first observation is the wide variation in the estimated TBW change with each %BM loss. Thus for a mass loss of ~3% (Figure 2a), the variation in range of calculated TBW change was -4 to –0.5L. For %BM losses of -2.1%, -0.2% and +1.8% BM, the respective ranges were -4 to +1L (Figure 2b), -1.5 to +1L (Figure 2c), and +0.2 to +2.8L (Figure 2d). This suggests either that there is a wide individual variability in this response or that the techniques for measuring changes in TBW during exercise are particularly inaccurate.

But more importantly, in contrast to the finding for the complete data set, in none of these 4 experiments was the BM loss significantly related to the change in TBW. This was true also for the three experiments in which there were 15 or more data points.

This analysis shows that the conclusion of Baker et al. (2009) that the change in body mass accurately predicts the change in TBW during exercise is an artefact of the manner in which they analysed their data and is probably confounded by the unusual manner in which they used different biological samples (urine or serum) to measure changes in TBW in the same individuals. Rather a more careful analysis of their data (Figures 2a-2d) shows that changes in BM during exercise were unable to accurately predict changes in TBW in their experiments.

We welcome the authors' views on these various issues.

HW Nolte

TD Noakes

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Chapter 8

Summary, general conclusions and recommendations

8.1 Introduction

The extent to which humans need to replace their fluid losses during exercise remains contentious despite more than 60 years of focused research. Unfortunately, apart from the inherent physiological risk associated with "under" or "over" hydration, providing an excess of water contributes to the burden of the payloads imposed on the dismounted foot soldier. By determining the optimal water requirements of soldiers, one can ensure their safety and physiological comfort while optimising their payload burdens. The correct water replacement strategy will provide safe hydration levels without affecting soldier performance, while potentially reducing the payload burden imposed on the modern soldier.

This debate has special relevance for the military since soldiers ingesting fluid *ad libitum* will drink less than those who are forced to drink in order to lose less than 2 % of their body mass during exercise. While it is now accepted that exercisers should not be encouraged to drink "as much as tolerable" there is still no consensus of the optimum rate of fluid ingestion during exercise. The modern emphasis is now on individualised drinking behaviours, these individualised strategies aim to limit "body water losses" to <2% by using changes in body mass as a surrogate for changes in TBW during exercise.

This altered emphasis has provided the opportunity to determine the optimal rates of fluid ingestion by military personnel. In turn, this could reduce the mass in the form of water, soldiers might need to carry on military missions. Accordingly the purpose of this body of research was to investigate the efficacy of *ad libitum* fluid replacement to maintain safe fluid balance in soldiers during prolonged (~4 hours) exercise.

8.2   Main findings

The main findings of this research effort, in relation to the objectives presented in the general introduction are:

1.   What are the rates of fluid ingestion freely chosen by soldiers during exercise?

The results of Chapter 2 were unable to detect any superiority of an *ad libitum* drinking regime (525 ml/hr) compared to a restricted regime (300ml/hr) on fluid balance and performance during selected soldiering tasks. However, we believe that drinking *ad libitum* is probably the more appropriate response even though there was no measurable benefit associated with the slightly higher rate of fluid intake under the conditions of this experiment. Contrary the results in Chapter 6 showed that subjects drank profusely during exercise, maintaining rates of fluid intake that exceed by far rates of fluid intake achieved by athletes in competition.

The mean *ad libitum* fluid intake of soldiers ranged from 383 ml/hr to 1264 ml/hr during the studies conducted for this thesis. These findings indicate the freely chosen rates of fluid ingestion and highlight that

these rates are highly variable due to its dependence on factors such as work rate, clothing configurations and environmental conditions.

2. **Are these freely chosen (*ad libitum*) rates of fluid ingestion sufficient to protect against major fluid and electrolyte imbalances as evaluated by TBW, serum [Na$^+$] and POsm?**

The results from Chapter 4 indicate that soldiers participating in a 16 km route march regulated their serum [Na$^+$] and POsm within the normal range while drinking "*ad libitum*" at a mean rate of 383 ml/hr even though this drinking rate replaced only 61% of their measured hourly body mass loss (626 ml/hr). In Chapter 5 we showed that there were no significant changes to TBW, USG or UOsm in any of the subjects despite an average body mass loss of 1.3 kg (1.98%). The results in Chapter 6 showed that despite significant sweat losses, possibly resulting in unreplaced sodium losses in excess of 240 mmol, subjects increased their serum [Na$^+$] by drinking only water *ad libitum* during exercise. Thus a*d libitum* fluid ingestion was sufficient to protect against any fluid and electrolyte imbalances by maintaining POsm, serum [Na$^+$] and TBW despite mean body mass losses ranging from 1.4% to 3.8% during the studies conducted for this thesis.

This is compatible with the finding that sodium ingestion is not required to maintain serum [Na$^+$] during exercise. Rather it is the inappropriate regulation of the TBW that determines the extent to which the serum [Na$^+$] falls during prolonged exercise. This is because the serum [Na$^+$] and not the body mass is the homeostatically regulated variable during exercise. In contrast drinking to stay "ahead of thirst" by "drinking as

much as tolerable" must cause serum [Na$^+$] to fall if renal free water clearance is insufficient to prevent an increase in TBW. This occurs when arginine vasopressin (ADH) secretion is not appropriately suppressed by an increasing serum osmolality. In contrast our data show that an increase in TBW produced a fall in serum [Na$^+$] whereas a fall in TBW produced a rise in serum [Na$^+$]. This data is compatible with our findings that an increase in body mass (and hence TBW) is the major determinant of exercise-associated hyponatraemia (EAH).

3. **Are these freely chosen (*ad libitum*) rates of fluid ingestion sufficient to maintain safe thermoregulation during exercise?**

The results shown in Chapter 4 indicates that the relatively low core body temperatures measured in our subjects indicate that none was under extreme physiological stress or suffering from excessive thermal strain. Accordingly we conclude that their *ad libitum* fluid intake was adequate to maintain a safe thermoregulation during the march for the particular environmental conditions. Chapter 5 indicated that there was no relationship between % body mass loss or fluid intake and the post-exercise core temperature. Instead core temperatures were homeostatically regulated within a normal range unrelated to the degree of mass loss during exercise. Most importantly the results from Chapter 6, recorded under extreme hot and dry environmental conditions, indicate that although the subjects drank profusely during exercise their high rates of fluid intake did not explain their low body temperatures. Furthermore, all completed the study successfully and none presented with either the signs or symptoms of "heat illness".

The lower temperatures in these subjects would be explained by the relatively lower exercise intensities and hence metabolic rates that can be sustained for exercise lasting ~ 4 hours. In addition it is clear that the core temperatures fluctuate according to the pacing of each individual. The observed behaviour during the march was that of individuals performing periods of exercise followed by periods of rest, typically stopping, sitting down (in shade when available) and drinking water. The patterns of the core temperatures follow these periods of increased or decreased exertion.

Accordingly we conclude that no relationship was found between the rates of fluid consumption and mean peak core body temperatures during exercise in any of the studies conducted for this thesis. Thus *ad libitum* fluid intakes (between 60-70% of sweat losses) were adequate to maintain safe thermoregulation during exercise for the particular environmental conditions despite significant body mass losses. Our findings that some selected humans are able to perform competitive exercise in severe environmental conditions are indeed compatible with the historical interpretation that humans are the mammals with the greatest capacity for exercising in extreme heat and that this adaptation must have evolutionary significance.

4. **Can changes in body mass be used as an accurate surrogate measure for changes in TBW during prolonged exercise?**

The first conclusion of Chapter 4 was that changes in body mass did not accurately predict changes in TBW in these soldiers that lost 1.4% of their body mass during exercise. The results of this study however

did not exclude the possibility that greater levels of body mass loss may not be detrimental to either health or performance in those who drink to prevent the development of thirst during exercise. Chapter 5 showed no significant changes in TBW despite an average body mass loss of 1.3 kg (1.98%). Thus although the subjects developed "voluntary dehydration" as classically described they did not show a decrease in TBW and so were not "dehydrated". Instead TBW increased marginally by about 197 g during the route march.

The results from Chapter 6 indicate that a magnitude of body mass loss of nearly 4%, almost double that considered desirable during exercise, was clearly the homeostatically-regulated response of the subjects who drank *ad libitum* during exercise. Similarly in these subjects changes in body mass were not related exactly to changes in TBW according to a 1:1 relationship.

The debate of whether the change in body mass (in kg) during exercise can be used as a 1:1 predictor of the change (in litres) in TBW is crucially important since it raises the question of whether or not there is a body fluid reserve of perhaps up to 2 litres that may not require replacement in order to insure that whole body fluid homeostasis is maintained during exercise. Thus we conclude that changes in body mass does not accurately nor reliably predict changes in TBW during prolonged exercise and should not be used as a surrogate for fluid balance change. The results supports the theory that certain body mass loss during exercise may, at least theoretically, not contribute to body water losses including

metabolic water production, substrate oxidation and the release of water associated with the storage of glycogen.

Until recently it was advised that full replacement of body mass losses should be achieved during exercise. Currently this position has been revised so that the newest ACSM Position Stand proposes that the mass loss during exercise should not exceed 2% of the starting body mass. The findings of this research effort do not support this prescription and indicates that large changes in body mass did not cause changes in TBW that is associated with deleterious physiological changes.

The study raises questions about the validity of the term "voluntary dehydration" that was first coined more than 60 years ago. Indeed this study invites a more thorough interrogation of the use of the term "dehydration" which should be used only when there is a proven reduction in TBW and not, as this study shows, merely a reduction in body mass during exercise.

8.3   Recommendations for research and practice

Further the following recommendations for future research:

1. TBW as determined by the diluted isotope method should be used to assess fluid changes during prolonged exercise together with serum $[Na^+]$ when possible.
2. When applying the diluted isotope method (deuterium oxide) to assess acute changes in TBW a concurrent TBW measure by

the tracer oxygen-18 should be performed where possible. This will allay anecdotal fears that a second deuterium oxide dose might result in an over estimation of the post-exercise TBW. Unfortunately this might seldom be possible due to the exorbitant cost of the oxygen-18 tracer.

3. While corrections for increased isotope loss during the post-exercise TBW measures proofed of almost no significance under the experimental conditions of this study it is still recommended as the prudent approach for future studies. Increased isotope loss during post-exercise TBW measures might be more pronounced according to the magnitude of excess post-exercise oxygen consumption and the current study have not investigated this effect under more prolonged and/or higher intensity exercise.

4. More data points should be gathered to increase the confidence levels of the data collected during this study in order to assess the effects of significant body mass loss (>2%) on TBW and serum $[Na^+]$ during prolonged exercise.

5. Controlled laboratory studies should be conducted to investigate the amount of water that is associated with the storage of glycogen and which become available to the total body water pool during prolonged exercise. While we and others assume that water contributing to the total body water pool in this manner is indeed considered by the deuterium oxide tracer there are currently no definitive studies that have investigated this particular question.

Further the following recommendations for practice:

6. Fluid replacement to match body mass loss during exercise should not be advocated, and is not necessary to prevent voluntary dehydration.

7. The term dehydration should be used only when there is a proven reduction in TBW and not, as this study shows, merely a reduction in body mass during exercise.

8. *Ad libitum* fluid intake should be the fluid replacement strategy of choice during exercise.

9. *Ad libitum* fluid intake is sufficient to maintain safe serum $[Na^+]$ levels without the supplementation of sodium during exercise through fluid intake.

Appendices

LETTER TO THE EDITOR

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

Timothy D. Noakes · Heinrich W. Nolte

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 (ΔBM) 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 ΔBM and the change in TBW (ΔTBW) 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 ΔBM and ΔTBW when their four experiments were analysed separately.

Instead the real focus of our letter was to contest an apparent 1:1 relationship between the ΔBM (in grams) and ΔTBW (in mllilitres) 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 ΔBM of up to 1,000 g was required before it was possible to detect any ΔTBW. In our letter, we explained why it is logical to assume that some of the ΔBM 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 ΔBM 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 ΔBM during exercise and the extent to which that mass loss reflects any ΔTBW. Or conversely the extent to which other sources contributes to the ΔBM 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 ΔBM and the ΔTBW so we fully agree that "the use of ΔBM" 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

Nicholas Tam, MSc,* Heinrich W. Nolte, MA,† and Timothy D. Noakes, MBChB, MD, DSc*

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 ± 0.6 kg; $P < 0.000$ and 56 km; -2.5 ± 1.1 kg; $P < 0.000$). Total body water was reduced in the 56-km race (-1.4 ± 1.1 kg; $P < 0.001$). A negative linear relationship was found between percentage change (%Δ) in TBW and %Δ 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 ± 8.2 mOsm/kg H$_2$O; $P < 0.05$ and 5.4 ± 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.[1,2] 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.[3]

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.[4] 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%.[4] 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.[5–7] 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[8] 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[9] and Maughan et al[10] have presented data to support this interpretation.

In contrast, Montain[11] and Baker et al[12] 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

methodologies and statistical techniques and therefore its validity in supporting their conclusions.[13]

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,[14] 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.[15]

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.[16,17]

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).[18] 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^+] 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, Kremsmunster, 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^+]) and potassium ([K^+]) (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_2O.[19] The use of the stable isotope D_2O allows for the most accurate quantification of changes in TBW during exercise.[20] This allows a more accurate determination if all the BM lost during exercise is because of a reduction in TBW.[9,10,21–23]

An initial approximately 1.5-mL saliva sample was collected to calculate baseline D_2O 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°C. Before analysis, samples were prepared by filtration through sterile syringe filters into 1-mL glass vials.

Each D_2O dose was dispensed from a 4% stock solution that was prepared by mixing appropriate amounts of 99% D_2O (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 D_2O administration is known to be appropriate.[19,23–33] All urine excreted during the equilibration period was collected and analyzed to account for any isotope loss when calculating TBW.

Saliva D_2O 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[34] with a modified correction factor for nonaqueous hydrogen exchange at 1.04 (4%).[28,34]

Calculations

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

$$\Delta\%BM = \left(\left(BM_{post-race} - BM_{pre-race}\right)/BM_{pre-race}\right) \times 100$$

Equation 2: Change in plasma volume (PV)[35] was calculated as follows:

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

Equation 3: The Davies method was used to calculate TBW[36] as follows:

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

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); Ea, enrichment of diluted dose; Et, enrichment of water used to dilute the dose; Ep, enrichment of baseline sample; Es, enrichment of postdose sample; 1.04, correction factor for over estimation of TBW by the use of D_2O.[37]

Equation 4: Percentage change (%Δ) in TBW was calculated as follows:

$$\%\Delta TBW = \left(TBW_{post-race}/BM_{post-race} \times 100\right) - \left(TBW_{pre-race}/BM_{pre-race} \times 100\right)$$

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 ± 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 ± 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 ± 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 ± 180 mL/h (range, 87-792 mL/h) and 538 ± 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 [K^+] and [Na^+] were observed during the races (Table 2). Body mass decreased by 1.4 ± 0.6 kg and 2.5 ± 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 (Δ) and %Δ in BM loss in the 2 races (Figure 1; $P < 0.001$). There were no significant changes in %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 (±SD)	Minimum	Maximum	Mean (±SD)	Minimum	Maximum
Age, y	31.7 ± 11.0	20	56	40.6 ± 10.6	28	54
Prerace body mass, kg	70.0 ± 8.2	57.1	89.4	69.9 ± 8.6	58.4	83.7
Body fat, %	18.1 ± 4.2	11.4	25.6	15.3 ± 4.6	8.2	24.4
Predicted race time, h	2.05 ± 0.38	1.5	2.8	5.32 ± 0.89	3.8	6.3
Actual race time, h	2.15 ± 0.41	1.5	3.0	5.66 ± 1.06	4.1	6.9
Fluid intake, mL	689.3 ± 365.6	150	1400	3186.3 ± 2254.3	400	7700
Drinking rate, mL/h	326.3 ± 180.0	87	792	538.4 ± 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$^+$], 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$^+$], 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$_2$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)

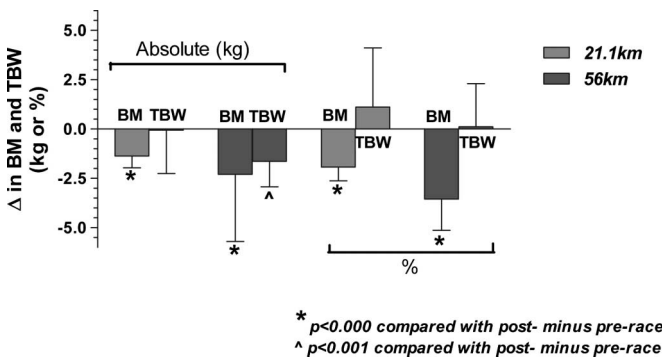Values are presented as mean ± SD.
[K$^+$], plasma potassium concentration; [Na$^+$], plasma sodium concentration; POsm, plasma osmolality; [TP], total protein concentration.
#$P < 0.000$ versus prerace; $$P < 0.01$ versus prerace; *$P < 0.05$ versus prerace; ^$P < 0.001$ versus prerace.

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$^+$] (Figure 4) in both races; however, the %Δ in BM significantly correlated to postrace [Na$^+$] 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$_2$O and plasma [Na$^+$], 135-145 mmol/L)[38] applicable for both Figures 4 and 5.

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

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$^+$] and [K$^+$] within the normal range for athletes completing a standard marathon established by Kratz et al[39] 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$^+$] during exercise in the apparently normal population.[40,41]

Ad libitum drinking was associated with a significant reduction in BM in both races. This condition was termed "voluntary dehydration" by Adolph[42] 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,[43–45] 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."[46]

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
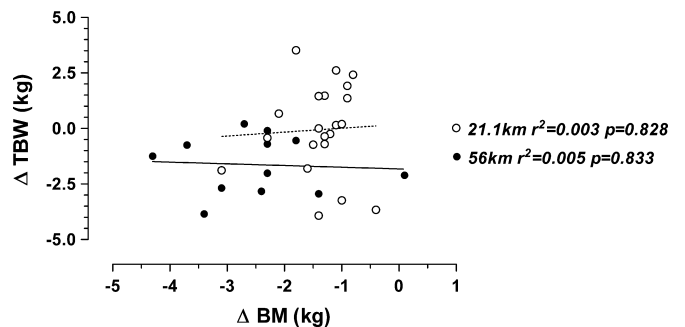

FIGURE 1. The prerace to postrace absolute and percentage changes in (%) body mass (BM) and total body water (TBW) for both 21-km and 56-km runners. *$P < 0.000$ compared with postrace minus prerace. ^$P < 0.001$ compared with postrace minus prerace.

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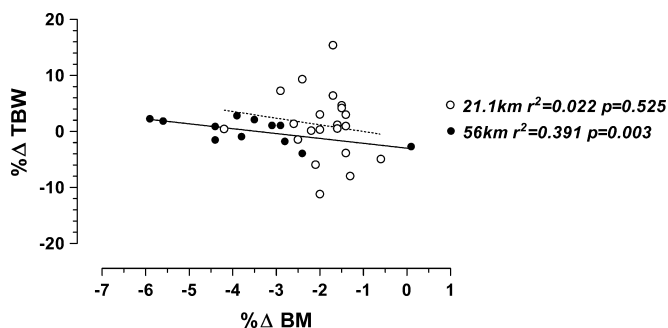
FIGURE 3. Significant correlation between percentage changes in (%Δ) body mass (BM) and total body water (TBW) in the 56-km runners but not in the 21.1-km runners.

between BM and TBW loss suggested by the likes of Maughan et al[10] and Pastene et al.[9] Our findings disagree with those of Baker et al, who concluded that changes in TBW and BM tracked each other in a linear fashion.[12] 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.[13]

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[14,18] support this interpretation, as indeed do the data of Baker et al when analyzed appropriately.[12,13]

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[47] 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.[48]

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.[10,21] 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.[10,49]

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.[2,50] Although changes in BM may be useful to predict fluid homeostasis in certain clinical scenarios at rest,[51] during exercise the homeostatic controls that protect POsm may require a decrease in BM without any negative physiological consequences.[3,52] 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."[44,45] 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.[53,54] 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.[16,17]

Others have reported a dissociation between BM loss and TBW. Colt et al[55] 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
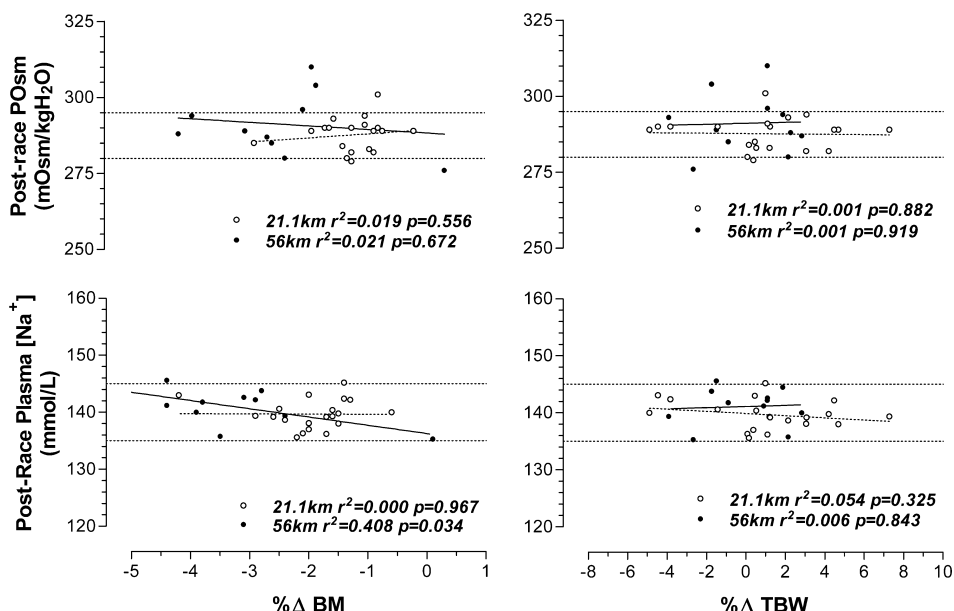
FIGURE 4. The relationship between percentage changes in (%Δ) body mass (BM) and total body water (TBW) in relation to postrace plasma osmality (POsm) and plasma sodium concentrations ([Na+]) in both 21.1-km and 56-km runners.
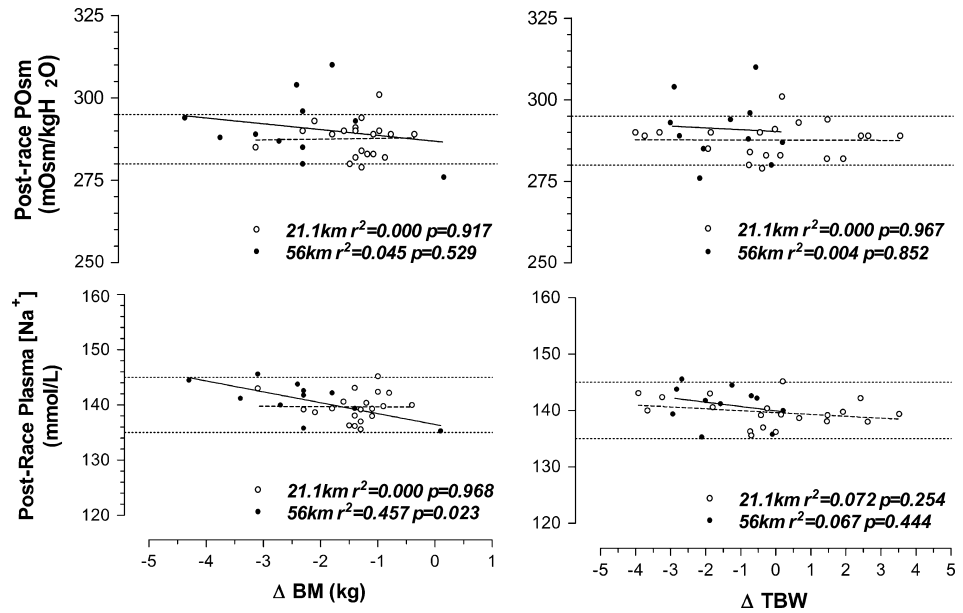
FIGURE 5. The relationship between absolute changes (Δ) (kg) in body mass (BM) and total body water (TBW) in relation to postrace plasma osmality (POsm) and plasma sodium concentrations ([Na⁺]) in both 21.1-km and 56-km runners.

runners, where we observed ~1.9% BM loss with a 1.1% increase in TBW. Similarly, this trend is shown when comparing the changes of both absolute and %Δ 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.[9,56,57]

The only study that has critically assessed changes in BM, muscle glycogen content, and TBW was performed by Olsson and Saltin in 1970.[21] They demonstrated that 3 to 4 g of water may be complexed with each gram of glycogen stored in muscle (and the liver).[21] 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.[14,21,58–60] 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.[39] 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 (~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.[61,62]

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.[63] 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 [~1.4 kg (1.9%) and ~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[3,49,61] that the body primarily defends POsm and plasma [Na$^+$]—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.

ACKNOWLEDGMENTS

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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 ($[Na^+]$), plasma potassium ($[K^+]$) 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 $[Na^+]$ 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 $[Na^+]$ 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 loosing 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 premixed 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):

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

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; **Ea** = enrichment of diluted dose; **Et** = enrichment of tap water used to dilute the dose; **Ep** = enrichment of baseline sample; **Es** = 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:

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

Equation 3:

Percent of body mass (%Δ 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):

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

Equation 5:

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

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

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 ± 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 ± 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 ± 9.2 years (range: 33 - 61) with a mean baseline weight (2-days pre) of 72.2 ± 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 (±1.99) from 2-days pre-race to immediately pre-race but decreased ~4 kg (±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$_2$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
mean		*11.5*	*72.2*	*73.4*	*69.6* #*	*1.1*	*-3.1*	*-3.7*	*1.5*	*-4.1$*	*-5.0$*
±SD		*1.0*	*9.0*	*8.8*	*7.6*	*2.1*	*1.2*	*2.7*	*3.1*	*1.2*	*3.5*

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; $$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
Plasma [K+] (mmol/L)	5.0 ± 0.5 (n= 8)	4.9 ± 0.6 (n= 9)	5.1 ± 0.7 (n= 8)
Plasma [Na+] (mmol/L)	140.0 ± 2.0 (n= 8)	137.3 ± 2.2 (n= 9)	138.5 ± 4.3 (n= 8)
Plasma Osmolality (mosm/kgH$_2$O)	286.1 ± 9.7 (n= 8)	298.2 ± 7.4 (n= 9)	292.9 ± 8.5 (n= 8)
Plasma Protein (g/L)	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)							
	Fluid	CHO A	CHO B	Cola	CHO C	Other	Total (L)	Rate (ml/hr)
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
mean	1697	835	683	1089	500	340	4.50	422
±SD	816	823	427	688		169	1.23	103

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	-
mean	*42.8*	*43.6*	*40.9*	*-1.0*	*-2.5*	*-2.5*	*60.1*	*58.8*	*58.8*	*-0.6*	*-2.0*	*-1.4*
±SD	*5.35*	*6.09*	*5.40*	*1.11*	*0.91*	*0.80*	*2.48*	*5.72*	*4.37*	*4.44*	*1.76*	*4.92*

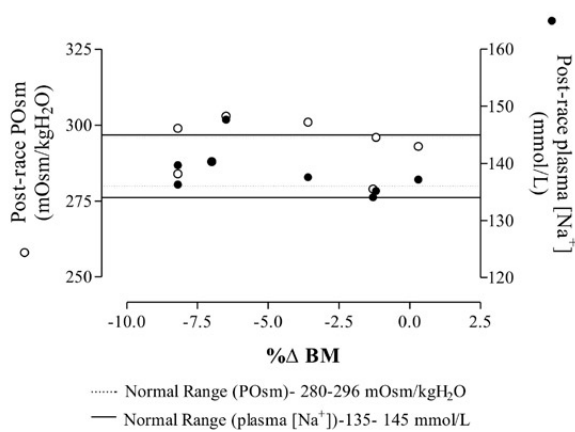TBW- Total body water; A- 2 Days Pre-race; B- Pre-race; C- Post-race; Δ-change; (-) – data unavailable

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

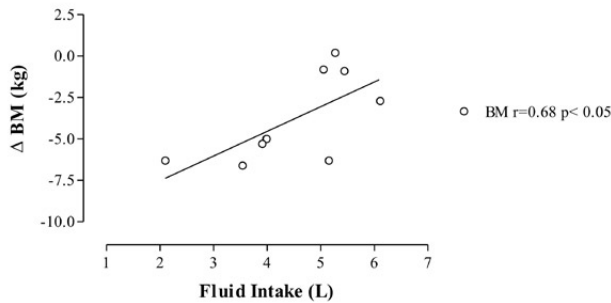
Fig. 3. *Changes (Δ) in body mass (B-C) (BM) in relation to total fluid intake (liters)*
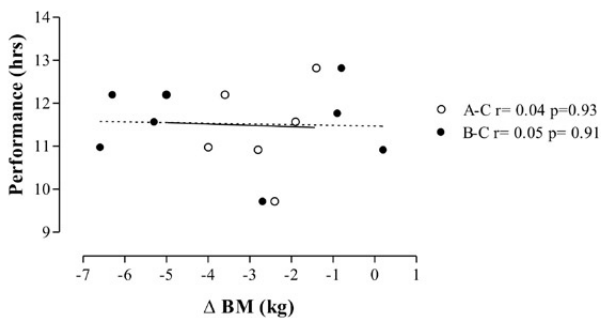

Fig. 4. *Non-significant correlation between change in body mass (ΔBM) and performance (race time) (hours) for both 2-days pre-race minus post-race (A-C) and immediately pre-race minus post-race (B-C) (n = 8)*
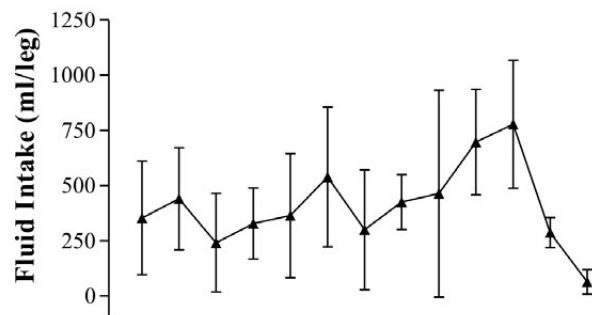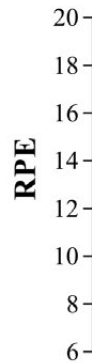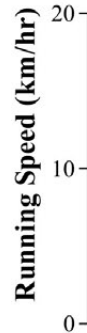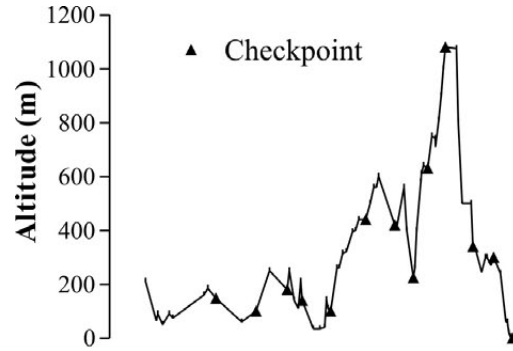

Fig. 2. *The race profile in distance (checkpoints) set against change altitude (m); subject's running speed (km/hr); their ratings of perceived exertion (RPE) and fluid intake from the last checkpoint to the specified checkpoint (ml/leg)*

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 masss 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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