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

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

Nolte H, Noakes TD, van Vuuren B. 2010. Ad libitum Fluid Replacement in Military Personnel during a 4-Hour Route March. *Medicine and Science in Sports and Exercise,* 42(9): 1675-1680.*

*Referencing format in the text and list applied as required by Medicine and Science in Sports and Exercise.

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

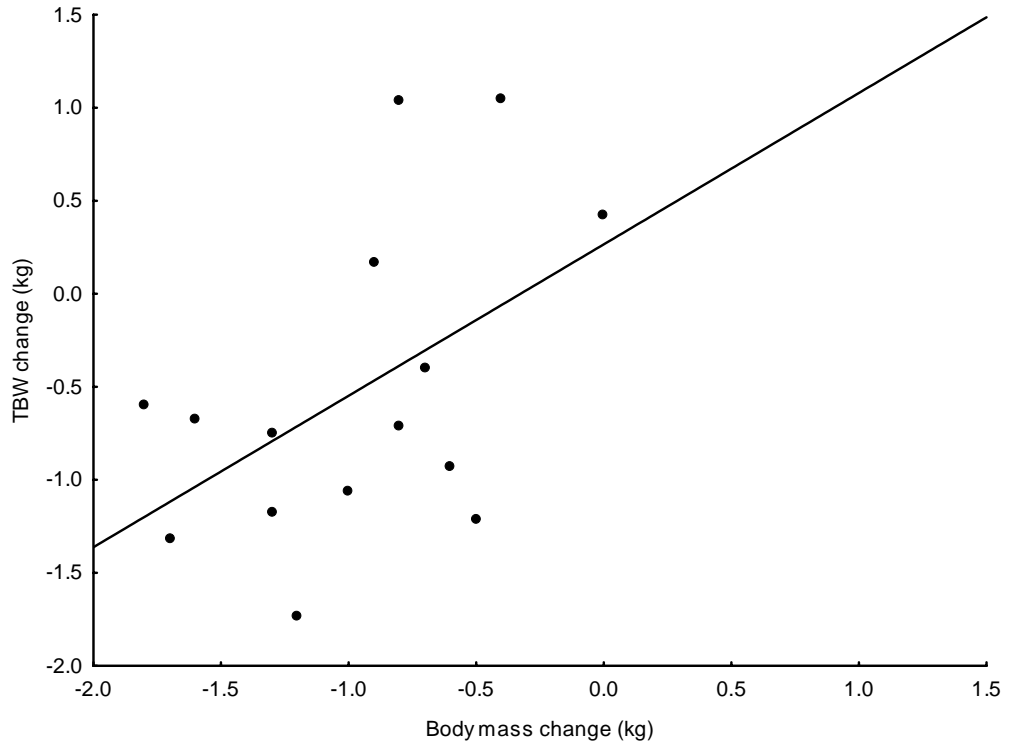**Figure 1:** The relationship between changes in body mass and TBW
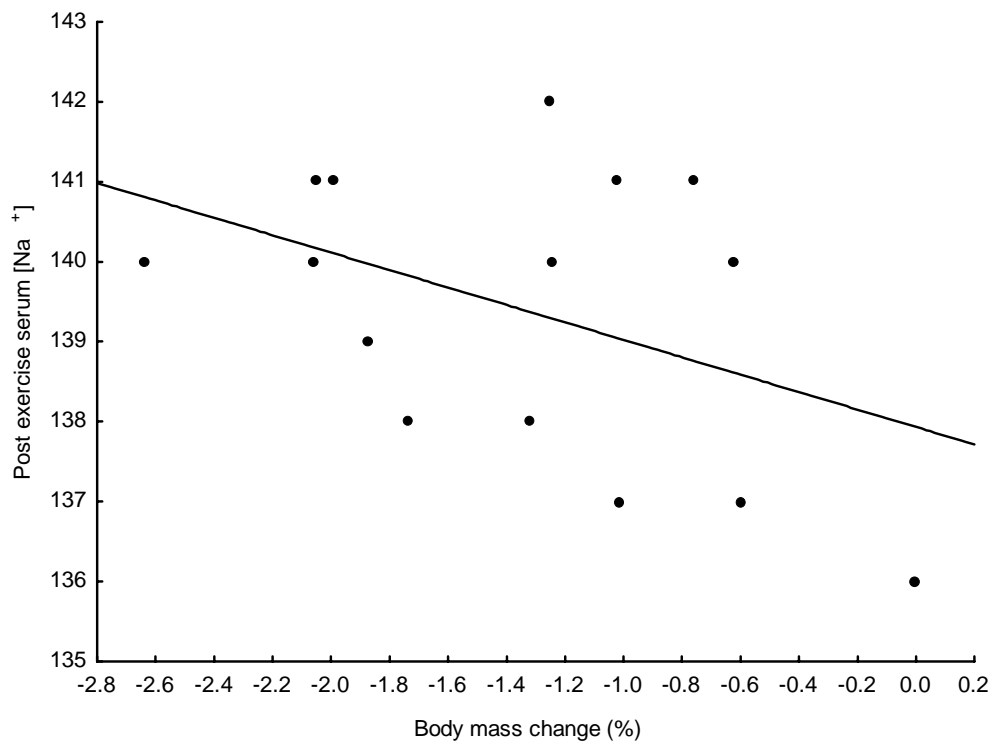
(p>0.05, r = -0.49)

Figure 2: Relationship between post-exercise serum [Na$^+$] and body mass change during exercise (p>0.05, r = -0.42)
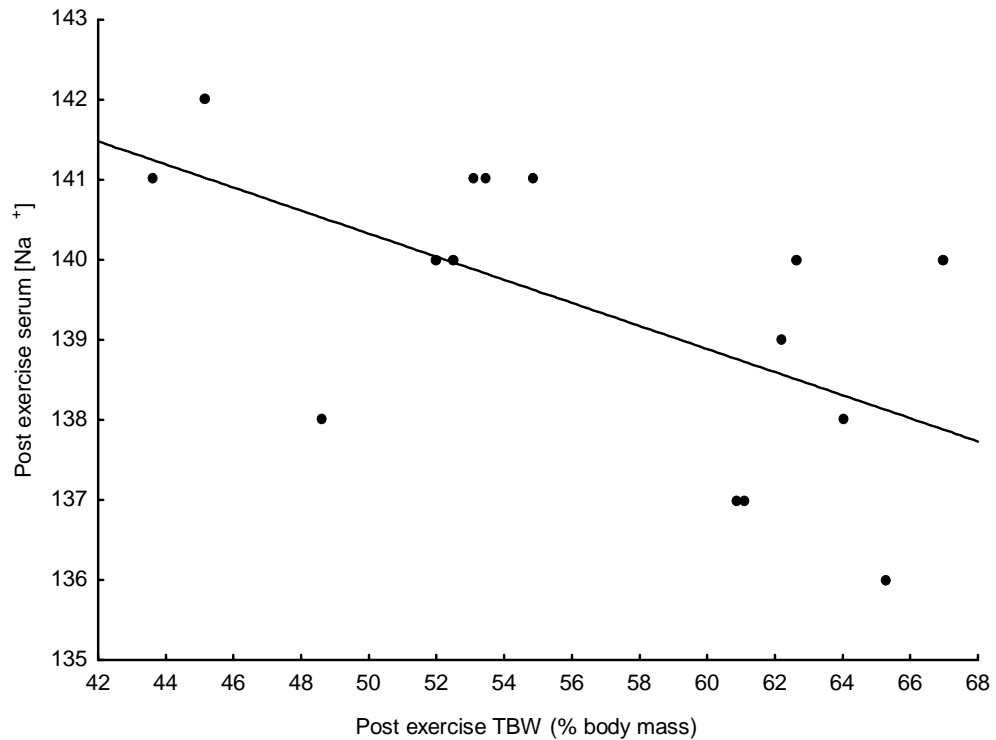
Figure 3: Relationship between post-exercise serum [Na$^+$] and post exercise % TBW (p<0.05, r = -0.59)

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.

4.5 References

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