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THE MAINTENANCE OF BODY FLUID HOMEOSTASIS DURING EXERCISE WHEN DRINKING AD LIBITUM

by

Nicholas Tam

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*UCT/MRC Research Unit for Exercise Science and Sports Medicine,
Department of Human Biology, Faculty of Health Sciences*
UNIVERSITY OF CAPE TOWN

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Supervisor:

Timothy D. Noakes OMS, MBChB MD DSc

Discovery Chair of Exercise and Sports Science

UCT/MRC Research Unit for Exercise Science and Sports Medicine, Department of Human Biology,
Faculty of Health Sciences, University of Cape Town

University of Cape Town

Faculty of Health Sciences, Department of Human Biology,
UCT/MRC Research Unit of Exercise Science and Sports Medicine

DECLARATION

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LIST OF SCIENTIFIC PRESENTATIONS FROM THIS THESIS:

a) ARTICLES IN INTERNATIONAL PEER-REVIEWED JOURNALS:

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LIST OF SYMBOLS AND ABBREVIATIONS

ACSM	:	American College of Sports Medicine
ANOVA	:	Analysis of variance
ANP	:	Atrial Natriuretic Peptide
AVP	:	Arginine-vasopressin
BM	:	Body mass
BMI	:	Body mass index
Δ	:	Change in
CV	:	Co-efficient of variation
D₂O	:	Deuterium oxide
EAH	:	Exercise- associated hyponatremia
EAHE	:	Exercise-associated hyponatremic encephalopathy
ICC	:	Intra-class correlation co-efficient
IMMDA	:	International Marathon Medical Directors Association
POsm	:	Plasma osmolality (mOsm/kgH ₂ O)
PV	:	Plasma volume
[K⁺]	:	Potassium concentration (mmol/L)
RWL	:	Respiratory water loss
[Na⁺]	:	Sodium concentration (mmol/L)
SIADH	:	Syndrome of inappropriate anti-diuretic hormone secretion
TBW	:	Total body water
[TP]	:	Total protein concentration (g/L)
UOsm	:	Urine osmolality (mOsm/kgH ₂ O)

ABSTRACT

The prescription of an optimal fluid intake during exercise has been a controversial subject in sports science over the past decade. Only recently has it evolved from “blanket” prescriptions to more individualised recommendations. Currently the American College of Sports Medicine (ACSM) advise that sufficient fluid should be drunk in order to ensure that body mass (BM) loss does not exceed >2% of starting BM in order to avoid exercise-associated medical complications. Historically BM changes have been used as a surrogate for fluid loss during exercise. It would be helpful to accurately determine fluid shifts in the body in order to provide physiologically appropriate fluid intake advice. The measurement of total body water (TBW) via deuterium oxide has been found to be the most accurate measure to detect changes in body fluid content. Thus the aim of this thesis was to understand body fluid homeostasis during exercise when drinking according to the dictates of thirst (*ad libitum*).

This thesis begins with a review of the literature examining the basis for fluid intake prescription with the use of BM, the concepts of “voluntary and involuntary dehydration” and the major routes by which the body is potentially able to gain and lose fluid during exercise.

We initially found that changes in TBW are more variable than BM at rest, although technical machine error accounted for a majority of the total error in the TBW measurement. Interestingly BM remains very stable at rest compared to the dynamic nature of changes in TBW both daily and weekly. We also found that measurements of both BM and TBW produce reproducible results at rest.

Our first finding was that with the adoption of an *ad libitum* fluid intake during exercise athletes participating in the study were able to finish races of varying distances without any medical complication along with a >2% BM loss. Which leads onto our second and more important finding that we have also demonstrated that despite a >2% BM loss, all of our subjects finished their respective races whilst maintaining plasma sodium concentration ($[Na^+]$) and plasma osmolality (POsm) within the normal range when drinking *ad libitum*. This finding demonstrates the reality of drinking in athletes competing in various types of foot races and that it is unnecessary to drink to maintain BM in order to successfully complete races of any distance

Thirdly and most pertinent finding was that associated with this >2% BM loss we measured TBW changes during these races and found that changes in BM do not track changes in TBW during real-life competition in athletes when drinking *ad libitum*. This finding illustrates that to some extent, sweat losses during exercise are offset by internal water sources associated with metabolic water formation and water associated with glycogen storage ensuring the maintenance of body fluid homeostasis.

It was also noted that athletes performing the best often experience the greatest BM loss during the 21.1km and we found a similar trend in the 56km race. We have suggested that this can be explained by both behavioural and physiological reasons. Lastly all athletes successfully completed their respective races without encountering any exercise-associated medical complications with the adoption of an *ad libitum* fluid intake approach.

The outcomes from this thesis support the prescription of more physiologically appropriate advice for fluid intake prescription during exercise. We hope that these

studies will provide adequate corroboration that during exercise together with an *ad libitum* approach, athletes are able to maintain adequate hydration (maintenance of POsm and plasma [Na⁺]) regardless of significant decreases in BM, which is often associated with superior performance in some athletes.

University Of Cape Town

CHAPTER 1

Introduction and Scope of the Thesis:

**THE QUANTIFICATION OF BODY FLUID HOMEOSTASIS
DURING EXERCISE**

University Of Cape Town

1.1) General Introduction:

Mindful of complications such as exercise induced heatstroke, those competing in ultra-endurance events are encouraged to be vigilant regarding their fluid intake. Athletes are advised to avoid sustaining a decrease in body mass (BM) during exercise as this “dehydration” is perceived to be detrimental to performance and even increase the probability that heat related illnesses can occur^{103;104}.

In 1996 the American College of Sports Medicine (ACSM) published a position stand providing guidelines on exercise and fluid replacement²⁰. Their recommendations encouraged athletes to ingest fluid equivalent to the amount of BM lost during exercise²⁰. A recent review by Beltrami *et al* (2008) investigating the recommendations subsequent to the advent of this position stand noted that there was an increase in the dissemination of advice in the academic literature that athletes should regulate their drinking in order to maintain BM. This conflicts with the established physiological principle that plasma osmolality (POsm) is the physiologically-protected variable which is regulated by the stimulation of thirst^{10;123}.

Since the adoption of these guidelines there had been a disturbing increase in the number of cases of exercise associated hyponatremia (EAH) and even death due to exercise associated hyponatremic encephalopathy (EAHE)⁸³. This troubling rise and lack of consensus on the cause led to the holding of the 1st International EAH Consensus Statement, on the aetiology and risk factors for EAH⁴³. EAH is often described as a life threatening and totally preventable condition, its predominant aetiology has been hypothesized as a dilutional hyponatremia associated with the syndrome of inappropriate anti-diuretic hormone secretion (SIADH)⁴⁴. This consensus lead to a general understanding and a directed focus in future research

into the aetiology and management of EAH. Subsequently the ACSM sort to rethink their recommendations for fluid replacement during exercise replacing the 1996 guidelines with new guidelines published in 2007. These new guidelines include the advice that athletes should drink to thirst (*ad libitum*) but should now strive to prevent a >2% BM loss during exercise¹⁰³.

It is evident that recent fluid replacement guidelines have evolved from blanket fluid replacement²⁰ to more individualised prescriptions¹⁰³, even though recommendations are still often disseminated to athletes participating in endurance events to replace fluids, based on the extent of BM lost¹³⁵. These prescriptions are encouraged because data obtained from tightly controlled laboratory trials have demonstrated that athletes who ingest fluid according to thirst or who restrict their fluid intake, do not maintain a constant BM during exercise^{37;135}. This is thought inappropriate because a decrease in BM is considered to be an indicator that the thirst mechanism is inadequate to properly replenish the body's water stores or return the body to a state of euhydration. It is argued that this apparently dehydrated state places athletes at risk of heat illness, ill health and impaired athletic performance^{88;103}.

It is commonly assumed and accepted that during any bout of exercise regardless of duration the amount of fluid loss is exactly equivalent to the BM loss¹⁰³. But this conflicts with the historical findings that athletes regularly lose 5-6% of their BM during ultra-endurance exercise lasting 5-24 hours, whilst maintaining proper fluid homeostasis and without developing any medical complications^{2;66}.

It should be apparent that fluid regulation is far more complex than can be assessed purely by gross BM changes before and after endurance exercise, which may very well overestimate the extent of body fluid losses^{55;73;100}. Thus it would be most

beneficial to investigate the physiological markers and the actual state of hydration in relation to changes in BM during endurance exercise.

1.2) Concepts in Hydration

Defining euhydration is complex because the body's water content varies as a sinusoidal rhythm. A common definition for euhydration^{37;88}: a BM that is relatively stable (within 0.45kg from day to day) with the maintenance of an adequate fluid intake to sustain normal urine volume and concentration. To this definition may be added more physiologically appropriate terms such as: a relatively stable TBW content alongside the maintenance of plasma sodium concentration ($[Na^+]$) and POsm, although these measurements are not as easily measured as BM⁸⁸. A majority of these variables seem appropriate during daily sedentary activity but during endurance exercise some of these variables may be altered in order to maintain the *milieu interior*²⁶.

The use of BM as a marker of hydration status during exercise begins perhaps with Adolph and Dill (1938) who reported that humans walking in the desert heat for several hours did not drink sufficiently to prevent some degree of BM loss during the exercise bout³. As a result of this observation the term "voluntary dehydration" was coined by researchers to describe a state of BM loss in the presence of an adequate fluid supply³⁸. This has been hypothesized to occur because of a time delay before sweat losses are fully replaced causing a temporary reduction in TBW. As a result it is argued that the thirst mechanism is inadequate to maintain body fluid homeostasis during exercise^{88;89}.

More recently Greenleaf (1992) used the term "involuntary dehydration" to describe the failure of the thirst mechanism to maintain baseline BM^{37;103}. Laboratory

experiments have demonstrated that “voluntary dehydration” (>2% BM loss) is associated with impaired aerobic exercise performance in temperate conditions¹⁰³. In contrast to these laboratory findings recent research conducted during ultra-endurance events has found that often the best performing athletes finish apparently the most “dehydrated” (>6% loss of BM)^{55;108;109}.

Interestingly a recent statement by Hew-Butler *et al* (2006) described this model of understanding as: “to assume the thirst drive would be an ‘inaccurate index’ of fluid balance during exercise would seem contradictory to the evolution of our species”⁴⁹. A pertinent statement which refers to the fact that researchers investigating fluid balance relationships during exercise often forget to consider the complex integration of the brain (i.e. hypothalamus) and how it governs the body’s *milieu interior* (maintenance of POsm and plasma [Na⁺]) in regards to the body’s ability (via osmoreceptors in the brain & baroreceptors in the heart) to maintain body fluid homeostasis via mechanisms developed during our evolution into terrestrial beings²⁶. These evolutionary capabilities have liberated us from having to continually seek water every moment of the day²⁶.

It seems that fluid-associated BM loss could be defined as a voluntary dehydration until the level of water loss alters the *milieu interior* causing the exercising body to stop exercising as occurs in the more severe stages of dehydration as illustrated by Adolph in 1947². However whilst severe dehydration should be avoided to prevent a decrease in exercise performance and to maintain overall health, it is evident that strictly adhering to replacing all BM loss with fluid intake during exercise will contribute to a state of body fluid overload resulting in a multitude of problems such as gastric discomfort, nausea and possibly causing decrements in performance and more severely EAH^{83;118-121}.

This life threatening condition has arisen because some researchers have not questioned critically the use of BM as an absolute indicator of body hydration status during exercise. This has long been a contentious issue when considering the inability for BM to account for various factors affecting the body's fluid homeostasis^{45;49;108;109;118}.

There are many factors that can influence the body's hydration status during exercise and that would contribute to the TBW pool. These include gains in TBW via routes through eating, drinking, metabolic water formation (water as a product of fuel metabolism) and glycogen-associated water liberation (water released from the bonds formed when glycogen is stored in muscle and liver) or factors associated with the loss of TBW such as respiratory and cutaneous water loss, gastrointestinal loss and renal water clearance^{15;56;73;75;87;88;90;93;103;111}. All need to be considered if the changes in TBW during exercise are to be understood properly.

1.3) Dynamics of Body Fluid Balance

What is lost?

i) Renal water clearance:

This is the loss of water through the process of filtration in the kidneys which are the primary controllers of water balance in the body. The kidneys are controlled by various systems such as the renin-angiotensin-aldosterone system and anti-diuretic hormones including arginine vasopressin (AVP) and atrial natriuretic peptide (ANP)^{21;127}. Through these hormonal actions there would be either an increase or decrease in the re-uptake of fluid passing through the kidneys tubules resulting in an increase or decrease in the concentration of urine excreted.

This wholly depends on the state of hydration of the person, the greater the fluid intake the greater the fluid volume excreted as urine and vice versa. This is tightly controlled in order to maintain body fluid homeostasis to prevent either over hydration or water loss resulting in severe dehydration⁴⁹.

When exercising various mechanisms work to prevent perturbations in fluid homeostasis. It has been observed that the body is unable to maximally suppress AVP secretion during exercise so that a state of relative fluid retention occurs during and sometime after exercise⁴⁶. Under these circumstances any relative overdrinking combined with unsuppressed AVP secretion will greatly influence the probability for the development of EAH, hence a dilutional hyponatremia⁴⁴.

The average urine clearance for normal individuals is 1- 2 litres per day but can range from 0.5 – 4 litres^{73;113}. It has been stated that there is little reduction in urine flow rate during prolonged exercise but in more strenuous exercise a reduction of 20-60% has been reported⁵³. Thus renal water clearance during prolonged exercise could be considered to be highly individual but suppressed during prolonged exercise through elevated AVP secretion.

ii) Respiratory water loss

Respiratory water loss (RWL) is the loss of water associated with respiration. Daily RWL for individuals at rest in temperate climates are approximately 400ml and can reach 1500ml/day when performing hard work in dry air⁷⁵. Water losses from this route are a function of respiratory minute volume and the vapour pressure gradient between ambient air and the lung surface according to the following equation⁷⁵:

m_e = rate of evaporative water loss [g/min]

VO_2 = oxygen uptake in [L/min (Standard Temperature and Pressure)]

P_a = water vapour pressure [mmHg]

Mitchell *et al* (1972) described the rate of water loss through the respiratory tract as roughly 2-5g/min during exercise exceeding 1.5 L/min (VO_2) in a dry environment with a vapour pressure of 10 mmHg. There is no associated solute (electrolyte) loss from this means of fluid loss^{73;75}. This loss is said to roughly equal the release of metabolic water as a result of substrate breakdown^{73;103}.

iii) Gastrointestinal water loss

The normal loss of water through faecal excretion is ~100–200 ml a day, excluding the gross mass of the actual stool. Most fluid ingested is reabsorbed by the small intestine and the colon^{57;73;103}. Faecal water loss does not usually occur in the normal “healthy” individual during exercise^{57;103}. Thus fluid loss via this route during prolonged exercise should not account for much.

Interestingly Ladell (1955) first hypothesized a possible fluid reserve of up to 2 litres in the body that may not require fluid replacement in order to ensure that whole body fluid homeostasis is maintained⁶⁶. This has led to the hypothesis of a possible fluid volume that exists in the gut and can be drawn upon to offset some fluid losses from the body. This would explain why some BM losses of up to 3% may not carry any consequence on physiological function or performance during prolonged

exercise^{73;77;109}. Consequently this possible fluid store when assimilated at cellular level will offset a fluid deficit.

iv) Cutaneous water loss

Cutaneous water loss plays a major thermoregulatory role during exercise due its cooling effect through evaporation but at the cost of fluid loss¹⁰⁴. Sweat is water secreted through sweat glands as a hypotonic fluid (when compared to plasma); the rate of sweat loss is affected by various factors and biological variables such as the exercise intensity and ambient temperature. The secretion of sweat consists mainly of water, urea and sodium at a concentration that varies depending on the habitual sodium intake and the rate of urinary sodium loss (since losses in sweat and urine must balance the sodium intake).

The average person sweats between 0.5- 2.0 L/hr. This is due to a variety of factors, the most important factors being metabolic rate (includes the size of the person and their predisposition to sweating), temperature and humidity¹⁰³. Currently theoretical sweat loss is most appropriately determined through this equation⁷³:

$$\text{Sweat Loss} = (\text{body mass loss}) + (\text{ingested fluid}) - (\text{substrate oxidation}) + (\text{metabolic water}) - (\text{respiratory water loss})$$

Analysis of sweat rates and collection of sweat is a difficult task due to the function of sweat as a thermoregulatory control mechanism utilizing evaporative heat loss¹¹⁴. Thus in order to collect sweat it is necessary to prevent evaporation of the sweat collected (thus hampering cooling) and prevent contamination of sampling^{67;114}. Various techniques have been used but none has been shown to be scientifically sound for measuring whole body sweat rates. Methods that have been

used include closed-pouch collection⁴², sweat patches, whole body wash downs, whole-body sweat collection in a large plastic frame¹¹⁴ and more recently collection via technical absorbent materials that can define regional sweat rates and sweat electrolyte concentrations⁴¹.

It seems all have their shortcomings but the ability to collect the whole-body sweat to measure rates is logically the most reproducible because it does not rely on a specific area to estimate whole body sweat rates since regional sweat rates vary. New advances in this field have prompted interest in the neuro-humoral control of sweating¹³². Further investigations should be conducted to elucidate the exact mechanisms which that may be involved in the regulatory factors that would contribute towards sweat regulation and whole body fluid homeostasis during exercise.

What is gained?

i) Food and fluid consumption

Maintaining adequate hydration and energy replacement is necessary for optimizing sporting performance and health during prolonged exercise⁷². Specifically for ultra-endurance exercise athletes should consume enough energy and fluid to maintain and prevent decrements in performance or increase their risk of heat-illness⁹⁸.

As mentioned previously the prevention of both extremes (no fluid intake and overconsumption) should be avoided. The most apparent and preventable development has been EAH due to overzealous fluid consumption associated with SIADH⁴⁴. The most probable explanation for the recent appearance of EAH in ultra-endurance sporting events has been the promotion of fluid replacement equal to BM

loss or to drink to “stay ahead of thirst” or “as much as tolerable” in order to maintain health and prevent any decrements in performance⁷⁹.

Monitoring fluid and food consumption in race settings is difficult and cumbersome due to the nature of competitive races in which subjects are competing and as a result of psychological and logistical factors that hamper data collection. These include the difficulty of determining exact quantities of fluid consumed because not all the fluid in a measured volume may be ingested, but could be used for cooling of the head and body. A similar difficulty is experienced because of large numbers of athletes participating. This makes it difficult to identify subjects in an experiment and to monitor their fluid usage.

It is often easier to use the fluid recall method which involves the recall of the total amount of fluid ingested post-race^{108;109}. This is an indirect and subjective report of fluid consumption and thus not totally reliable but practically useful. It would be most beneficial to determine the fluid intake habits of athletes during ultra-endurance competition and especially to establish whether an *ad libitum* drinking approach is adequate to prevent the development of serious medical complications, allowing athletes to complete these events adequately hydrated.

Recent research has found that adopting an *ad libitum* approach to general food and fluid consumption adequately maintains day-to-day euhydration in elite Kenyan athletes with high daily- training demands³³. Following this, it will be of great interest to determine whether the body is indeed in a state of dehydration during athletic competition when athletes will be eating and drinking *ad libitum*.

ii) Metabolic water formation

Metabolic water is an inevitable end-product of the oxidation of fats, carbohydrates and proteins when broken down to produce the energy needed to perform work. Metabolic water formation is most commonly calculated using stoichiometric equations for substrate oxidation such that 1g of carbohydrate oxidised will result in the 0.6ml water formation⁷³. In regards to fat oxidation 1g of fat will result in the generation of 1.3ml of water^{19;73}. An invaluable consideration when working out the formation of metabolic water is that the rate of substrate oxidation is dependent on the work intensity and that the preference for fuel for oxidation is individual for each athlete⁹³.

Pivarnik *et al* (1984) calculated that the metabolic water formation produced during endurance exercise would be of minimal help in maintaining the plasma volume⁹³. However the researchers did not state whether metabolic water formation would replace the intracellular volume and not directly contribute to extra-cellular fluid re-hydration⁷³. Although metabolic water production did not provide an adequate supply to replenish the extra-cellular volume this does not mean that it did not contribute to the net gain in the TBW pool⁹³.

Additionally a study conducted during a 90km cross-country ski race calculated that there was a possibility of 2kg of endogenous water liberation, where contributions were accounted for 1kg from fat and carbohydrate oxidation and 1kg of water contributed from glycogen store mobilization thus an addition of 2 litres of fluid was added to the body's hydration status and further an additional irreversible 2kg loss of BM in the athletes post-exercise⁹⁷.

The 2007 ACSM Guidelines for Exercise and Fluid Replacement state that metabolic water formation does not produce a net water gain since it merely offsets RWL¹⁰³. It would be incorrect to ignore the contribution these sources contribute to body fluid homeostasis during competition because environmental and individual factors change and so will RWL along with the rate of metabolic water production^{45;97;100}.

iii) Glycogen associated water stores

Widely acknowledged as a stored constituent with glycogen storage, and often associated with the noticeable increase in BM when a high carbohydrate diet is ingested, is the water associated with glycogen storage. Still a contentious issue in the field of physiology is the exact amount of water associated with glycogen storage in the muscle and the liver.

However it is widely understood and taught that glycogen units are stored in an osmotically inactive state in the human body¹²². The reason for this would be to prevent great alterations to the body's cellular solute concentration, in order to prevent significant changes in cell osmotic pressure. Thus water associated with the storage of glycogen should not affect the state of fluid homeostasis due to the "bound" nature of the solute it is joined to until the stored glycogen is broken down. Thus water released from this breakdown should contribute to offset the fluid lost from the body.

It was in 1906 that Zuntz and his colleagues proposed the widely acknowledged ratio of 3g of water associated with each gram of glycogen but they did not directly measure this. Instead they calculated the relationship from data presented by Pavy in 1860 who at the time was experimenting on sugar (carbohydrate) formation in the

liver^{91;136}. It would take some years until methods were developed that possessed a greater degree of precision.

Progressing Gamble *et al* (1923) studied the chemical changes resulting from a fast illustrated this trend³⁵. An observation during the early days of fasting was of “a very striking loss of body water, largely extracellular in origin”. When the fast was broken by giving carbohydrate, in energy content far below the caloric needs, a gain in BM was accompanied by retention of water. Similarly Hoelzel (1928) noted on himself the biochemical phenomenon of an increased mass gain with an increase in carbohydrate consumption concomitantly with oedema due to water retention⁵².

But it was researchers studying glycogen storage in the liver, Puckett and Wiley (1932); McBride *et al* (1941); Mackay and Bergmann (1932) and Bridge and Bridges (1931; 1932)^{12;13;70;71;74;95} who propelled the field forward by performing experiments that enabled them to determine liver glycogen concentrations and associated water storage. Puckett and Wiley found that 2.4g of water was stored with 1g of glycogen in albino rats with varying glycogen contents in their liver and concluded by postulating that the liver needed be considered a possible site for water retention when fluid balance is studied⁹⁵. MacKay and Bergman established in rabbits that there was a direct proportion in which water is stored with glycogen storage in the liver. They concluded that although their results did not directly support the 3g of water to 1g of glycogen theory, their data did not oppose it either⁷⁰. Whilst McBride and colleagues concluded after experimenting on a hundred rats, that during glycogenesis, as long as there is no change in non-glycogen solids of liver, there is 2.7g of water associated with 1g of glycogen storage⁷⁴. The association of greater than 2g of water was confirmed as the breakdown of 1g of glycogen released that amount of water.

The only study to investigate this complex relationship in humans was that of Olsson and Saltin (1970) who found a 2.4kg increase in BM and a 2.2 litre increase in TBW measured with the use of tritium (^3H) labelled water, which was assumed to be the result of glycogen storage in the muscles and the liver amounting to approximately 500g. They concluded that 3-4g of water would be associated with glycogen storage in the whole body, particularly in the liver and muscle⁸⁷.

This was disputed by Sherman *et al* (1982) whose data from Sprague-Dawley rats found an inconsistent ratio between storage of muscle glycogen and water¹¹¹. Although they disagreed with the findings of Olsson and Saltin (1970), their study only investigated muscle glycogen concentrations, utilized different experimental models and did not investigate TBW changes and whole body glycogen storage in muscle and liver as had the study of Olsson and Saltin.

1.4) **Total body water (TBW):**

In the 'normal' (70kg) human, 60% of BM (approximately 42L), with a range from 45 to 75% of BM comprises of TBW^{7;92}. TBW is the measure of the body's entire water content representing what would be considered the true hydration state at the time of sampling assuming the subjects have normal blood reference values and are in good health. Body fluids composed of the largest amount of water are: cerebrospinal fluid and bone marrow fluid (are composed of 99% water), blood plasma (85%) and the brain (75%)⁹².

Body fluid homeostasis is essential for electrolytic, acid-base and thermal balance and is the medium in which bodily processes take place^{90;92;113}. Water turnover by the body ranges from 5-10% (3-5L) of TBW daily. Studies have observed that the

level of physical activity will promote a higher rate of water turnover in a healthy state because well-trained subjects have a higher water turnover as a result of the habitual exercise^{34;112}. TBW is regulated within ± 0.2 to 0.5% of BM at rest⁷. It has been stated that no one true absolute value for TBW can be assigned¹⁰⁵ although it has been proposed that a water deficit of greater than 2% of BM falls outside the “normal” TBW fluctuations. This statement might hold true for short exercise durations (<2 hours) and at rest but there has been little direct research to prove this statement.

The use of isotopic tracer methodology to measure the body's total water content has been the accepted gold standard for measurement of hydration¹¹³. The use of the stable isotope deuterium oxide (D_2O or 2H_2O) has emerged as the most acceptable tracer for modern use. Other tracers that can be considered include tritium (3H) or tritiated water (3H_2O) which is used less frequently in TBW studies because of its radioactive nature and a half-life spanning 12 years which could render it potentially hazardous¹⁹.

Initial studies found the optimum tracer equilibration period to be longer than 4-6 hours when sampling from urine⁶⁸. More recent research has found that tracers are distributed quite rapidly throughout the body water spaces. Thus an equilibration time of 2-4 hours has been found to be adequate when sampling with saliva, 2.5-5 hours for urine and 1.5- 6 hours for blood^{54;68;112}.

Samples are usually corrected for the exchange with non-aqueous hydrogen²³. Analysis is carried out with the use of a ratio mass spectrometer³⁹ for the stable isotopes and with a scintillation counter when analyzing tritiated samples^{19;69}. Colt *et al* (1978) utilized 3H during exercise and stated that 3H might not be the best tracer

to use because of the increase in ^3H space as the result of an increase in non-aqueous hydrogen-exchange in the post-exercise period¹⁹.

Precision with the use of D_2O tracers used to analyse TBW has found to have the accuracy of 0.5% according to the method of Halliday and Miller³⁹. Bartoli *et al* (1993) found that TBW measurements are repeatable with a ~4% CV of which 60% is inherent in the dilution technique⁷.

Although the use of D_2O is possibly the best method by which to assess TBW, the problem is the ability to attain instantaneous (quick) measurements. Because this involves the use of an isotope ratio mass spectrometer that determines the tracer's mass compared to the rest of the solution it is contained in, which is costly, cumbersome and inconvenient⁶⁸.

Previous research by Fusch *et al*³⁴ examined TBW and TBW turnover during a 7-day trek at a moderate altitude. They found TBW decrease by 2L and then stabilized over the first few days of the trek this was associated with a decrease of 0.8kg of BM over the entire trial³⁴. This leads us to further question, are changes in TBW equivalent to changes in BM during acute or prolonged durations of exercise? By obtaining TBW and POsm concurrently post-exercise it could be established whether an athlete's body fluid homeostasis really does change when drinking *ad libitum* and to determine to what extent does TBW have to change in order to alter POsm. Also whether there is a gram-to-millilitre relationship between changes in BM and TBW.

TBW has been investigated by O'Brien *et al*⁸⁶ who measured TBW via D_2O before and after an eight day, moderately cold-weather (1-3°C), military training exercise. They observed a significant decrease in TBW over the eight day study which they

believed was due to a noticeable decrease in lean BM and fat mass. They concluded that despite high activity levels, significant BM loss and negative energy balance, body fluid balance was maintained over the eight days. Thus adequate hydration was possible during such demanding activities. Similar findings were observed by Knechtle *et al* (2008) who used bio-electrical impedance to monitor athletes during a 1200km multi-day stage running race. Alongside a significant loss in BM there was a noted increase in %TBW, despite a decrease in skeletal muscle mass and fat mass. The authors further confirmed this finding with a cohort of female runners during a 100km ultra-endurance run. They found that despite a 2.2% decrease in BM, TBW content was maintained with an increase in %TBW⁶¹.

Recently Baker *et al*⁵ measured TBW utilizing the tracer methodology for pre-exercise TBW determination and concluded that TBW changes track BM changes during exercise⁵. But various issues plagued their methodology for example the exercise protocol which lasted in total 2 hours but was intermittent and did not reproduce conditions present during prolonged endurance exercise. Their protocol also required the subjects to drink to maintain, decrease or increase their BM to absolute values which is not reflective of normal fluid intake behaviour and could be construed as BM and TBW manipulation with the use of forced fluid ingestion regimes for subsequent measurement of TBW. Lastly their TBW calculations initially calculated TBW with the tracer methodology but post-exercise TBW was not directly quantified instead they corrected for isotope loss, via sweat sample collection, breathe vapour, urine and non-aqueous hydrogen exchange. This would prove somewhat problematic because it would be necessary to assume that the whole body sweat and breathe D₂O concentration is the same and that whole body sweat loss is purely BM loss and that breathing rate was consistent over the entire exercise trial. The other errors in their methodology and analysis have been described⁸⁵.

Thus by tracking TBW changes with the diluted isotope method before and after exercise it would be possible for researchers to establish whether the amount of BM lost during exercise is exactly equivalent to the amount of fluid lost from TBW during exercise.

1.5) Body mass (BM) as a marker for fluid balance:

BM change is the most popular proxy for TBW balance because it is non-invasive and easily to administer^{57;103;113}. This is based on the assumption that a BM loss of 1g is equivalent to the loss of 1ml of water⁷³. The use of BM changes has been established because multitudes of studies have determined that a majority of water loss is achieved through excretory mechanisms such as urination and sweat loss which the latter being the major source of fluid loss during exercise, in order to regulate the body's temperature.

Other clinically applicable reasons are the use of BM in chronic clinical scenarios, in these conditions it has been found that the use of BM is an accurate and reliable day-to-day measure. But it seems that these changes in BM are more closely associated to regulation of fat mass than fluid balance^{1;16}.

Although during acute exercise the differences in BM and TBW are assumed to be directly proportional. This is not necessarily true during ultra-endurance exercise for bodily functions such as mass lost due to the use of endogenous fuel sources and water complexed during glycogen storage are not taken into consideration as irreversible^{73;108;109;118}.

Fluid consumed during exercise does not usually equate to the fluid “lost” during exercise. This “dehydration” is considered to place the athlete at risk of heatstroke²⁰. But this “loss” is a result of the use of BM changes during exercise on the assumption that all the BM lost during exercise is a resultant of fluid loss alone. This may be true for acute and intermittent bouts of exercise but not necessarily for prolonged exercise. Consequently is often translated into fluid replacement prescription for endurance exercise resulting in errors in estimation of hydration status and possibly encouraging overzealous fluid consumption⁵⁵.

Potential confounding factors that affect the accuracy of these measures is fluid loss as a result bowel movements, clothing mass, acute BM gain due to carbohydrate loading and food and fluid consumption could alter the readings in obtaining a reliable measure of true baseline BM. This could be carried over as an error in measuring body fluid homeostasis⁷³. Other issues involving the accuracy of the scale used to measure BM is that the ground must be level and the scale calibrated

Concern should be taken when using BM to explain changes in body fluid balance especially during prolonged endurance exercise. Physiological cues such as the thirst mechanism should not be ignored at the ease of utilizing BM changes. Physiologically the body is unable to determine BM and track changes involved in acute physical mass loss unlike more appropriate markers such as serum $[Na^+]$ and $POsm$ (influencing the thirst mechanism) which have been established as the main indicators to body fluid homeostasis^{45;48;126}.

Often observed in laboratory studies is that a >2% BM loss is associated with decrements in exercise performance. However BM changes have been tracked over a 12- and 24- hour marathon in which researchers found that there was a significant decrease in BM of 2.9% (ranging from 0 to - 6.5%) for the 12-hour race and 5.1%

(ranging from -0.8 to -11.4%) for the 24-hour race. It was noted that BM fell during the first 8 hours of exercise and from there on BM fluctuated about a new “baseline” value⁵⁵.

That study amongst many others should discourage the concern that BM must be maintained during exercise through excessive fluid intake. Further Dill *et al* hypothesized in 1933²⁷ that humans do not defend BM during exercise but rather protect POsm. It has been observed that BM loss can occur without a decrease in TBW, thus questioning the maintenance of BM to maintain fluid homeostasis during endurance exercise²⁷.

Yet the use of BM changes as an absolute indicator of hydration status is still widely advocated because it is a practical and convenient measurement that can be used in the laboratory and field setting^{57;73;103;113}. The question remains whether it should be used as a justified marker for fluid replacement guidelines to maintain body fluid homeostasis for athletes during and after exercise.

1.6) Fluid balance controls

Classically the main fluid balance control in the human body is the hormone AVP alongside hormones aldosterone and ANP. Although recently interleukin-6 and oxytocin have also been found to potentially play a role in control of fluid balance during exercise in humans^{47;50}. AVP predominately affects the fluid reuptake rate by the kidney¹²⁶. Whilst aldosterone exerts its effects on the body to either conserve (increase re-absorption from the body via sodium conservation, concomitant fluid reuptake) or liberate fluid for excretion (vice versa) in response to changes in blood

pressure and volume, thus ensuring body fluid homeostasis with the maintenance of blood biochemical markers of POsm and plasma $[\text{Na}^+]^{127}$.

Hormone collection and analysis is costly. Hence it is practical and inexpensive to assess body fluid homeostasis according to proxies which these hormones respond. A variety of blood biochemical measures are often used to assess body fluid homeostasis such as blood POsm and electrolyte (sodium and potassium) concentrations. These markers are often measured because their concentrations reflect the actual state of hydration that the body uses to assess the net flux of fluid in and out of the circulatory system which allows for the body to remain in optimum functional state.

Kratz *et al* have last updated reference values for various haematological and blood biochemical markers in regards to normal laboratory reference values of these ⁶³. For hydration both before and after prolonged exercise they have proposed other values⁶⁴. This allows us to understand the deviations from the normal resting values in which athletes completing a marathon without requiring some sort of medical assistance would display what could be considered 'normal' values.

i) **Plasma osmolality (POsm)**

POsm defined as the concentration of a specific solution expressed in milliosmoles of solute particles per kilogram of water (mOsm/kgH₂O). In this the case it is the concentration of solute particles bathed in the blood⁴. It is most appropriately calculated as¹²⁶:

$$\text{Plasma Osmolality (mOsm/kgH}_2\text{O)} = 2 \times [\text{Na}^+] + [\text{BUN}] + [\text{glucose}]$$

[Na⁺] – plasma sodium concentration (mmol/L)

[BUN] – bread urea nitrogen (mmol/L)

[glucose] – plasma glucose concentration (mmol/L)

Sodium is the main electrolyte constituent in the extra-cellular fluid, thus plasma $[Na^+]$ largely dictates POsm which in turn regulates cell size. Body fluid homeostasis is achieved through neuro-endocrine regulation in which osmoreceptors located in the organum vasculosum of the lamina terminalis and the subfornical organ in the hypothalamus detect changes in POsm (1- 2%)¹²⁶. An increase in POsm will stimulate the posterior pituitary gland secretion of AVP¹²⁷. The macula densa in the kidney also senses blood osmolality. Renin secretion is adjusted in order to modulate POsm. Renin is used to convert angiotensinogen (present in the liver) to angiotensin I, which subsequently is converted into angiotensin II by angiotensin converting enzyme (present in the capillaries of the lungs). Angiotensin II exerts global affects, triggering aldosterone release from the adrenal cortex, direct vasoconstriction, and thirst behaviours originating in the hypothalamus. This complex system regulates overall fluid homeostasis^{127;128}.

The POsm increases as a curvilinear of exercise intensity and a change in PV during exercise^{21;127}. Thus AVP concentrations rise as a linear function of increases in POsm increasing the stimulus for thirst concomitantly with an increase in renal re-absorption to maintain in fluid homeostasis. This occurs when the POsm rises above 5-10 mOsm/kgH₂O above normal or when TBW decreases by 1.7 – 3.5%

The measurement of POsm is the most valid measures of hydration status compared to urine osmolality which has a delayed response as a marker of hydration^{4;45}. Popowski *et al* (2001)⁹⁴ determined if POsm could accurately identify euhydration and found it to accurately reflect acute changes in hydration status(via BM loss) as compared to urine osmolality which lags behind POsm readings when

measuring hydration status during acute dehydration. It has been established that the mean reference value for normal people at rest ranges from 280-296 mOsm/kgH₂O⁶³. Reference values for POsm has observed to range from 273-317mOsm/kgH₂O immediately after endurance exercise after running a marathon⁶⁴. POsm should be used in order to scientifically determine the body hydration status at anytime.

ii) ***Plasma sodium concentration (plasma [Na⁺]):***

Since plasma [Na⁺] is largely responsible for dictating the osmotic pressure of the body, any significant disturbances during rest and specifically during long distance endurance events can produce significant illness^{48;65;82;101;121}. Dysnatremias can be the result of overdrinking or the excessive intake of consumption of sodium tablets in response to fears of severe dehydration and decreases in performance. These dysnatremias are also associated with delayed recovery in runners who collapse after prolonged exercise⁴⁸

Although sodium is lost in urine and sweat (in smaller amounts) it has been hypothesized that the body is able to activate or inactivate various endogenous stores of sodium in order to maintain homeostasis of plasma [Na⁺]¹³¹. Normal reference values for plasma [Na⁺] have been established as 135-145 mmol/L for the general population⁶⁴. Immediately after a marathon or prolonged exercise the values have been established as ranging from 134-149 mmol/L but much lower values can occur in EAH⁶⁴.

Because plasma [Na⁺] is an easily measurable and important marker of body fluid homeostasis, certain researchers have insisted that in order to prevent the body's

levels of sodium from decreasing during exercise and preventing EAH, that sodium should be replaced during exercise in order to correct for any sweat and urine sodium losses^{103;104}. But it has been found that significant plasma $[Na^+]$ decreases are due to the increase in fluid consumption resulting in fluid retention of the body during prolonged exercise and therefore diluting the plasma $[Na^+]$ levels. Consequently it has been found that the use of oral sodium supplementation during prolonged exercise is unnecessary to maintain the plasma $[Na^+]^{51;124}$. Rather it is the importance of preventing the overconsumption of fluid.

Thus it is not the loss of sodium through sweat but the dilution of the body's sodium content due to overhydration concomitantly with the inability to maximally suppress AVP that causes EAH¹⁰². The use of plasma $[Na^+]$ as a marker of the body's hydration is a valid indicator due to the measurement ease with the use of portable analyzers and its close relationship with POsm enabling rapid and accurate determination of the body's fluid balance state.

1.7) Summary:

The safety and efficacy of past and present ACSM Guidelines for Fluid Replacement have been questioned^{20;103}. After presenting the current understanding of euhydration^{37;38}, we have proposed a more appropriate definition of euhydration in relation to physiological variables such as POsm⁴⁹. Having considered the general gains and losses of water through the human body we have noted that water may be released from internal stores during exercise. This would add to the body's overall hydration especially from water associated with glycogen storage. Our previous and continued research has laid the ground to establish a more physiologically appropriate model of fluid replacement during exercise^{49;78;80}.

Two fundamental questions require continued attention. The first is to determine whether *ad libitum* fluid consumption during exercise will maintain body fluid homeostasis. Second is to find out whether BM loss equates to body fluid loss (TBW) during exercise when drinking to the physiological stimulus of thirst.

Currently evidence found during ultra-distance exercise has proven that it is normal to lose >2% BM^{55;108;109}. It seems that performance is neither compromised during competition that the athletes losing the greatest BM tend to perform better than their counterparts who lose the least or even gain BM during exercise.

Hew-Butler *et al* (2007) noted that despite a 3.8% loss of BM there was maintenance of plasma [Na⁺] and PV from pre-race to post-race during the South Africa Ironman triathlon. This is indicative of the body's ability to control fluid homeostasis even when there is a substantial loss of BM⁴⁵. As mentioned before, it is largely the state of plasma [Na⁺] which determines POsm thus stimulating the release of AVP and other fluid regulating hormones to regulate fluid excretion, it is

not any change in BM that dictates the body's response to fluid overload or dehydration^{49;127;128}.

TBW is considered the most accurate indicator of body fluid homeostasis⁸⁶. The use of tracer methodology allows the determination of TBW with accuracy and thus the measurement of real changes in hydration status. The relationships between direct changes in BM and TBW have yet to be studied during ultra-endurance exercise. Studies have noted the changes in BM before and after races and found that the markers of fluid balance pre- and post-exercise do not usually change as dramatically as does BM¹⁰⁰.

Nolte *et al* (2010) and Knechtle *et al* (2008) found that that BM decreased from pre- to post-exercise but percentage TBW rose indicating that TBW content remained relatively stable despite a reduction in BM⁶⁰. This was speculated to be due to the release and formation of endogenous water stores from various pools. These studies therefore provided some evidence suggesting that BM lost during prolonged exercise is not equivalent to the loss of TBW^{90;100}. Whilst Baker *et al*⁵ attempted to directly quantify changes in BM versus TBW changes and concluded that the relationship was accurate and precise, but their study appears to be flawed⁸⁵. Rogers *et al* studied the water budgets of athletes during ultra-endurance exercise and concluded that endogenous water gains may aid in offsetting the total amount of water loss even in the presence of BM loss¹⁰⁰.

A decade later some researchers have yet to acknowledge that the body might remain in relative fluid homeostasis before, during and after exercise regardless of BM fluctuations when consuming food and fluid *ad libitum*. Therefore further research should be conducted in fluid balance during exercise in order to establish

more physiological based guidelines that will allow athletes to complete athletic competition without medical complications.

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1.8) Hypotheses

- 1.) When drinking *ad libitum*, athletes will demonstrate variable fluid intake and will develop significant loss in BM.
- 2.) Body fluid homeostasis is unaffected during exercise even when BM a loss >2% occurs
- 3.) BM loss does not equal changes in TBW during exercise in those drinking *ad libitum*
- 4.) Significant BM loss will not be associated with a decrease in performance.

1.9) Study Objectives

- 1.) To determine whether *ad libitum* fluid intake will enable athletes to complete races in a normal state of hydration regardless of the extent of BM loss
- 2.) To measure indicators of body fluid homeostasis and their relationship to the extent of BM loss
- 3.) Determine whether BM loss exactly equates to change in TBW during exercise
- 4.) Determine whether BM loss is associated with altered racing performance.

1.10) Structure of this thesis

This thesis comprises six chapters and focuses on the use of D₂O to measure TBW changes in order to monitor body fluid homeostasis at rest in the laboratory setting and before and after two races over three distances: the 2007 PUFfeR, an 80km off-road ultra-marathon foot race and the 2009 Two Oceans Marathon, consisting of a half-marathon (21.1km) and a 56km ultra-marathon on-road foot race.

Chapter one is titled “Quantification of hydration status during exercise” it is a review of the current understanding, knowledge and controversies around the prescription of fluid replacement during exercise. More specifically with the use of BM to estimate changes in TBW during exercise, the routes of fluid loss and gain in the human body during exercise and discusses more physiologically appropriate measurements for quantifying body fluid homeostasis. More specifically it proposes the need to compare changes in BM and TBW during exercise to determine whether BM can be used as a proxy for changes in TBW

The second chapter investigates TBW and BM changes at rest and their relationship to other commonly used blood biochemical measures of body fluid homeostasis. This study allowed us to monitor the variability associated with the measurement of TBW and BM in a controlled sedentary environment with a double dose D₂O protocol under resting conditions.

Chapter three is titled, “Fluid intake and changes in blood biochemistry, running speed and BM during an 80km mountain trail race”. This manuscript has been published in *Medicina Sportiva* (2009) 13: 108-115 and tracks our initial attempt to quantify TBW over an ultra-endurance mountain trail-race. More importantly we were successful in tracking individual fluid intakes of athletes over an entire ultra-

endurance off-road competition thus establishing whether athletes ingest adequate amounts of fluid to maintain fluid homeostasis when drinking *ad libitum* under these conditions.

The final study was conducted during the 2009 Two Oceans Marathon and comprises two chapters, Chapter 4 examines the “Changes in total body water content during a race of 21.1km in athletes drinking *ad libitum*” and Chapter 5 deals with the “Changes in total body water content during a race of 56km in athletes drinking *ad libitum*”. This study was performed to address the main crux of this thesis which was to determine whether the changes in TBW equal changes in BM during prolonged exercise. The Two Oceans Marathon provided a unique setting since we were able to monitor athletes running both a shorter 21.1km (half-marathon) and more gruelling 56km (ultra-marathon) road running race on the same day. This allowed us to determine if fluid balance changes are similar during shorter and more prolonged ultra- endurance exercise.

Finally Chapter 6 represents concluding remarks, a synthesis of the studies and novel findings added to the body of literature on the physiology of fluid balance during prolonged exercise. With this advancement in the knowledge of body fluid homeostasis during exercise we are able to develop the most physiologically effective fluid replacement guidelines. Further we are able to discuss future directions in this ongoing challenge of prescribing optimum fluid intake with the aid of improving athlete performance but whilst avoiding potentially life-threatening conditions such as EAH and EAHE.

CHAPTER 2

**TOTAL BODY WATER VARIABILITY AT REST MEASURED
WITH A SHORT-DURATION DOUBLE DOSING PROTOCOL OF
DEUTERIUM OXIDE**

University Of Cape Town

2.1) Abstract:

Introduction: To measure changes in body mass (BM), total body water (TBW), plasma osmolality (POsm), plasma sodium concentrations ($[Na^+]$) at rest and assess both the daily and weekly variability associated with the measurement of TBW.

Methods: 15 subjects were tested on a morning and afternoon session over three trials/weeks. BM, TBW, POsm, plasma $[Na^+]$, plasma potassium ($[K^+]$) and plasma total protein ($[TP]$) concentrations were measured.

Results: No significant differences were found with BM and TBW measurements over the entire study. BM remained stable across the three trials with a 0.86% coefficient of variation (CV) whilst the TBW had a CV of 5.9% across the three trials. A significant relationship was found between TBW and BM content ($R=0.90$, $P<0.0001$) as well as between POsm and absolute TBW and BM. Intra-class correlation coefficients found the measurement were repeatable over the three trials for both BM and TBW ($R=0.84$ and $R=0.93$ respectively).

Conclusion: Both BM and TBW do not differ significantly daily and weekly in healthy free-living individuals. TBW is more variable at rest than BM which illustrates that BM does not necessarily track the changes in TBW at rest. We conclude that both measures can be determined reliably and with accuracy at rest.

Key words: fluid balance, deuterium oxide, hydration status.

2.2) Introduction:

The 2007 American College of Sports Medicine's (ACSM) Exercise and Fluid Replacement Guidelines, advise athletes to avoid a >2% BM loss during exercise to ensure they maintain their health and optimise their sporting performance ¹⁰³. The validity of these guidelines, however, are disputed by the finding that top finishers participating in ultra-marathons and Ironman triathlons, routinely exhibit greater BM losses than less competitive athletes. In addition a majority of athletes who lose >2% BM finish these races with no apparent health consequences ^{55;108;109}.

Gross BM changes and various other indirect measurements might be inadequate predictors of overall body fluid balance as metabolic water release from fuel oxidation and water bound to glycogen during storage is released are not often taken into account. Nor is the actual irreversible mass lost from substrate oxidation considered. Thus measurements of changes in BM could possibly lead to an overestimation of the true extent of fluid loss. These considerations indicate the necessity of a more direct and scientifically precise measurement ^{73;113}. In order for efficient and concise prescription of fluid intake requirements during exercise, it is necessary to attempt to accurately measure body fluid balance by changes in TBW rather than to grossly estimate and model fluid loss on the basis of indirect methods such as changes in BM. In addition it is necessary to include studies of athletes involved in out of laboratory competitive sport.

The work of Rogers *et al* (1997) ¹⁰⁰ calculated the water budget of triathletes by considering the gross variables determining fluid balance. They calculated that athletes who ate and drank *ad libitum* replaced almost 90% of their total water loss when competing in a multisport ultra-triathlon incurring a net loss of only about 1.9%

of their initial BM. Interestingly athletes still completed the event in good health and with a significant BM loss (4.6%).

Maughan *et al* (2007) supported the analysis of Rogers *et al* (1997) and also developed a theoretical analysis of the dynamics of body fluid balance during exercise. They examine substrate oxidation, respiratory water loss, cutaneous water loss (sweat), water loss in urine and faeces, food and fluid ingestion, metabolic water formation and water associated with glycogen storage. Their review exposed issues associated with these often simplified explanations and stated that a significant amount of BM loss (1-3%) may occur without any apparent impairment to body fluid homeostasis⁷³.

These observations of the body's ability to lose significant amounts of BM without a significant change in hydration status, warrants the study of body fluid stores (TBW) to determine whether BM changes adequately define the dynamics of body fluid homeostasis.

These data will further confirm our suspicion that fluid intake stimulated by thirst will allow athletes to safely complete endurance events if they drink according to the physiologically controlled stimulation of thirst and not "stay ahead of thirst". Measuring TBW is commonly achieved with the use of the stable isotope deuterium oxide (D₂O)^{7;9;28;39;107}. D₂O is able to equilibrate freely with the body's water contents. It is non-hazardous since it is not radioactive^{28;39;106} and is an accurate measure of TBW (±0.5%) using the method of Halliday and Miller (1977)³⁹.

D₂O dosing protocols are often used to measure body composition or to measure body water turnover with the method of single dosages with repeated measures between extended periods for example one dose every week. The weekly variability

of this method has been established ^{7:54:58}. However it would be interesting to investigate the daily (between morning and afternoon) and weekly variability of TBW when a double dosing protocol is used. The time between measurements over a single trial will be reflective of the time in which athletes would complete prolonged exercise.

Therefore in order for further investigation into the dynamics of TBW changes during exercise to be accomplished, our aims for this study were to investigate the precision and the variability (between the trial and session) of fluid balance at rest measuring TBW with the use of a double D₂O dose over a single day.

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2.3) Materials and Methods:

Subjects: Fifteen healthy individuals were recruited and gave their informed consent and approval for this study was obtained from the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town and was performed according to the World Medical Organisation's Declaration of Helsinki (Seoul, 2008).

Experimental Procedures: All subjects visited the laboratory on three occasions and were requested to follow the same routine (e.g. working and exercise habits and foods consumed) 24-hours prior to each visit. Each trial was separated by a minimum of seven days.

Trials 1, 2 & 3:

On the morning of the trial the subjects reported to the laboratory after eating a breakfast, kept constant for each trial, at least an hour before the beginning of the trial. After emptying their bladder, height and BM was measured in minimal attire without shoes on a calibrated electronic digital scale on a flat level surface (Clover Scales (Pty) Ltd: TCS-A300) (to the nearest 0.01kg). Height was measured with a stadiometer.

Pre-dose saliva (2ml) and blood (10ml) samples were then collected. Blood samples were collected from an antecubital vein within two minutes of the subject being seated to determine plasma total protein ([TP]), plasma sodium ([Na⁺]) and potassium ([K⁺]) concentrations and plasma osmolality (POsm). Saliva samples were collected with a cotton wool swab and subjects were requested not to eat or

drink for 30 minutes prior to this collection. Subjects then salivated a few times, rinsed the mouth and swallowed the saliva prior to providing a sample for analysis.

The subjects then orally ingested their first dose of deuterium oxide (D₂O) solution (dosage 0.05g/kg) as determined by their initial BM. When sampling D₂O in saliva, a minimum of two hours is required for complete equilibration of D₂O to occur within the TBW pool^{14;18;23;24;24;29;54;58;69;106;112;117;130;134}. During the equilibration period the subjects were free to perform daily habitual tasks but refrained from any exercise or activities that would increase the rate of body fluid turnover.

Two hours after the initial D₂O dose, a second saliva sample was obtained. The subjects were then free to consume food and fluid *ad libitum* for the next 5 hours and portions/volumes were recorded with the use of food diaries to insure that similar foods were eaten during all three trials. The subjects were instructed to maintain the same, consistent behavioural patterns associated with their daily activities (including food and fluid consumption) on all trial days for a period of seven hours post-D₂O dosing.

Seven hours after the initial D₂O ingestion the subjects returned to the laboratory, where they repeated the protocol described above. This sequence was repeated two more times (trials 2 and 3).

Blood Biochemical Analyses: 10 ml venous blood samples were collected from the antecubital vein on the occasions previously described, into lithium- heparin Vacutainer (Becton Dickinson, Rutherford, NJ) tubes. Blood samples were centrifuged at 3000g for 10 minutes at 4°C. Plasma was extracted and then stored at -20°C until analysed for: plasma [Na⁺] and [K⁺] (EasyLyte PLUS Na/K/Cl analyzer,

Medica Corporation); POsm (Osmomat 030 Cryoscopic osmometer, Gonotec) (CV- 2.86%); and plasma [TP] (Roche P-Module, Biuret) (CV- 2.1%).

Measurement of Total Body Water (TBW): TBW was calculated with the diluted isotope method using D_2O ⁵⁴. A ~1.5ml saliva sample was collected for use to calculate pre-dose deuterium abundance (as well as increased D_2O abundance due to prior doses) and after a 2-hour equilibration period to determine TBW on two separate occasions (morning and afternoon) over each of the three trials. Saliva was collected by placing a sterile cotton wool swab into each subject's mouth with sterile tweezers. Each subject was then 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 10ml sterile syringe. All saliva was then extracted by compressing the cotton swab against the head of the syringe this allowed all collected fluid to flow directly into a cryotube. Samples were immediately sealed and frozen (-80°C). The samples were prepared before analysis by filtration through sterile, single-use syringe filters into glass 1ml vials which were then sealed and sent for analysis.

The dose of D_2O used for TBW measurement was administered as individualized doses immediately following baseline saliva sampling. Each D_2O dose was dispensed from a 4% weight-to-weight stock solution that was created by mixing required amounts of 99% D_2O (Cambridge Isotope Laboratories Inc., MA.) and distilled water. The pre-mixed D_2O 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 BM 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.

Food and fluid consumption was prohibited during the 2-hour equilibration. A 2-hour equilibration period following D₂O administration has shown to be appropriate^{14;18;23;24;29;54;58;69;106;112;117;134}. All urine produced during this equilibration period was collected and analysed to account for any isotope loss. The D₂O enrichment and volume of these urine samples were accounted for in subsequent TBW calculations.

TBW determination was obtained through measurement of D₂O enrichment in saliva samples, measured by the Stable Light Isotope Laboratory (Department of Archaeology, University of Cape Town, South Africa) by pyrolysis in a Thermo Finnigan TC/EA (Thermo Fisher Scientific Inc. Waltham, MA, USA) with a SpectraSYSTEM® AS3000 Autosampler (Thermo Fisher Scientific Inc. Waltham, MA, USA), coupled via a Thermo Conflo III (Thermo Fisher Scientific Inc. Waltham, MA, USA) to a Thermo Delta XP stable light isotope mass spectrometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). Samples were run against internal laboratory standards and were measured at intervals throughout the run to ensure consistency. The results were normalised against and reported relative to the international standards (relative standard deviation <2%). TBW was calculated utilizing the Halliday and Miller method³⁹ with a modified correction factor for non-aqueous hydrogen exchange at 1.04 (4%)^{23;39}.

Calculations:

Equation 1: The Davies method was used to calculate the total body water (TBW) in kilograms (kg)²²:

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 (g); **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 D₂O⁹⁶.

Equation 2: Total body water (kg) was attained and percentages of total body water (TBW) were calculated as:

Statistical Analysis: Data were analysed and reported using means ± standard deviations. To determine the variability of the measures in question, repeated measures ANOVAs were used (time x trial). Intra-class correlation co-efficients (ICC) and co-efficients of variations (CV) were also calculated. All statistical tests were analysed using the Statistica 8 Software Programme™ (Tulsa, OK) and correlations with the use of Prism 3 (GraphPad Software Inc., La Jolla, CA). Statistical significance is accepted when $p < 0.05$.

2.4) Results:

Fifteen subjects volunteered to participate in this study (10 males and 5 females) (Table 2.1). The mean age of the cohort was 25 ± 4 years (range: 20-34). Average BM over the entire trial was calculated to be 69.9 ± 11.8 kg (range: 50.2-92.4) with an average TBW content of 42.8 ± 8.2 kg or L (range: 29.9 – 60.2) and percentage TBW content of $60.5 \pm 3.6\%$ (range: 54.0 – 67.2). Body mass index (BMI) was calculated to be 23 (normal range: 20-25) which reveals that subject group were of a normal healthy phenotype.

Table 2.1: General characteristics of the sample population

	Mean \pm SD (%)	Min	Max
Sample Number	15	-	-
Females	5 (33)	-	-
Males	10 (67)	-	-
Age (years)	25 ± 4	20	34
Body mass (kg)	69.9 ± 11.8	50.2	92.4
Height (cm)	173.5 ± 9.1	155	191
BMI (kg/m ²)	23.1 ± 2.5	18.7	26.6
Total body water (kg)	42.8 ± 8.2	29.9	60.2

BMI- Body mass index

There were no significant differences between BM, TBW and percentage TBW between the study's three trials ($P=0.72$, $P=0.47$ and $P=0.43$ respectively) (Table 2.2). Further no other significant differences were found between the blood biochemical markers of hydration over the entire trial (POsm, $P=0.06$ and plasma $[Na^+]$, $P=0.17$) (Table 2.3).

Table 2.2: Body mass and absolute (kg) and percentage (%) total body water over the three trials in the morning and afternoon

	Trial 1			Trial 2			Trial 3		
	n	Morning	Afternoon	n	Morning	Afternoon	n	Morning	Afternoon
BM (kg)	15	70.1 ± 12.1	70.0 ± 12.1	15	69.8 ± 11.7	69.8 ± 11.8	14	69.5 ± 12.1	69.5 ± 12.0
TBW (kg)	15	41.9 ± 9.0	42.6 ± 9.6	15	42.6 ± 7.5	42.7 ± 7.6	14	41.7 ± 7.7	42.5 ± 8.6
TBW (%)	15	59.4 ± 4.5	60.5 ± 5.1	15	61.0 ± 4.4	61.5 ± 6.3	14	60.1 ± 5.4	61.1 ± 5.1

BM- Body mass; **TBW-** Total body water

Within subject CVs were calculated for BM over the entire trial and for the morning and afternoon measurements over the three trials (0.86%, 0.94% and 0.85% respectively). Similarly within subject CVs were calculated for TBW over the period mentioned previously (5.9%, 5.5% and 6.3% respectively). ICCs for both BM and TBW over the entire trial were both found to be reliable (R=0.84 and R=0.93 respectively). When ICCs were calculated to assess the morning and afternoon BM over the three trials (R=0.97 and R=0.90 respectively) and TBW (R=0.95 and R=0.74) measures were found to be reliable except the afternoon TBW measure fell short of being highly reliable⁸.

Table 2.3: Blood biochemistry and indicators of hydration status over the three trials in the morning and afternoon sessions

	Trial 1			Trial 2			Trial 3		
	n	Morning	Afternoon	n	Morning	Afternoon	n	Morning	Afternoon
Plasma [K⁺]	14	4.2 ± 0.8	3.8 ± 0.3	15	4.0 ± 0.4	3.7 ± 0.2	13	4.2 ± 0.7	3.8 ± 0.3
Plasma [Na⁺]	14	139.2 ± 2.4	139.7 ± 2.4	15	139.0 ± 2.6	139.3 ± 4.0	13	137.7 ± 2.9	139.8 ± 2.8
POsm	14	291.7 ± 6.1	290.1 ± 4.7	15	288.9 ± 5.8	288.5 ± 4.9	13	290.0 ± 5.8	291.4 ± 5.1
Plasma [TP]	14	80.8 ± 5.0	77.3 ± 18.4	15	81.0 ± 6.3	80.7 ± 5.6	13	81.1 ± 5.2	80.3 ± 5.5

[K⁺]- Plasma potassium concentration (mmol/L); **[Na⁺]** - Plasma sodium concentration (mmol/L); **POsm-** Plasma osmolality (mOsm/kgH₂O); **[TP]**- Plasma total protein concentration (g/L).

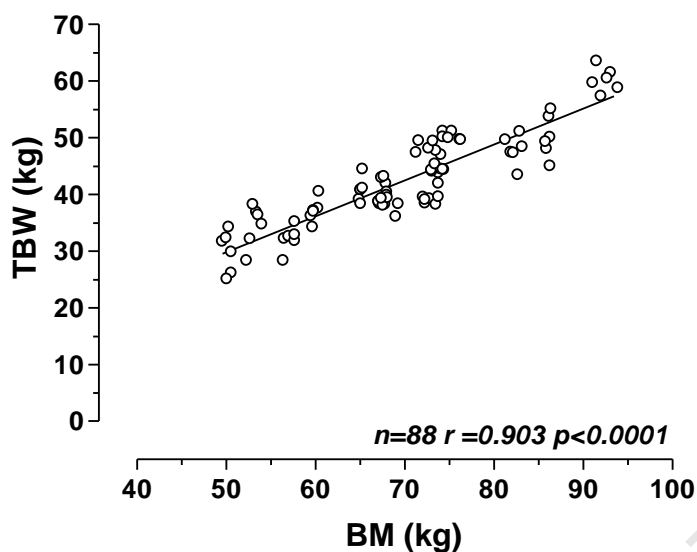


Figure 2.1 The significant relationship between total body water (TBW) and body mass (BM) of all subjects over the two time periods and three trials (n = 88)

A significant relationship was found between TBW and BM ($P < 0.0001$) (Figure 2.1). Likewise a correlation between POsm and both TBW and BM was found to be significant ($P < 0.001$ and $P < 0.0001$ respectively) (Figure 2.2). No significant relationship was found between plasma $[Na^+]$ and both BM and TBW. The dotted lines represent the normal ranges for both markers (POsm: 280-296mOsm/kgH₂O and plasma $[Na^+]$: 135-145mmol/L)⁶³. Eight POsm values fell marginally out of the normal range whereas four plasma $[Na^+]$ measurements fell below the normal range.

Individual changes over the three trials between TBW and BM are presented in Figure 2.3. A mean decrease was calculated in BM (-0.031kg) and slight increase in TBW (0.58kg). The correlation between the change in BM and TBW over all three trials was found to be not significant.

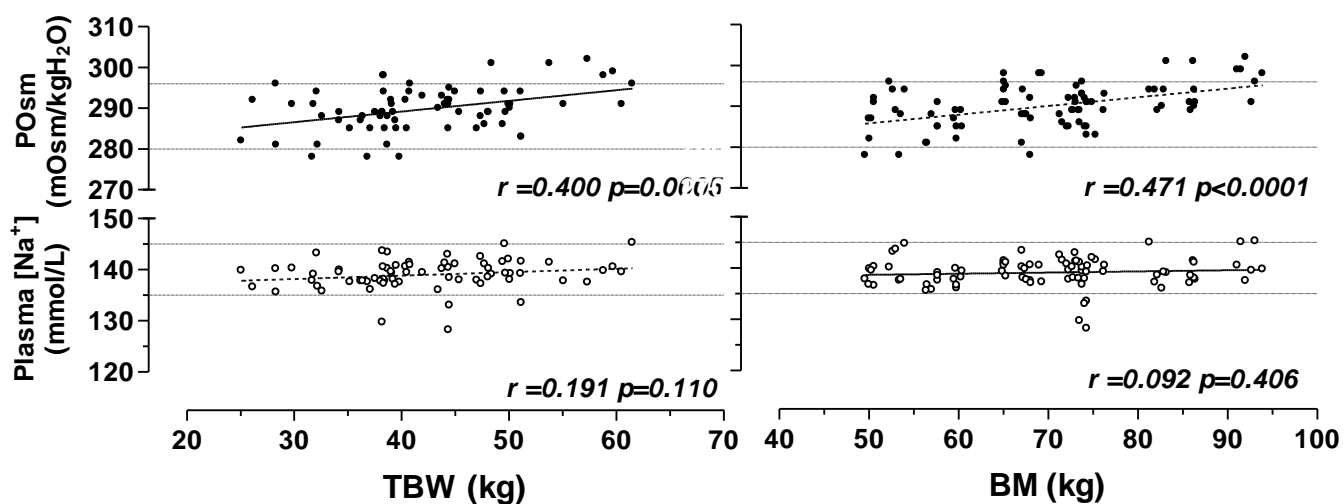


Figure 2.2 Significant correlations with plasma osmolality (POsm) but not plasma sodium concentrations ($[Na^+]$) with total body water (TBW) or body mass (BM) (n=71)

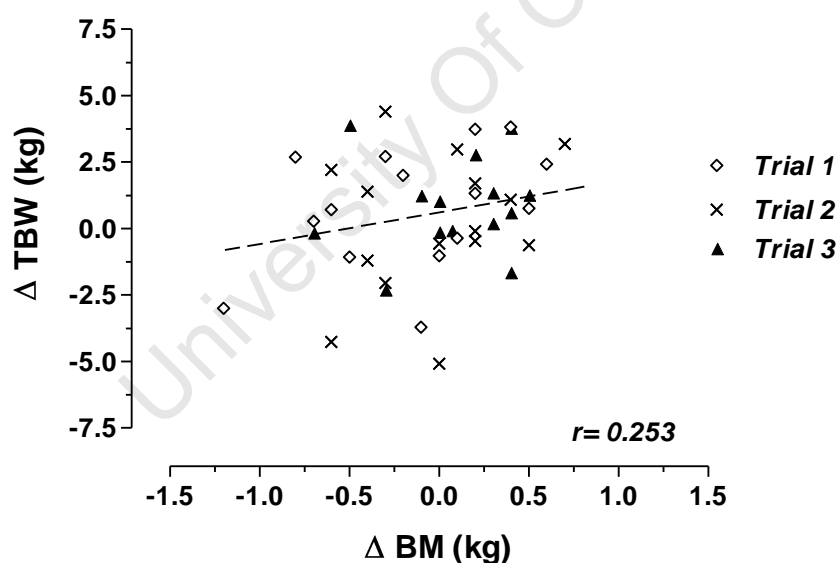


Figure 2.3 Individual values for changes (Δ) in body mass (BM) plotted against the changes in total body water (TBW). Each symbol represents the different trials.

2.5) Discussion:

Our main finding from this study was that neither TBW nor BM changed significantly during the entire study when measured in the morning or afternoon sessions (Table 2.2). We found greater variability associated with the measure of TBW (5.9%) than of BM (0.95%) over the three trial periods (total of six measurements). This TBW finding was slightly out of agreement with the findings of Bartoli *et al* (1993) who measured TBW over four separate occasions together with BM and underwater weighing⁷. The CV for BM (0.7%) had remarkable agreement with our data. Whereas the CV value for TBW in that study was only 4%.

It has been stated that TBW measurements are of dubious repeatability with error of measurement for a single sample having a CV value of 3%. This illustrates the inherent variability associated with machine error rather than with biological variability. In view of this it appears that our results for TBW are acceptable together with acceptable ICCs for BM and TBW (R=0.84 and R=0.93 respectively).

TBW variability associated with both morning and afternoon sessions did not differ significantly (P=0.86) but the associated CV was found to be different between the two sessions (5.5% and 6.3% respectively). It is apparent that the morning session produced a smaller CV for the TBW measurement than the afternoon session. This may have been associated with the inability to control for the subject's movement between each testing session (morning and afternoon sessions) and also between each trial. Other explanations could be a tracer accumulation effecting the measurement from morning to afternoon²⁵. With regards to methodological error the process of collection of "baseline" samples of saliva prior to the ingestion of the D₂O dose would correct for any unnecessary tracer miscalculation since background "baseline" content is known.

This finding illustrates the difficulty in using TBW values to define a specific value for euhydration. Rather the most physiologically appropriate measure to establish adequate hydration would be the measurement of plasma $[Na^+]$ and/or POsm in order to accurately quantify adequate hydration^{45;49;94}.

Our second finding was that no significant differences occurred in the biochemical markers of hydration status over the entire trial illustrating that adequate hydration was maintained during the testing period (Figure 2.2, Table 2.3) despite some variability in the TBW measurement. This suggests that the body fluid homeostasis was maintained even though there was some variation in the TBW measurement.

Lastly previous research involving repeated measures of TBW have found both positive and negative relationships between TBW and BM^{7;14;106}. To date only five studies have investigated repeated measures of TBW with the use of D_2O ^{7;14;32;106;107}. All studies except that of Faller *et al* (1955) found that the changes of TBW and BM were positively correlated. We did not find a significant relationship between the daily changes (differences between afternoon and morning measurements) of TBW over the trial period ($P=0.09$). Despite this we did find a positive trend between changes in BM and TBW (Figure 2.3) and a significant relationship between the TBW content and BM (Figure 2.1). The latter illustrates the phenomenon that 60% of the human body is water⁹².

The objective of this thesis is to utilize the measure of TBW to provide us with an understanding of the current state of fluid balance during exercise and compare this to BM, the often used proxy for body fluid homeostasis. The theoretical basis behind the use of BM to assess hydration status is because BM comprises primarily (60%) of TBW and any acute change in BM would be assumed to be due to fluid shifts

since TBW is one of the most dynamic component in physiological systems. Often BM may be a valid proxy measure for TBW over prolonged periods especially at rest⁴⁰. But this does not mean it is also valid during exercise with consideration of changes in substrate loss and glycogen bound water release⁷³. Thus it would be advantageous to observe whether the changes in BM would track changes in TBW during exercise (Figure 2.2).

Thus the aim of this study was to assess the daily and weekly variability of BM and TBW. A greater understanding of the dynamics of fluid balance at rest might provide explanations with regards to the more complex dynamics occurring during exercise. Along with other measures of hydration status TBW was assessed twice with the use of two doses of D₂O tracer per trial. We chose to mimic the protocol similar to that which we would use in the other studies reported in this thesis in order to determine the accuracy of the method.

Strengths and weaknesses of the study: The strengths of this study include the use of D₂O to quantify changes in TBW at rest. Furthermore, our ability to trace TBW twice on the same day provided us with insight about the accuracy of measurement of acute changes in TBW over the three week trial period. The weaknesses of this study include the inability to control for what our subjects drank and ate over the entire trial period and even between morning and afternoon testing sessions, although we requested that they maintain the similar dietary requirements during testing sessions. Thus we were unable to appropriately deduce what specific changes in TBW and BM were produced by variations in diet. It would have been interesting to monitor changes in body composition to further provide insight into the origins of these changes in BM and TBW. Nevertheless When using the stable isotope D₂O 60% of the TBW variation is inherent in technical machine error and a smaller proportion to biological and sampling error.

2.6) Conclusion:

In summary this study has found that BM and TBW do not significantly change when measured daily (morning and afternoon) and weekly (three trials). It was found that BM remains clearly more stable than TBW at rest (CV values of 0.8% and 5.9% respectively). Regardless of the dynamic nature of TBW it is evident that POsm and plasma $[\text{Na}^+]$ remained within the normal range for the duration of the study. Thus the body's fluid homeostasis was maintained even though there was some apparent change in TBW. We conclude that the measurement of TBW is reproducible under these conditions but is subject to a variation of ~6%. Most of this variation is inherent in the technical machine error with the measurement of the D₂O method.

Therefore further studies should investigate whether the relationships found at rest are evident during exercise. Other studies that might provide further insight of physiological measurement would be to monitor body fluid homeostasis during exercise with change in BM and TBW. Additionally the collection of body composition and nutritive data will add to elucidate the dynamics of changes in body fluid homeostasis produced during exercise and further interrogate these controversial guidelines for fluid intake during exercise.

CHAPTER 3

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

University Of Cape Town

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3.1) Abstract:

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

Methods: Nine 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 BM, TBW, plasma osmolality (POsm), plasma sodium ($[Na^+]$), plasma potassium ($[K^+]$) and plasma total protein ([TP]) 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 from pre-race to post-race (-3.7 ± 2.7 kg; $p < 0.01$). Whereas TBW decreased on average by -2.5 ± 0.9 kg (range: -1.5 - -3.6 kg) between both 2-days pre-race to post-race and pre-race and post-race. 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- 628ml/hr) and total body mass loss varied substantially between individuals. POsm and plasma $[Na^+]$ 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 POsm and $[Na^+]$ concentrations are well regulated despite large changes in BM. The limited data of TBW showed that this measure did not significantly change. 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.

3.2) Introduction:

Current fluid replacement guidelines advise athletes to consume enough fluid in order to prevent a >2% decrease in BM during exercise¹⁰³. 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 BM losses in ultra-distance events like the Ironman triathlon or ultra-marathon races, in which some successful athletes can incur BM losses >6-8% without apparent detriment to their health or performance^{55;108;109}. 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 BM, running speed, rate 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 BM during the race. In contrast blood electrolyte measures especially plasma $[Na^+]$ and POsm would vary less since these are the biological variables that are homeostatically regulated during exercise⁴⁶. Finally we sought to evaluate the observation that athletes losing the most BM during the race will exhibit the best performance whilst drinking and eating *ad libitum* during an 80km off-road running race.

3.3) **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 on an electronic digital scale (Beurer GS32) (to the nearest 0.1kg) 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) after each had emptied their bladder.

Blood Biochemical Analyses: 10ml 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 BM. 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 (175ml or 350ml) 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 ~6km. 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 TBW took place 2-days before; immediately before and after the race with the use of D₂O dosage.

Measurement of Total Body Water (TBW): TBW was calculated with the diluted isotope method using D₂O. A ~1.5ml saliva sample was used to calculate natural D₂O abundance (as well as increased D₂O abundance due to prior doses) and after a 2-hour equilibration period to determine TBW on three separate occasions: 1) 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 10ml 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 D₂O administration has been seen to be appropriate^{14;18;23;24;29;54;58;69;106;112;117;134}. All urine produced during this equilibration period was collected to account for any isotope loss. The D₂O enrichment and volume of these urine samples were accounted for in subsequent TBW calculations.

The dose of D₂O used for TBW measurement was administered as individualized doses immediately following baseline saliva sampling. Each D₂O dose was dispensed from a 4% weight-to-weight stock solution that was created by mixing required amounts of 99% D₂O (Cambridge Isotope Laboratories Inc., MA.) and distilled water. The pre-mixed D₂O 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 0.05g/kg body mass 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 D₂O 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%). TBW was calculated utilizing the Halliday and Miller method³⁹ with a modified correction factor for non-aqueous hydrogen exchange at 1.04 (4%)^{23;39}.

Calculations:

Equation 1: The Davies method was used to calculate the total body water (TBW) in kilograms (kg)²²:

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 D₂O⁹⁶.

Equation 2: TBW (kg) was attained and percentages of total body water (%ΔTBW) were calculated as:

Equation 3: Percent of BM (%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: Performance Analysis: Running speed (km/hr) was calculated as an average over the entire race and over each leg between twelve checkpoints:

Rating of Perceived Exertion (RPE): RPE was noted using the Borg (6-20) category ratio scale¹¹. 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 correlations, repeated measures ANOVAs and post-hoc analysis with Tukeys HSD. Pearson's correlations were calculated in Prism 3 (GraphPad Software, Inc., La Jolla, CA). Where applicable, all data are presented as means \pm standard deviations (SD) including the range of values. Statistical significance was accepted when $p < 0.05$.

University Of Cape Town

3.4) 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 3.1). 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 ~60km, but completed BM 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 BM (2-days pre-race) of 72.2 ± 9.0 kg (range: 61.3 - 88.6) (Table 3.1).

The individual data for BM measurements including the relative and absolute changes during the course of the experiment are detailed in Table 3.1. BM increased ~1.05kg (± 1.99) from 2-days pre-race to immediately pre-race but decreased ~4kg (± 2.55) (5% BM) during the course of the race ($P < 0.05$).

Table 3.1: 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	12.20	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	12.20	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^s	-5.0^s
\pmSD		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; ^s $P < 0.05$ when compared to A-B; (-) - Unavailable data.

Plasma potassium (K^+), sodium (Na^+) and protein concentrations did not change significantly during the race, nor did the POsm (Table 3.2). Furthermore, no significant correlations existed between percent change in BM with either post-race plasma [Na^+] or POsm (Figure 3.1). The dotted lines indicate the normal range of POsm (280 – 296 mOsm/kgH₂O) while the solid lines denote the normal range of plasma [Na^+] (135 – 145 mmol/L)⁶³. Only one plasma [Na^+] concentration fell outside the normal range whereas three plasma osmolalities were above the normal range.

Table 3.2: Blood biochemical measures of runners participating in an 80km ultra endurance trail race (mean \pm SD)

	2-Days Pre-race	Pre-race	Post-race
Plasma [K^+] (mmol/L)	5.0 \pm 0.5 (n= 8)	4.9 \pm 0.6 (n= 9)	5.1 \pm 0.7 (n= 8)
Plasma [Na^+] (mmol/L)	140 \pm 2.0 (n= 8)	137.3 \pm 2.2 (n= 9)	138.5 \pm 4.3 (n= 8)
Plasma Osmolality (mOsm/kgH₂O)	286.1 \pm 9.7 (n= 8)	298.2 \pm 7.4 (n= 9)	292.9 \pm 8.5 (n= 8)
Plasma Protein (g/L)	71 \pm 3.3 (n= 8)	73 \pm 2.8 (n= 8)	75 \pm 5.1 (n= 8)

[Na^+] – sodium concentration; [K^+] – potassium concentration

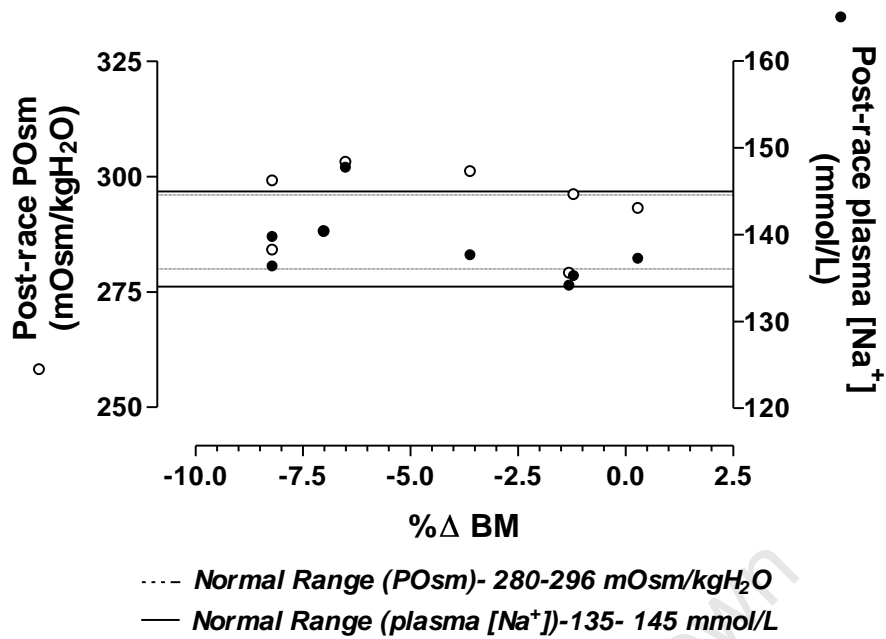


Figure 3.1 Percentage change (%Δ) in body mass (BM) in relation to post-race plasma osmolality (POsm) and sodium concentrations ([Na⁺])

Table 3.3 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 litres (mean 4.50 litres). The rates of fluid intake varied from 327 to 628 ml/ hr (mean 422 ml/hr).

Table 3.3: Fluid intake of runners participating in an 80km ultra endurance trail race

Subject	Fluid Intake (ml)							Rate (ml/hr)
	Water	CHO A	CHO B	Cola	CHO C	Other	Total (L)	
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 – Litre; **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

The data obtained resulted in only two complete sets of data for all three TBW measurements (Table 3.4). 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 3.4: 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

Figure 3.2 illustrates the race profile and elevation (altitude- metres); average running speed (km/hr) of the group between checkpoints; average RPE and average fluid intake (ml/leg) of the group at each checkpoint with bars indicating standard deviation.

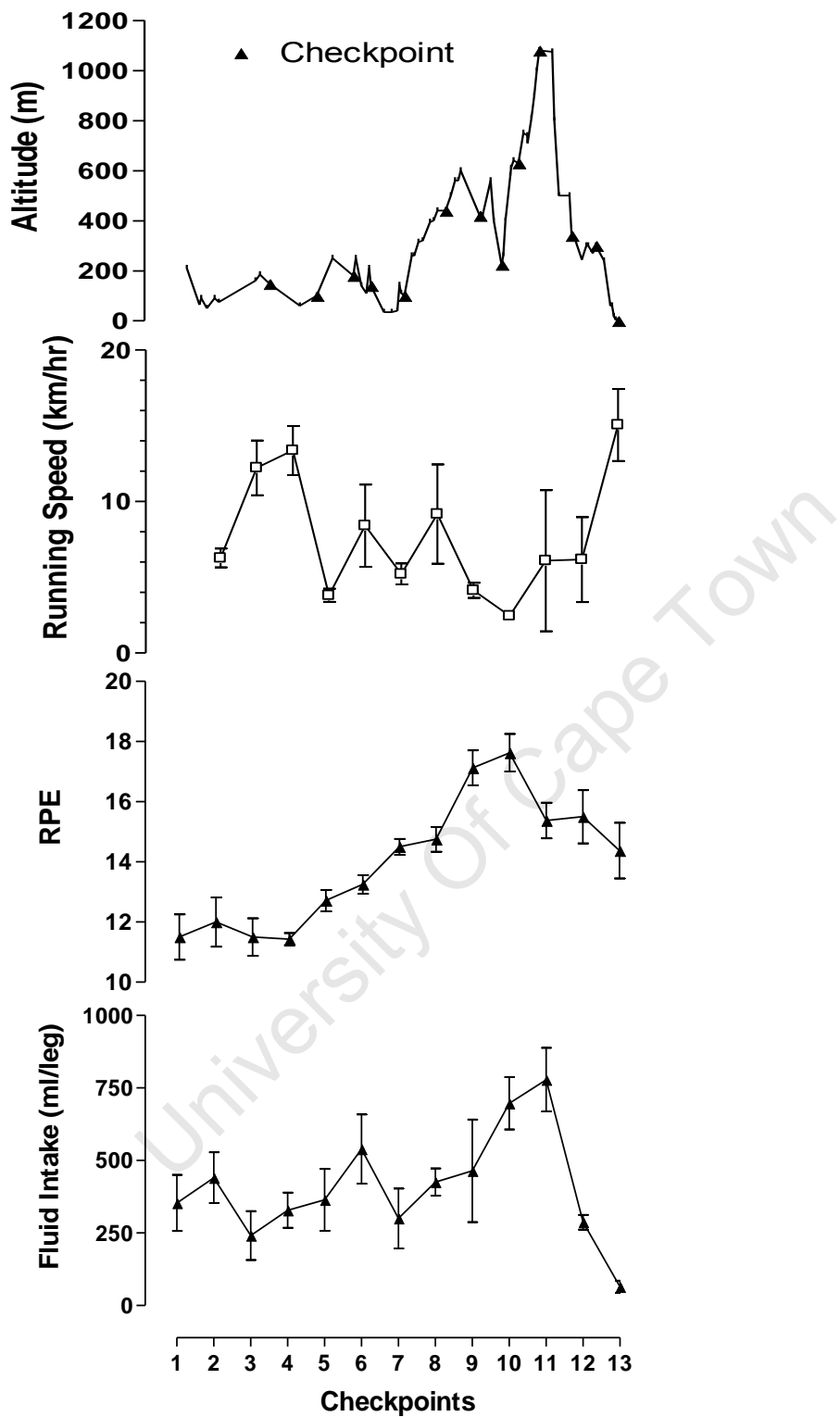


Figure 3.2 The race profile in distance (checkpoints) set against change altitude (m); subject's running speed (km/hr); their ratings of perceived exertion (RPE) and fluid intake from the last checkpoint to the specified checkpoint (ml/leg).

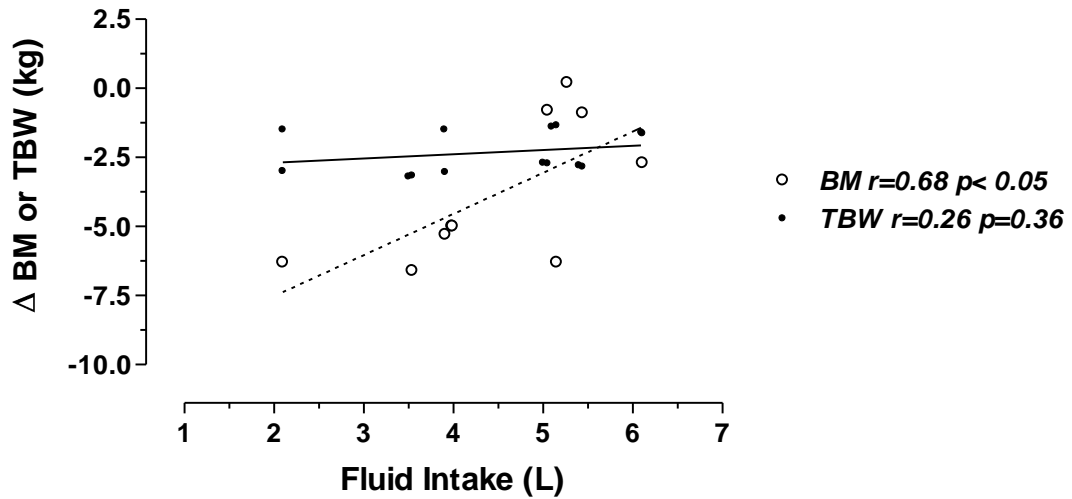


Figure 3.3 Changes (Δ) in body mass (B-C) (BM) in relation to total fluid intake (litres).

There was a significant correlation between percentage change in BM and fluid intake so that those athletes who ingested the most fluid had the smallest reductions in BM during the race and vice versa (Figure 3.3).

There was also no correlation between performance and change in BM from 2-days pre-race to post-race and immediately pre-race to post-race (Figure 3.4).

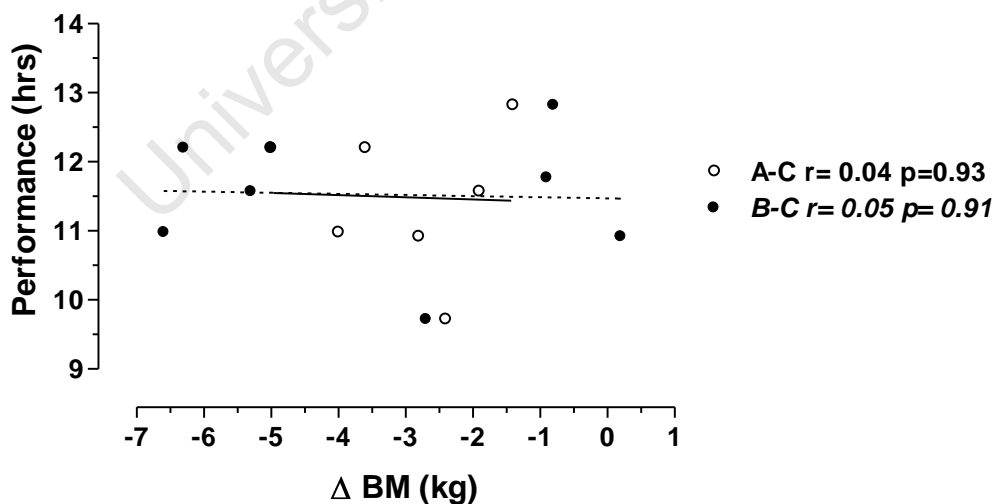


Figure 3.4 Non-significant correlation between change in body mass (Δ BM) and performance (race time) (hours) for both 2-days pre-race minus post-race (A-C) and immediately pre-race minus post-race (B-C) ($n = 8$)

3.5) Discussion:

The most pertinent finding of this study is that with an *ad libitum* drinking approach, rates of fluid intake were relatively similar between subjects and were at the lower range of the current ACSM¹⁰³ and USA Track and Field/ IMMUDA⁷⁸. These data therefore confirm that moderate drinking according to the dictates of thirst appropriately protects athletes from encountering fluid associated medical problems⁴⁹. Importantly no athlete drank to excess, thereby avoiding a significant gain in BM and developing EAHE as can occur when athletes are encouraged to drink to “stay ahead of thirst” or “as much as tolerable” and demonstrates that *ad libitum* drinking appropriately protects athletes against EAH⁸³.

All finishers were asymptomatic despite BM losses >2% in six subjects and >6% in five subjects. This again confirms our previous findings that athletes may complete ultra-distance races with a BM loss >6%, yet be completely asymptomatic^{55;108;109}. This conflicts with the theory that any BM loss >2% is inevitably injurious to health or performance during exercise¹⁰³.

Interestingly overdrinking may be associated with the more inexperienced runner since any athlete entering this race needs to demonstrate some pedigree in both ultra-distance running on both trail and the road (and could only run if he or she had completed an ultra-distance race within the past year and to have some sort of trail running experience). 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 fluids to finish in good health without over-consuming.

The second important finding was that plasma $[Na^+]$ and POsm were regulated within the adjusted normal range established by Kratz et al (2002)⁶⁴ even in subjects

who lost >2% of BM during the race (Figure 3.1; Table 3.2). As previously shown by us⁸³ these variables are homeostatically regulated within the normal range despite quite large differences in %BM loss^{45,49}. This suggests that athletes are able to regulate their POsm even when they lose >2% of their BM during prolonged exercise⁴⁶.

The third finding from limited data obtained from TBW measurement by D₂O analysis revealed a small but highly variable (range 1.5–3.6L) decrease in TBW content from 2-days pre-race to post-race (Table 3.4). This is interesting to note because a majority of the athletes finished appropriately hydrated (as indicated by their normal POsm) and without, any clinical signs or symptoms suggesting ill-health. The same could be noted with the data set obtained from immediately pre-race to post-race (B-C).

These findings on a limited number of subjects encourages us to further assess the relationship between BM 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* (2009)⁵ who ran subjects intermittently for a period of 2 hours for each running session. Although it could be argued that 2 hours of intermittent exercise is sufficiently long to observe such findings as were found with some our subjects, the data of Baker *et al*⁵ maybe more comparable to exercise of a short duration (e.g. half- marathon, football matches etc.). The methods and conclusions of Baker *et al* have recently been questioned⁸⁵.

While changes in BM may be useful to predict fluid homeostasis in certain clinical scenarios at rest and over short episodes of exercise (<2 hours)¹²⁹, during exercise the protection of POsm and the plasma [Na⁺] may in some cases require a

concomitant decrease in body water content and hence in BM without causing any apparent disruption of bodily function or athletic performance.

This is the opposite of the expected outcome if a BM loss >2% causes a progressive impairment in running performance¹⁰³. In contrast we found no such relationship although we noted that subjects were able to complete the race without experiencing any symptoms even though their BM loss exceeded 2% of starting BM. However the 2 fastest runners lost between 2-3% of their BM which again exceeds the recommended optimum weight loss according to currently accepted drinking guidelines (Figure 3.4). The recent study of Kao *et al* (2008)⁵⁵ reported that athletes completing 12- and 24-hour ultra-marathons can finish without adverse effects to their health even when the BM loss of some exceeded 7%. More importantly in that study there was a significant relationship between percentage BM loss and performance so that athletes who lost the greatest mass completed the greatest distance in the 24-hour race.

Strengths and weaknesses of the study: The strengths of this study include the use of D₂O to quantify changes in TBW. 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 D₂O 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. In addition subjects are not keen to participate in research studies during competitive events.

Future directions: A larger cohort of ultra-endurance athletes should be investigated, to make these preliminary findings more robust. Furthermore, to better understand fluid balance despite BM loss in athletes participating in ultra-endurance exercise, fluid regulatory hormones such as arginine vasopressin, aldosterone and natriuretic peptides should be evaluated along with measurement of TBW using the D₂O dilution method. Hopefully with a larger cohort, it may be possible to document associations between changes in TBW versus the maintenance of POsm and plasma [Na⁺] in sporting events where there are a larger number of entrants in order to increase the likelihood of voluntary participation.

University Of Cape Town

3.6) Conclusion:

In summary, this study found that *ad libitum* drinking rates between 300 – 650 ml/hr were associated with protection of POsm and plasma electrolyte homeostasis despite a range of BM losses in athletes competing in an 80km mountain trail race. 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 behaviour 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 BM loss during the race was 5%. Despite changes in BM exceeding 2%, no subject developed medical complications such as heatstroke. Indeed no subject complained of any symptoms after the race. Finally these data support the adoption of *ad libitum* drinking guidelines during exercise since performance was not apparently negatively affected by BM loss >2%.

CHAPTER 4

CHANGES IN TOTAL BODY WATER CONTENT DURING A RUNNING RACE OF 21.1KM IN ATHLETES DRINKING *AD LIBITUM*

University Of Cape Town

4.1) Abstract:

Objective: To measure changes in body mass, total body water and blood biochemical variables in athletes drinking *ad libitum* during a 21.1km foot race.

Setting: Two Oceans Marathon 21.1km foot race.

Main Outcome Measurements: 21 participants were measured for body mass (BM), total body water (TBW), plasma osmolality (POsm), plasma sodium ($[Na^+]$), plasma potassium ($[K^+]$) and plasma total protein ([TP]) concentrations immediately before and after these races. Fluid intake was recorded from recall after the race. Subjects were advised to drink according to the dictates of thirst, that is, *ad libitum*.

Results: Significant BM loss occurred during the race ($-1.4 \pm 0.6\text{kg}$; $P < 0.000$). TBW was reduced by $-0.06 \pm 2.0\text{kg}$ during the race. All biochemical measures remained within the normal range during the race. There was a significant positive correlation between performance time and BM loss ($R = 0.528$, $P = 0.014$).

Conclusion: Although TBW fell in both races the reduction was less than the fall in BM suggesting that all the BM loss is not the result purely of a drop in TBW. Despite significant reductions in both BM and TBW, plasma $[Na^+]$ and POsm was maintained in both races. These findings support the interpretation that the body primarily defends plasma $[Na^+]$ and POsm and not BM during exercise and that a reduction in BM can occur without an equivalent reduction in TBW during prolonged exercise.

Key words: fluid balance, body mass loss, exercise, deuterium oxide

4.2) Introduction:

Recently the American College of Sports Medicine (ACSM) has revised its drinking guidelines from advice that athletes should drink “as much as tolerable during exercise” advising that athletes should now drink according to the dictates of thirst provided any body mass (BM) loss during exercise does not exceed 2% of the starting BM^{20;103}. In contrast the drinking guidelines of the International Marathon Medical Directors Association (IMMDA) propose that athletes should drink to thirst regardless of the extent of BM loss but should not drink more than 800ml/hr in order to reduce the risk that exercise- associated hyponatremia (EAH) will develop⁴⁹.

Presently 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 an impaired exercise capacity³⁰. Whilst 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%³⁰. Rather, the evidence might be interpreted as proof for an opposite theory, namely that some BM loss during exercise may enhance performance especially in weight-bearing activities, particularly long distance running^{15;108;109}. Whilst the resolution of this debate requires additional studies, especially large numbers of competitors in out-of-laboratory exercise, a more direct question requiring an answer is the exact origin of the mass that is lost during exercise. For if most of this loss comes from sources that do not require replacement during exercise, then some degree of BM loss during exercise will occur without a change in TBW.

For example, already in 1978 Shephard *et al*¹¹⁰ proposed that BM loss might conceivably occur without any measurable reduction in TBW. This is because of irreversible substrate oxidation and the theoretical release of water complexed to

glycogen (~2kg) as the glycogen is metabolized during exercise. More recently Pastene *et al* (1996), Maughan *et al* (2007) and King *et al* (2008) have presented arguments to support this interpretation^{59;73;90}.

In contrast, Montain (2008) and Baker *et al* (2009) have concluded the opposite^{5;76}. Baker *et al* believe they have already established that all the BM lost during exercise can be explained by an equivalent gram-for-millilitre reduction in TBW. But we have raised questions about their methodologies and statistical techniques and have argued that their data presented so far do not support their conclusion⁸⁵.

This debate has important practical implications for competitive athletes. For if all the mass lost during exercise is due to a reduction in TBW alone and if a reduction in TBW impairs exercise performance, then drinking to thirst alone cannot be the optimal strategy. This is because subjects 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 suggests¹²⁵, then this drinking approach might be considered appropriate. In which case the presence of thirst, not of a reduced BM, might explain the impaired exercise performance in those who are forced to restrict their fluid intake during prolonged exercise¹⁰⁵.

Indeed there is no evidence to support the concept that during prolonged exercise BM is a physiologically regulated parameter. BM 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^{1;40}.

The use of the stable isotope deuterium oxide allows the most accurate quantification of changes in TBW during exercise⁶⁸. This allows the more accurate

determination of whether or not all the BM lost during exercise is due to a reduction in TBW^{73;87;90;106;133}.

We have recently shown that the BM loss during exercise exceeded the reduction in TBW content in soldiers performing a 16km route march in warm dry condition (-1kg in BM and -0.5kg in TBW)⁸⁴. Additionally we found in Chapter 3 in a smaller group of athletes during an 80km mountain trail race a similar trend (a decrease of ~2.5kg in TBW and ~3.5kg in BM). To extend these analyses we now report the findings of subjects competing in 21.1km and 56km races (see Chapter 5), lasting from 1.5 -7 hours. We hypothesise that BM loss would be greater in the 56km than in the 21.1km race but that in neither race would the change in BM accurately predict the change in TBW because of associated changes in reversible substrate oxidation and release of glycogen-bound water.

These findings have relevance to the theory that when drinking according to the dictates of thirst athletes drink less than their physiological requirement producing voluntary dehydration. We were also interested to determine whether change in BM is related in to performance during either race.

4.3) Materials and Methods:

Subjects: Media releases prior to the race invited entrants in the 2009 Two Oceans half- (21.1km) and ultra-marathon (56km) to participate in this study which had been approved by the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town. The study was conducted according to the Declaration of Helsinki (Seoul, 2008). Twenty-one athletes gave written informed consent to participate in this project. All the athletes were free to consume food and fluids *ad libitum* during the race.

Setting: The Two Oceans Marathon is a 56km ultra-distance road running race around the Western Cape Peninsula; the 21.1km race is run on a part of the 56km route. The event took place on a warm day with the dry bulb temperatures ranging from 18 – 24°C with 50-70% humidity. There was some cloud cover with a wind of 0.5 – 2 m/s. Official cut-off time was three hours for the 21.1km race.

Fluid Intake: Fluid intake was estimated from a food and fluid recall questionnaire administered by the researchers after the race. The recall consisted of enquiring about the amount of fluids (contained in sachets, cups and bottles of known volume) consumed at each station during either race. Fluids were available to the runners every 3km on the 21.1km route (8 aid stations).

Measurement of Body Mass (BM): Subjects were weighed to the nearest 0.01kg in racing attire without shoes on a calibrated electronic digital scale (Clover Scales (Pty) Ltd: TCS-A300) that had been placed on a flat, solid, stable surface after each athlete had emptied their bladder. Weighing took place 60 minutes prior to the race start (pre-race) and immediately upon completion of the race (post-race). Subjects were immediately dried with a towel prior to the post-race re-weighing.

Urine Osmolality (UOsm): Urine samples were collected prior to BM measurement when subjects were requested to void their bladder on the occasions described above. UOsm was measured in triplicate with the use of a portable refractometer (Osmocheck, Vitech Scientific, West Sussex, UK).

Blood Biochemical Analyses: 10ml venous blood samples were collected from the antecubital vein into lithium- heparin Vacuette (Greiner Bio-one International AG, Kremsmuenster, Austria) containers after the subjects were weighed before and after the race. During the blood drawing, subjects were seated. Blood samples were centrifuged at 3000G for 10 minutes at 4°C. Plasma was extracted and placed in eppendorf tubes, placed on ice then stored at -20°C until analysis for plasma total protein ([TP]) (Roche P-Module, Biuret) (CV- <2 %), sodium ([Na⁺]) and potassium ([K⁺]) (EasyLyte PLUS Na/K/Cl analyzer, Medica Corporation) concentrations and plasma osmolality (POsm) (Osmomat 030 Cryoscopic osmometer, Gonotec) (CV- 2.86%).

Measurement of Total Body Water (TBW): TBW was measured with the diluted isotope method using d D₂O⁵⁴. An initial ~1.5ml saliva sample was collected for use to calculate natural D₂O abundance (as well as increased D₂O abundance due to prior doses) and repeated after 2-hour equilibration periods to determine TBW on two separate occasions: 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 the use of sterile tweezers. Each subject was then 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; this allowed all the collected fluid to flow directly into a cryotube. Samples were

immediately sealed and frozen (-80°C). Before analysis samples were prepared by filtration through sterile, single-use syringe filters into 1ml glass vials then sealed and sent for analysis.

The doses of D₂O used for TBW measurement were administered as individualized doses immediately following baseline saliva sampling. Each D₂O dose was dispensed from a 4% weight-to-weight stock solution that was prepared by mixing appropriate amounts of 99% D₂O (Cambridge Isotope Laboratories Inc., MA., USA) and distilled water. The pre-mixed D₂O stock solution was decanted into an airtight container and sealed with duct tape to prevent fractionation during storage. Each participant received a dose of approximately 0.05g/kg BM with each dosage was pre-weighed to the nearest 0.001g. After each dose had been consumed, the dose bottle was immediately re-weighed to quantify the precise amount that had been ingested.

Food consumption was prohibited during the 2-hour equilibration period. For the first two hours after the race fluid ingestion was also restricted. A 2-hour equilibration period following D₂O administration is known to be appropriate^{14;18;23;24;29;54;58;69;106;112;117;134}. All urine excreted during this equilibration period was collected and analysed to account for any isotope loss. The D₂O enrichment and volume of these urine samples were accounted for in subsequent TBW calculations.

TBW determination was obtained through measurement of D₂O enrichment in the saliva samples, as measured by the Stable Light Isotope Laboratory (Department of Archaeology, University of Cape Town, South Africa) by pyrolysis in a Thermo Finnigan TC/EA (Thermo Fisher Scientific Inc. Waltham, MA., USA) with a SpectraSYSTEM® AS3000 Autosampler (Thermo Fisher Scientific Inc. Waltham, MA., USA), coupled via a Thermo Conflo III (Thermo Fisher Scientific Inc. Waltham,

MA., USA) to a Thermo Delta XP stable light isotope mass spectrometer (Thermo Fisher Scientific Inc. Waltham, MA., USA). Samples were run against internal laboratory standards and were measured at intervals throughout the run to ensure consistency. The results were normalised against and reported relative to the international standards (VSMOW) (relative standard deviation <2%). TBW was then calculated utilizing the Halliday and Miller method³⁹ with a modified correction factor for non-aqueous hydrogen exchange at 1.04 (4%)^{23;39}.

Calculations:

Equation 1: Percentage change (%Δ) of body mass (BM) lost or gained during the race was calculated as:

Equation 2: Change in plasma volume (PV) was calculated¹²³:

Equation 3: Performance was measured as finishing times and running speed (km/hr). Running speed was calculated as an average over the entire race:

Equation 4: The Davies method was used to calculate the total body water (TBW) in kilograms (kg)²²:

Whereas: **A** = amount of dose solution drunk (g); **a** = amount of dose solution diluted in T (g); **T** = amount of tap water in which 'a' was diluted (g); **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 D₂O⁹⁶.

Equation 5: TBW (kg) was attained and %ΔTBW was calculated as:

Statistical analysis: Data were analyzed using the STATISTICA version 8 (StatSoft Tulsa, OK) statistical program and Prism 3 (GraphPad Software Inc., La Jolla, CA) using Pearson's correlations, students T-test. Where applicable, all data are presented as means ± standard deviations (SD) alongside the range of values. Statistical significance was accepted when p < 0.05.

4.4) Results:

All 21 runners (12 females and 9 males successfully completed the race with a mean finishing time of 2.2 ± 0.4 hours (range: 1.5 – 3 hours) (Table 4.1). The mean age of these runners was 31.7 ± 11.0 years (range: 20 – 56) (Table 4.1) with a mean body fat percentage of $\sim 18.1 \pm 4.2\%$ (range: 11.4 – 25.6) (Table 4.1). With an *ad libitum* drinking approach the runners mean fluid consumption over the entire 21.1km was 689.29 ± 365.60 ml (range: 150 – 1400ml). The athletes displayed a continuum in drinking behaviour with a mean drinking rate of 326.3 ± 180.0 ml/hr (range: 87.4 – 791.6).

Table 4.1: General characteristics of runners participating in the 21.1km road race (n=21)

Variable	21.1km		
	Mean \pm SD	Min	Max
Age (years)	31.7 ± 11.0	20	56
Pre-race body mass (kg)	70.0 ± 8.2	57.1	89.4
Body Fat (%)	18.1 ± 4.2	11.4	25.6
Predicted race time (hrs)	2.1 ± 0.4	1.5	2.8
Actual Race time (hrs)	2.2 ± 0.4	1.5	3.0
Fluid intake (ml)	689.3 ± 365.6	150	1400
Drinking Rate (ml/hr)	326.3 ± 180.0	87.4	791.6

Pre- and post-race blood biochemistry measures are detailed in Table 4.2. Only twenty blood samples were collected post-race (as one subject developed post-race hypotension and we were unable to acquire adequate blood through venipuncture). POsm, Plasma $[K^+]$ and $[Na^+]$ did not change significantly during the race, but plasma [TP] and percentage change in PV (as calculated from the change in [TP]) increased significantly ($P < 0.0001$) (Figure 4.2) .

Table 4.2: Changes in measured parameters of the runners participating in the 21.1km road race

Variable	21.1km					
	<i>n</i>	<i>Pre-race</i> (mean ± SD)	<i>n</i>	<i>Post-race</i> (mean ± SD)	Δ (mean ± SD)	<i>P</i> - value
Plasma [K ⁺] (mmol/L)	21	4.1 ± 0.3	20	4.5 ± 0.4	0.3 ± 0.5	NS
Plasma [Na ⁺] (mmol/L)	21	137.7 ± 3.5	20	139.7 ± 2.5	1.9 ± 4.5	NS
POsm (mOsm/kgH ₂ O)	21	289.7 ± 6.7	20	287.7 ± 5.4	-1.8 ± 5.3	NS
Plasma [TP] (g/L)	21	73.9 ± 3.4	20	78.0 ± 3.8	4.4 ± 3.5	< 0.0001
UOsm (mOsm/ kgH ₂ O)	21	614.8 ± 287.2	21	568.0 ± 225.5	-46.7 ± 352.4	NS
Body mass (kg)	21	70.0 ± 8.2	21	68.62 ± 7.9	-1.36 ± 0.6	< 0.0001
Body mass (%)	21	100.0	21	98.05 ± 0.8	-1.93 ± 0.7	< 0.0001
Total Body Water (kg)	21	40.6 ± 7.2	21	40.55 ± 7.0	-0.06 ± 2.0	NS
Total Body Water (%)	21	57.9 ± 6.8	21	59.0 ± 6.8	1.11 ± 3.0	NS

[K⁺] – Plasma potassium concentration; [Na⁺] – Plasma sodium concentration; **POsm**- Plasma osmolality; **[TP]**- Plasma total protein concentration; **UOsm**- Urine osmolality; Δ – Change (post- minus pre-race); **NS** – Not significant.

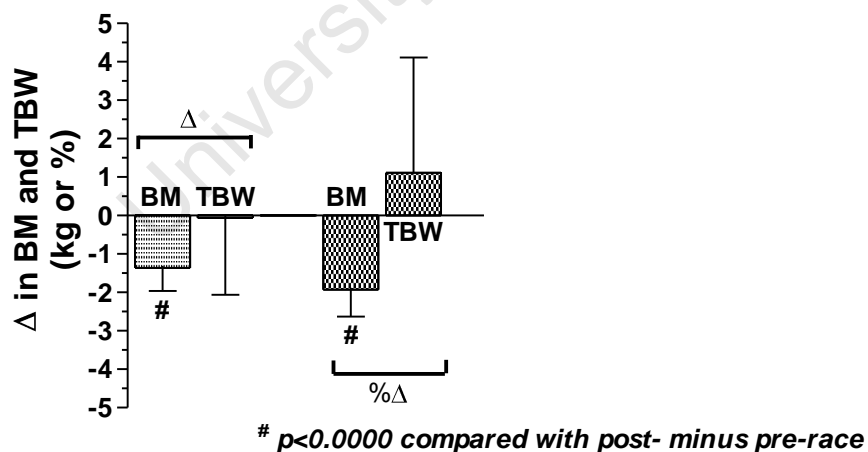


Figure 4.1 Changes (Δ) in both absolute (kg) and percentage (%) in body mass (BM) and total body water (TBW) during a 21.1km foot race.

Both absolute and % Δ BM significantly decreased $\sim 1.36\text{kg}$ (± 0.6) and $\sim 1.93\%$ (± 0.7) respectively ($P < 0.0000$). Whereas absolute TBW did not decrease significantly ($-0.43 \pm 2.6\text{L}$) nor was the increase in % Δ TBW significant ($0.39 \pm 4.0\%$) (Table 4.2) (Figure 4.2). There was no association when comparing absolute changes in TBW with changes in BM ($r^2 = 0.003$, $p = 0.828$) or between percentage change in TBW and BM ($r^2 = 0.03$, $p = 0.485$) during this race (Figure 4.3).

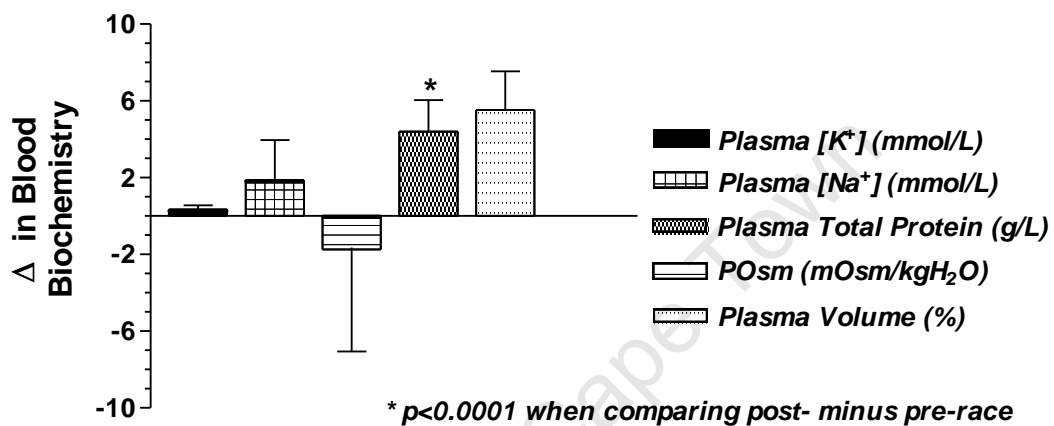


Figure 4.2 Changes in blood biochemistry in athletes participating in a 21.1km road race

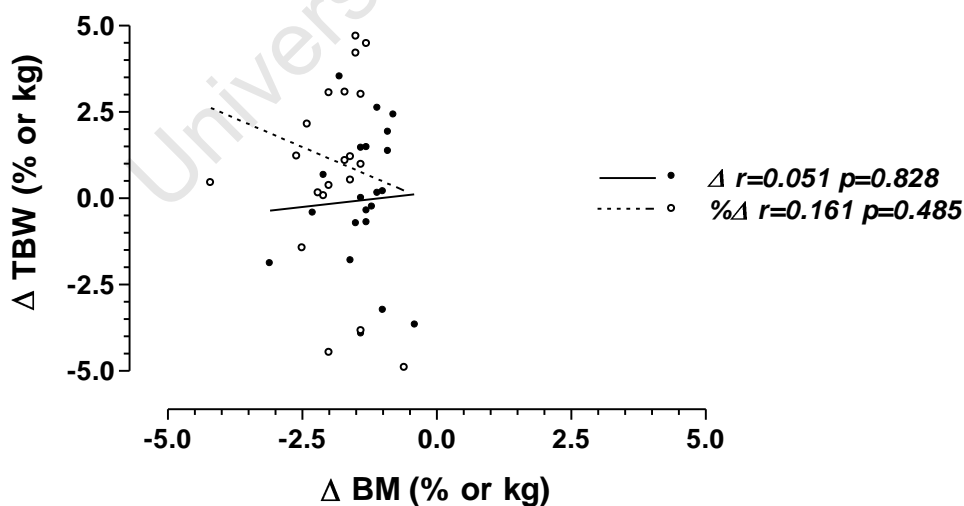


Figure 4.3 The non-significant relationship between both absolute (kg) and percentage (%) change in body mass (BM) and total body water (TBW) in athletes during a 21.1km foot race

When comparing the values obtained from plasma $[\text{Na}^+]$ and POsm values for both the pre- and post-race condition a significant relationship was found in the post-race measure ($P < 0.05$) but not in the pre-race measurements (Figure 4.4).

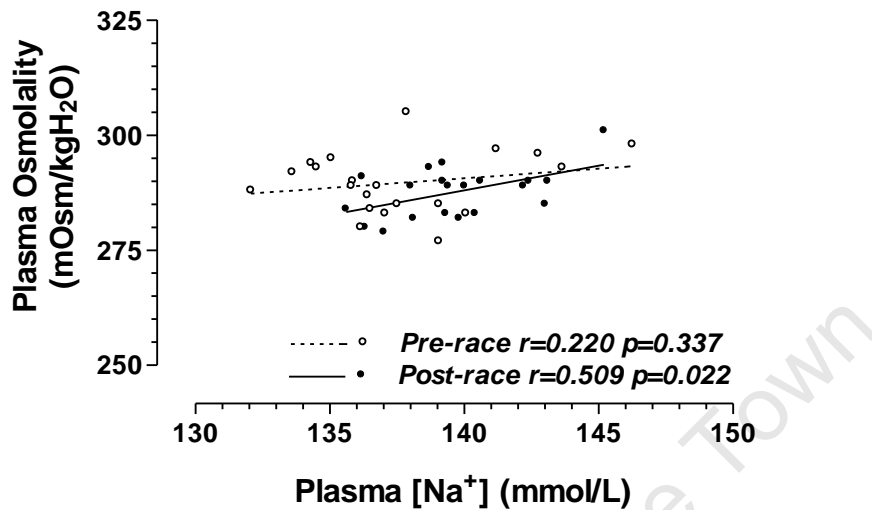


Figure 4.4 The correlations between plasma sodium concentration ($[\text{Na}^+]$) and plasma osmolality (POsm) either pre-race or post-race.

No significant correlations existed between the $\% \Delta \text{TBW}$ and $\% \Delta \text{BM}$ when compared to either post-race plasma $[\text{Na}^+]$ or POsm (Figure 4.5). The dotted lines indicate the normal range of POsm (280 – 296 mOsm/kgH₂O) while the solid lines denote the normal range of plasma $[\text{Na}^+]$ (135 – 145 mmol/L)⁶³. One plasma $[\text{Na}^+]$ marginally fell outside the normal range and one POsm was above the normal range (Figure 4.5).

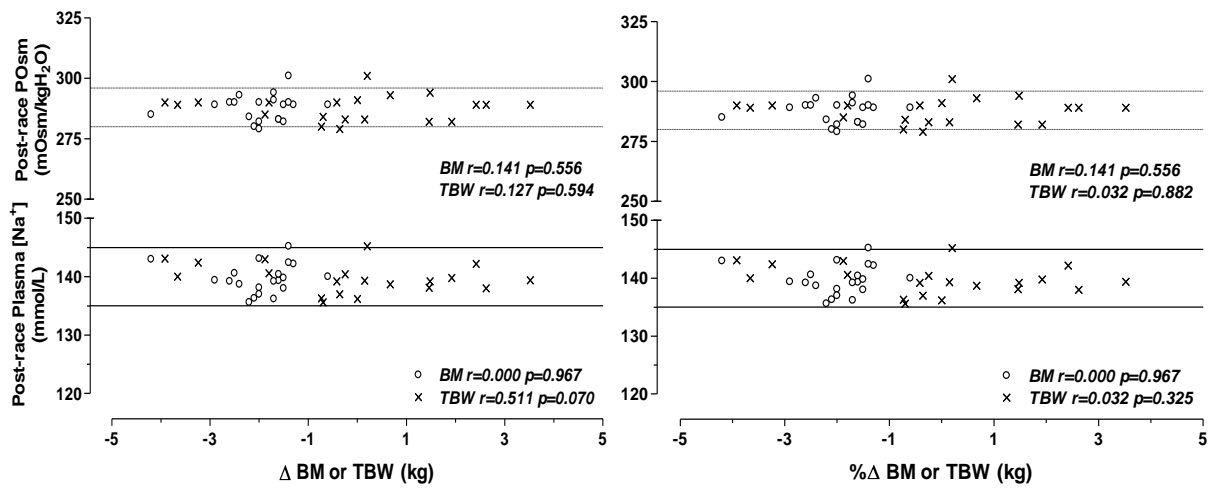


Figure 4.5 Post-race plasma osmolality (POsm) and sodium ([Na⁺]) compared to changes in either absolute (kg) or percentage (%) body mass (BM) and total body water (TBW).

When comparing the change in BM (kg) and race performance (hours) a significant correlation was found ($P < 0.05$), so that athletes who completed the race in the fastest time lost the most BM.

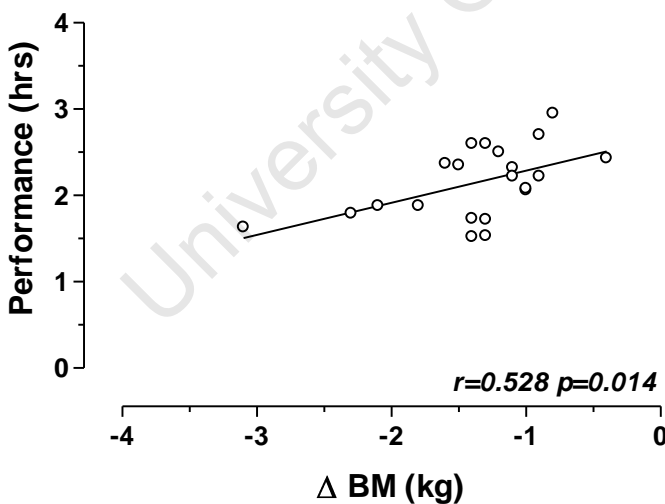


Figure 4.6 The significant association between the change in body mass (BM) and race time/performance.

4.5) Discussion:

The first relevant finding was that the athletes demonstrated a variable total fluid intake and rate of fluid intake (Table 4.1) illustrating individualistic requirements for fluid intake during exercise. But on average their fluid intakes fell into the lower range of recommended volumes^{78;103}. All the athletes in this cohort completed the race within in the time period required (3 hour cut-off) and without any serious medical complications (i.e. exercise induced heatstroke or post-exercise collapse).

These data are essentially the same as in Chapter 3 confirming our preliminary conclusion that fluid intake according to the dictates of thirst will ensure the maintenance of body fluid homeostasis during exercise (Table 4.2). Thus together with the support of findings from Chapter 3, we would further suggest that the athletes participating in races of this or shorter distance may find an *ad libitum* approach to fluid intake an appropriate strategy for safe and successful race completion.

We again found that all the athletes completed the race with plasma $[Na^+]$ and POsm well within the normal laboratory reference ranges despite significant BM loss (Figure 4.5)⁶³. This further emphasises the body's innate regulation of plasma electrolyte concentration and POsm in order to maintain fluid homeostasis and again we show that BM is not homeostatically-regulated during exercise but rather changes in order to insure that plasma $[Na^+]$ and POsm are regulated within a safe range. Interestingly it seems that moderate drinking to thirst seems to be appropriate to maintain the recommendations set by the 2007 ACSM position stand over this 21.1km foot race¹⁰³.

The third and perhaps most pertinent find in this study was that a ~1.4kg significant loss in BM occurred over the half-marathon (Figure 4.1). This was associated with a non-significant loss in TBW of ~0.06kg and non-significant changes in plasma $[Na^+]$, $[K^+]$ and POsm but with a significant increase in plasma [TP] (Figure 4.2). UOsm also decreased during the race (Table 4.2). This decrease in BM would be accounted for by both the loss of some TBW (Figure 4.3) and irreversible fuel oxidation. The post-race blood biochemical markers of fluid homeostasis remained within the normal accepted clinical ranges⁶⁴.

These data extend our findings from Chapter 3 that BM loss does not equate to TBW loss, during 2-3 hours of exercise. This is confirmed with the maintenance of plasma $[Na^+]$ and $[K^+]$, POsm and a mild decrease in UOsm. The small decrease in UOsm pre- to post-race possibly indicated that this race was not of sufficient duration to induce a maximal suppression of renal free water excretion suggesting that fluid balance was not significantly compromised by the -1.9kg change in BM and the insignificant change in TBW⁹⁴. Had there been a significant reduction in TBW then it would be expected that urine free water clearance would be reduced and urine osmolality would rise. But this did not happen indicating that urine free water clearance was not maximally suppressed during exercise suggesting that body fluid homeostasis was not seriously threatened a race of this distance whilst drinking to thirst.

Other researchers including Nolte *et al* (2010) and Colt *et al* (1978) have also found a decrease in BM associated with a minimal change in TBW in athletes running 10 miles^{19;84}. Although Colt and colleagues concluded that their use of tritium to determine TBW undermined the certainty of their findings, however jointly these observations support the hypothesis that significant BM can occur with a non-significant loss in TBW during an acute bout of exercise in which plasma $[Na^+]$ and

POsm is maintained but BM is lost (Table 4.2) (Figure 4.1). This suggests that body fluid homeostasis during exercise is appropriately regulated in those who drink according to thirst during exercise but that this regulation is highly individualized.

The half-marathon is of a shorter distance and duration to its longer counterparts (races above >42.2km races). In addition the intensity at which these athletes run and demographic of the population participating is varied which may explain some variances seen in this cohort. The variance is further increased by with inherent variability associated with the technique itself as discussed in Chapter 2.

Curiously no significant relationship was found when comparing both the absolute and percentage changes in TBW and BM ($r^2=0.003$, $p=0.828$; $r^2=0.026$, $p=0.485$ respectively) (Figure 4.3). Interestingly our data disagree with the findings of the laboratory study of Baker et al (2009) who concluded that BM changes linearly tracked the changes in TBW over exercise⁵. This would probably be a comparable field study trial since on average this study's exercise bout lasted on average 2.15 hours (Table 4.1) and Baker et al (2009) consisted of a 2 hour trial of intermittent running resulting in only 105 minutes of actual exercise.

Elsewhere we have argued that their data do not support their conclusions⁸⁵. Rather we have concluded that their data found no relationship between changes in TBW and BM during the 105 minutes of interval running. Indeed their data for each of the BM losses that they studied were remarkably similar to those we report for the 21km race. Just as we found here, they also showed a wide scatter for changes in TBW for any BM loss (Figure 2 in Nolte and Noakes (2009))⁸⁵.

Our last relevant finding was that a greater loss of BM was correlated with a faster performance over the half-marathon (Figure 4.6; $r^2=0.278$, $p=0.01$). Although not

entirely novel this finding has not been previously described to our knowledge over a shorter exercise duration and distance and supports previous studies that have established this relationship over both Ironman triathlons and ultra-endurance running races^{55;108;109}.

It would seem apparent that athletic performance is associated with a greater decrease in BM during competition which conflicts with the current ACSM position stand that athletes should be encouraged to prevent a >2% decrease in BM during exercise in order to maintain performance. This could be a result of many reasons for example behavioural actions such as the fastest athletes don't have time to consume as much fluid as slower athletes, therefore lose more BM and physiological actions such as the maintenance of body fluid homeostasis, thus a conservative fluid intake despite significant BM loss. Rather our current and previous findings have shown that this is not the case and have raised the possibility that it is the presence of thirst rather than of a specific degree of BM loss that causes an impaired exercise performance^{104;105}.

4.6) Conclusion:

This study has shown that BM lost does not equate to a loss in TBW in athletes completing a half-marathon. But despite BM loss there was the maintenance of plasma $[Na^+]$ and POsm. Further our data illustrates that better performance time is associated with a greater loss in BM which disputes one of the ACSM's logical reasons for their conclusion that athletes drinking should be sufficient to prevent a >2% BM loss during exercise.

Interestingly we have found that our data for *ad libitum* fluid intake fell into the ACSM recommendations to prevent >2% BM loss. *Ad libitum* fluid intakes were in the lower range of those guidelines. These findings reveal the accuracy of the human body's intrinsic ability to maintain fluid homeostasis whilst being able to perform work optimally when drinking according to the dictates of thirst.

CHAPTER 5

CHANGES IN TOTAL BODY WATER CONTENT OVER A 56KM RUNNING RACE IN ATHLETES DRINKING *AD LIBITUM*

University Of Cape Town

5.1) Abstract:

Objective: To measure changes in body mass, total body water and blood biochemical variables in athletes drinking *ad libitum* during a 56km foot race.

Setting: 2009 Two Oceans 56km Ultra-marathon.

Main Outcome Measurements: 12 participants were measured for body mass (BM), total body water (TBW), plasma osmolality (POsm), plasma sodium ($[Na^+]$), plasma potassium ($[K^+]$) and plasma total protein ([TP]) concentrations immediately before and after these races. Fluid intake was recorded from recall after the race. Subjects were advised to drink according to the dictates of thirst (*ad libitum*).

Results: Significant BM loss occurred during the race (-2.5 ± 1.1 kg; $P < 0.000$). TBW was reduced significantly during the race (-1.4 ± 1.1 kg, $P < 0.001$). A negative linear relationship was found between percentage change in ($\% \Delta$) TBW and BM in these athletes ($r^2 = 0.4$; $P < 0.01$). POsm and plasma [TP] increased significantly during the race (6.8 ± 8.2 mOsm/kgH₂O; $P < 0.05$ and 5.4 ± 4.4 g/L; $P < 0.01$ respectively) but all other biochemical measures were within the normal range. There were significant correlations between post-race plasma $[Na^+]$ and $\% \Delta$ BM ($r^2 = 0.4$; $P < 0.05$) and Δ BM ($r^2 = 0.5$; $P < 0.05$) in these athletes.

Conclusion: Despite a significant reduction in BM, the reduction in TBW was less during the race denoting that not all BM loss was due to a reduction in TBW. The maintenance of plasma $[Na^+]$ and POsm was observed with an *ad libitum* fluid intake. Collectively these findings support the hypothesis that the body preserves plasma $[Na^+]$ and POsm and not BM during exercise. A reduction in BM is not associated with an equivalent reduction in TBW during prolonged exercise.

Key words: fluid balance, ultra-endurance exercise, deuterium oxide.

5.2) Introduction:

The introduction supporting the rationale of this study during the 2009 Two Oceans 56km ultra-marathon can be found in Chapter 4.1.

5.3) Material and Methods:

The exact protocol was carried out as previously described in Chapter 4.3 except with a group of 56km athletes.

Subjects: Media releases prior to the race invited entrants in the 2009 Two Oceans ultra-marathon (56km) to participate in this study which had been approved by the Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town. The study was conducted according to the Declaration of Helsinki (Seoul, 2008). Thirteen athletes gave written informed consent to participate in this study. All the athletes were encouraged to consume food and fluids *ad libitum* during the race.

Setting: The Two Oceans Marathon is a 56km ultra-distance road running race around the Western Cape Peninsula. The event took place on a warm day with the dry bulb temperatures ranging from 18 – 24°C with 50-70% humidity (South African Weather Bureau). There was some cloud cover with a wind of 0.5 – 2 m.s⁻¹. Official cut-off time was seven hours for the 56km race.

Fluid Intake: Fluid intake was estimated from a food and fluid recall questionnaire administered by the researchers after the race. The recall consisted of enquiring about the amount of fluids (contained in sachets, cups and bottles of known volume)

consumed at each station during either race. Fluids were available to the runners every ~2km on the 56km route (32 aid stations).

Urine Osmolality (UOsm): Urine samples were collected prior to BM measurement when subjects were requested to empty their bladder on the occasions described above. UOsm was acquired in triplicate with the use of a portable refractometer (Osmocheck, Vitech Scientific, West Sussex, UK).

Measurement of Body Mass (BM): Subjects were weighed to the nearest 0.01 kg in racing attire without shoes on a calibrated electronic digital scale (Clover Scales (Pty) Ltd: TCS-A300) that had been placed on a flat, solid, stable surface after each had emptied their bladder. Weighing took place 60 minutes prior to race start (pre-race) and immediately upon completion of the race (post-race). Subjects were dried with a towel prior to the post-race re-weighing.

Blood Biochemical Analyses: 10ml venous blood samples were collected from the antecubital vein into lithium- heparin Vacuette (Greiner Bio-one International AG, Kremsmuenster, Austria) containers after the subjects were weighed before and after the race. During the blood drawing, subjects were seated. Blood samples were centrifuged at 3000G for 10 minutes at 4°C. Plasma was extracted and placed in eppendorf tubes, placed on ice then stored at -20°C until analysis for plasma total protein ([TP]) (Roche P-Module, Biuret) (CV- <2%), sodium ([Na⁺]), potassium ([K⁺]) (EasyLyte PLUS Na/K/Cl analyzer, Medica Corporation) concentrations and plasma osmolality (POsm) (Osmomat 030 Cryoscopic osmometer, Gonotec) (CV- 2.86%).

Measurement of Total Body Water (TBW): TBW was calculated with the diluted isotope method using D₂O⁵⁴. An initial ~1.5ml saliva sample was collected for use to calculate natural D₂O abundance (as well as increased D₂O abundance due to prior

doses) and repeated after 2-hour equilibration periods to determine TBW on two separate occasions: 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 the use of sterile tweezers. Each subject was then 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 10ml sterile syringe. All saliva was then extracted by compressing the cotton swab against the head of the syringe; this allowed all the collected fluid to flow directly into a cryotube. Samples were immediately sealed and frozen (-80°C). Before analysis samples were prepared by filtration through sterile, single-use syringe filters into 1ml glass vials then sealed and sent for analysis.

The doses of D_2O used for TBW measurement were administered as individualized doses immediately following baseline saliva sampling. Each D_2O dose was dispensed from a 4% weight-to-weight stock solution that was prepared by mixing appropriate amounts of 99% D_2O (Cambridge Isotope Laboratories Inc., MA.) and distilled water. The pre-mixed D_2O stock solution was decanted into an airtight container and sealed with duct tape to prevent fractionation during storage. Each participant received a dose of approximately 0.05g/kg BM with each dosage was pre-weighed to the nearest 0.001g. After each dose had been consumed, the dose bottle was immediately re-weighed to quantify the precise amount that had been ingested. Food consumption was prohibited during the 2-hour equilibration period. For the first two hours after the race 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 D_2O

administration is known to be appropriate^{14;18;23;24;29;54;58;69;106;112;117;134}. All urine excreted during this equilibration period was collected and analysed to account for any isotope loss. The D₂O enrichment and volume of these urine samples were accounted for in subsequent TBW calculations.

TBW determination was obtained through measurement of D₂O enrichment in the saliva samples, as measured by the Stable Light Isotope Laboratory (Department of Archaeology, University of Cape Town, South Africa) by pyrolysis in a Thermo Finnigan TC/EA (Thermo Fisher Scientific Inc. Waltham, MA, USA) with a SpectraSYSTEM® AS3000 Autosampler (Thermo Fisher Scientific Inc. Waltham, MA, USA), coupled via a Thermo Conflo III (Thermo Fisher Scientific Inc. Waltham, MA, USA) to a Thermo Delta XP stable light isotope mass spectrometer (Thermo Fisher Scientific Inc. Waltham, MA, USA). Samples were run against internal laboratory standards and were measured at intervals throughout the run to ensure consistency. The results were normalised against and reported relative to the international standards (VSMOW) (relative standard deviation <2%). TBW was then calculated utilizing the Halliday and Miller method³⁹ with a modified correction factor for non-aqueous hydrogen exchange at 1.04 (4%)^{23;39}.

Calculations:

Equation 1: Percentage change (%Δ) of body mass (BM) lost or gained during the race was calculated as:

Equation 2: Change in plasma volume (PV) was calculated ¹²³:

Equation 3: Performance was measured as finishing times and running speed (km/hr). Running speed was calculated as an average over the entire race:

Equation 4: The Davies method was used to calculate the total body water (TBW) in kilograms (kg)²²:

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 (g); **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 D₂O ⁹⁶.

Equation 5: TBW (kg) was attained and %ΔTBW was calculated as:

Statistical analysis: Data were analyzed using the STATISTICA version 8 (StatSoft Tulsa, OK) statistical program and Prism 3 (GraphPad Software Inc., La Jolla, CA) using Pearson's correlations, students T-test. Where applicable, all data were presented as means \pm standard deviations (SD) including the range of values. Statistical significance was accepted when $p < 0.05$.

University Of Cape Town

5.4) Results:

Twelve of the thirteen runners (3 females and 9 males) successfully completed the 56km ultra-marathon without requiring any medical attention. The runners completed the race with a mean finishing time of 5.65 ± 1.05 hours (range: 4.06 – 6.90) (Table 5.1). One male runner failed to finish the 56km race in the allotted time and was withdrawn from the study. Thus the mean age of the resultant 56km cohort was 40.6 ± 10.6 years (range: 28 -54) (Table 5.1). The subjects ingested a total of 3.19 ± 2.25 L with total volumes ranging from 0.4 – 7.7L. The rates of the runners' fluid intake varied from 99 – 1216ml/hr (mean of 538 ± 354 ml/hr) (Table 5.1).

Table 5.1: General characteristics of subjects participating in the 2009 Two Oceans 56km foot race (n=12)

Variable	56km		
	Mean \pm SD	Min	Max
Age (years)	41.6 ± 10.6	28.0	54.0
Pre-race body mass (kg)	69.9 ± 8.6	58.4	83.7
Body Fat (%)	15.3 ± 4.6	8.2	24.4
Predicted race time (hrs)	5.3 ± 0.9	3.8	6.3
Actual Race time (hrs)	5.7 ± 1.1	4.1	6.9
Fluid intake (ml)	3186 ± 2254	400	7700
Drinking Rate (ml/hr)	538 ± 354	98	1215

Plasma $[K^+]$ and $[Na^+]$ did not change significantly during the race but POsm and plasma [TP] increased significantly ($P < 0.05$ & $P < 0.01$ respectively) (Table 5.2) (Figure 5.2). Pre-race and post-race TBW and BM values are displayed in Table 5.2. A change in BM from pre- to post-race of -2.45 kg (± 1.2) (-3.6%) was experienced by the athletes (Figure 5.1) ($p < 0.0000$) alongside an absolute decrease in TBW of ~ 1.44 kg (± 1.1) ($P < 0.01$) (Figure 5.1).

Table 5.2: Body mass, total body water and markers of hydration status of runners participating in the 2009 Two Oceans 56km foot race.

Variable	56km					
	<i>n</i>	Pre-race (mean ± SD)	<i>n</i>	Post-race (mean ± SD)	Δ (mean ± SD)	<i>P</i> -value
Plasma [K ⁺] (mmol/L)	12	4.0 ± 0.5	11	4.7 ± 1.9	0.7 ± 2.0	NS
Plasma [Na ⁺] (mmol/L)	12	138.6 ± 2.2	11	141.1 ± 3.3	2.3 ± 3.0	NS
POsm (mOsm/kgH ₂ O)	12	284.6 ± 5.5	11	291.1 ± 9.9	6.8 ± 8.2	< 0.05
Plasma [TP] (g/L)	12	75.8 ± 2.0	11	81.5 ± 4.4	5.4 ± 4.4	< 0.01
UOsm (mOsm/kgH ₂ O)	12	620.0 ± 301.2	12	721.7 ± 322.9	101.7 ± 325.0	NS
Body mass (kg)	12	69.9 ± 8.6	12	67.4 ± 8.7	-2.5 ± 1.1	< 0.0000
Body mass (%)	12	100	12	96.5 ± 1.6	-3.6 ± 1.6	< 0.0000
Total Body Water (kg)	12	43.2 ± 7.4	12	41.6 ± 7.3	-1.44 ± 1.1	< 0.001
Total Body Water (%)	12	61.7 ± 5.7	12	61.5 ± 6.5	0.12 ± 2.2	NS

[K⁺] – Plasma potassium concentration; [Na⁺] – Plasma sodium concentration; **POsm**- Plasma osmolality; [TP]- Plasma total protein concentration; **UOsm** – Urine osmolality; Δ: comparing post-to pre-race

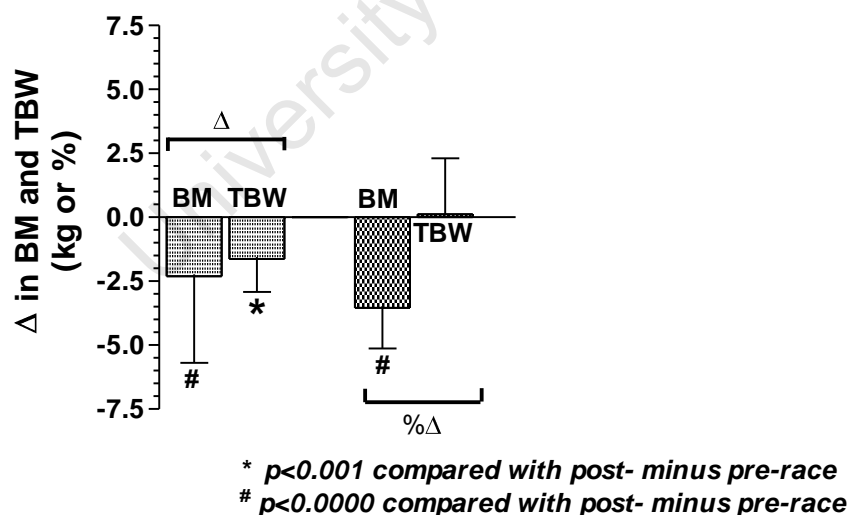


Figure 5.1 Both absolute change (Δ) and percentage change (%Δ) in body mass (BM) and total body water (TBW) in the 56km runners pre- to post-race.

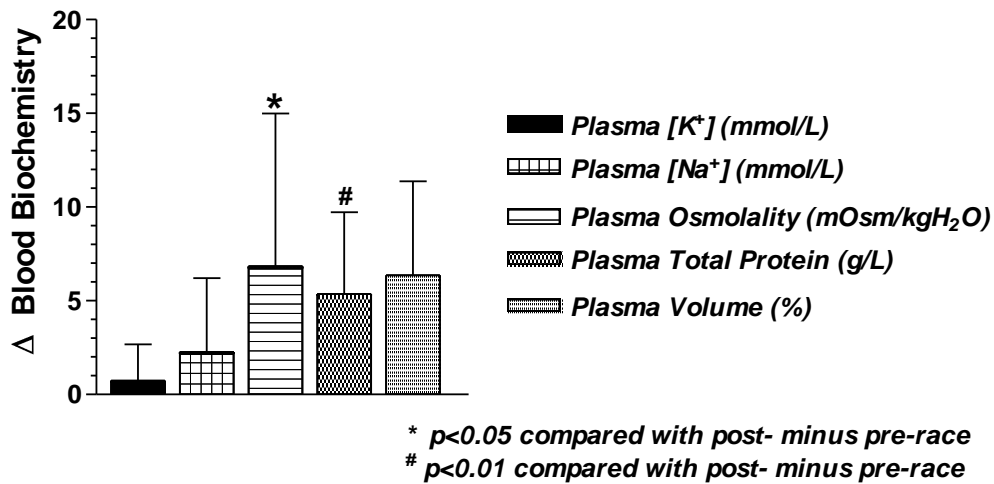


Figure 5.2 Changes in blood biochemical measures of hydration status in the runners participating in the 56km foot race

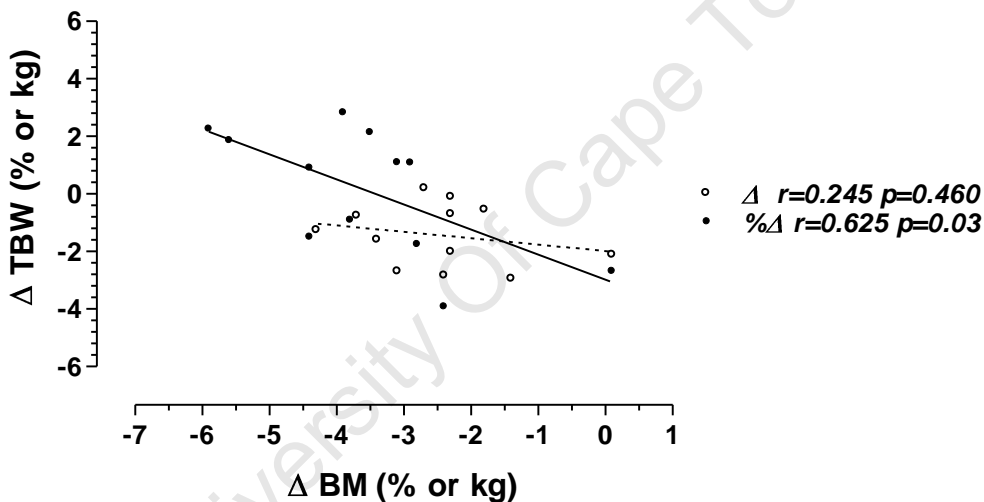


Figure 5.3 Non-significant correlation between absolute change (Δ) in body mass (BM) and total body water (TBW). A significant correlation was found between percentage change ($\% \Delta$) in BM and TBW ($P < 0.05$).

Figure 5.3 illustrates the correlation between either absolute and $\% \Delta$ TBW and BM. There was a significant correlation between $\% \Delta$ in BM and $\% \Delta$ TBW so that the 56km runners showed a decrease in $\% \text{BM}$ with a subsequent increase in $\% \text{TBW}$ (Figure 5.3; $p < 0.05$).

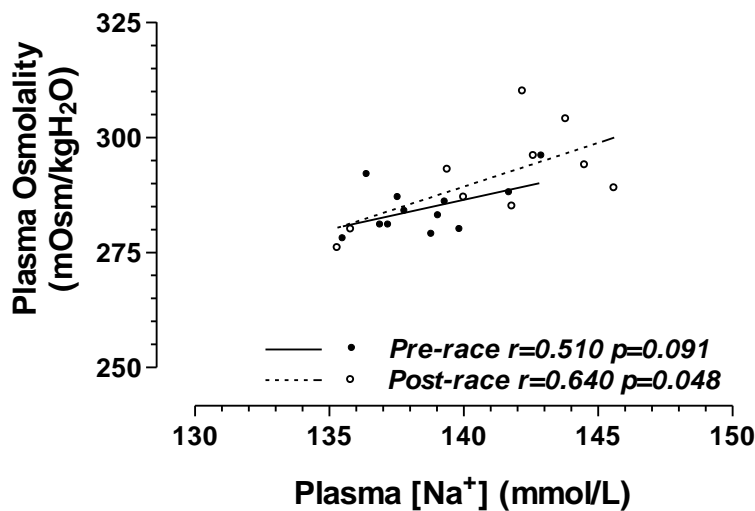


Figure 5.4 A significant correlation between plasma osmolality (POsm) and plasma sodium concentrations ([Na⁺]) in the post-race but not pre-race measures of the 56km runners.

The significant physiological relationship between POsm and plasma [Na⁺] was evident in this cohort for their post-races measures (Figure 5.4; $P < 0.05$). No significant relationships were found for their pre-race measures (Figure 5.4).

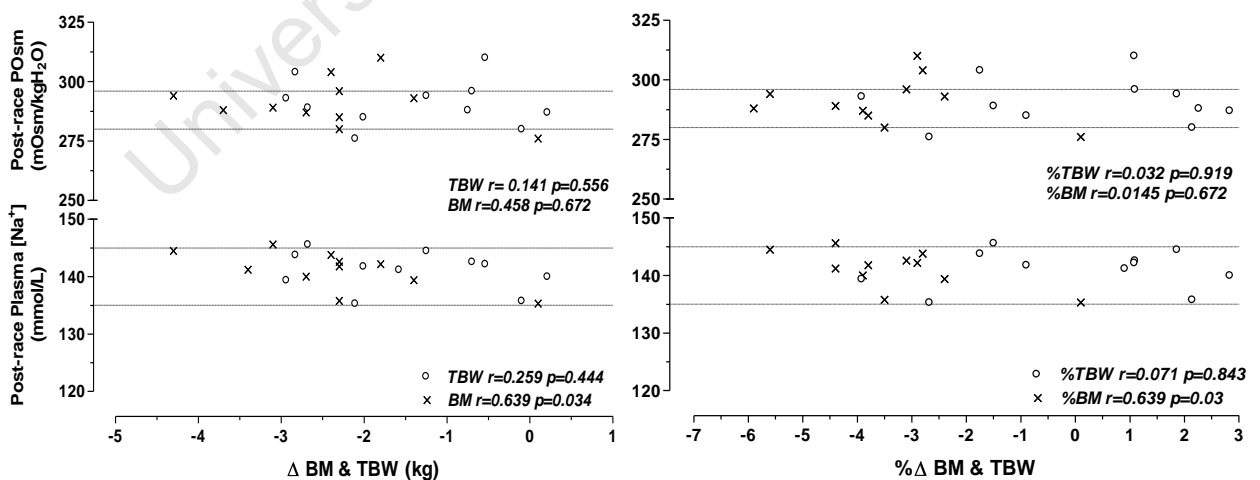


Figure 5.5 The relationship between percentage change (% Δ) and absolute change (Δ) in body mass (BM) and total body water (TBW) in relation to post-race plasma osmolality (POsm) and plasma sodium concentrations ([Na⁺])

Moreover, no significant correlations were found between both absolute and % Δ TBW and BM when compared to post-race POsm and plasma $[\text{Na}^+]$. Except with the absolute and % Δ BM and post-race plasma $[\text{Na}^+]$ ($p < 0.05$) a significant inverse relationship was found (Figure 5.5). The dotted lines indicate the normal range of POsm (280 – 296 mOsm/kgH₂O) while the solid lines denote the normal range of plasma $[\text{Na}^+]$ (135 – 145 mmol/L)⁶³. Only one runner's plasma $[\text{Na}^+]$ fell marginally outside the normal range whereas only three POsm measurements were above the normal range (Figure 5.5).

There was no significant association found between performance time and change in BM (Figure 5.6).

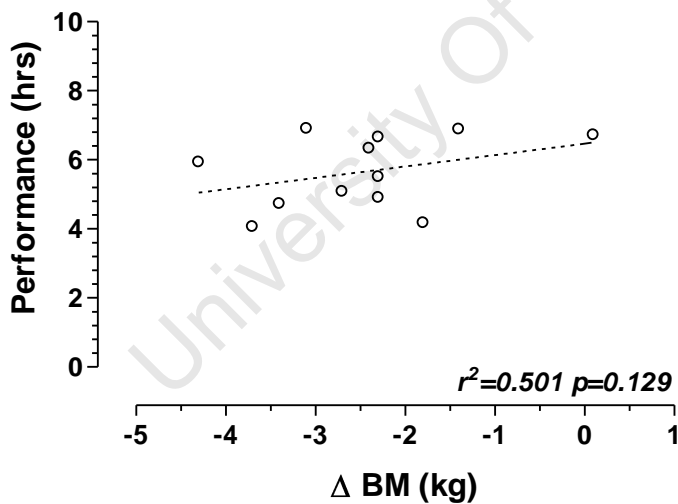


Figure 5.6 The relationship between absolute change in body mass (BM) and athlete performance measured as race time (hrs) in the 56km runners.

5.6) Discussion:

The first major finding of this study was that when subjects drank *ad libitum* during the race they maintained their plasma $[Na^+]$, $[K^+]$ and PO_{sm} within the normal accepted clinical range. This agrees with findings observed in both Chapter 3 and 4, which advances the established finding that abnormalities in fluid balance and not excessive losses of sodium chloride in sweat and urine explain variations beyond the normal range in blood $[Na^+]$ during exercise^{44;83}.

Ad libitum drinking was however associated with a significant reduction in BM. This condition was termed “voluntary dehydration” by Adolph in 1947² on the assumption that this BM loss is due to water losses that cause a reduction in TBW. But the authors did not actually measure changes in TBW in their study subjects, yet the term has been retained in the literature^{21;37;38} despite few attempts actually to measure concurrently exercise induced changes in BM and TBW. This is necessary to determine whether the condition of voluntary dehydration does indeed exist or whether it is another example of “christening by conjecture”^{31;115;116}.

Accordingly the second important finding of this study was that the changes in BM in all races exceeded the smaller changes in TBW indicating that water losses alone did not explain all the BM lost during exercise in these subjects. This finding is in keeping with the classic interpretations^{73;90;110}. Our findings disagree with those of Baker *et al* (2009) who concluded that changes in TBW and BM tracked each other in a linear fashion, a conclusion we have questioned⁸⁵ and previously discussed in Chapter 3 and 4 it has been shown that the authors data do not support their conclusions⁸⁵.

Thus an important conclusion is that changes in BM during exercise are not due purely to changes in TBW so that all the BM loss during exercise is not due solely to water loss. Our other recent studies support this interpretation as indeed do the data of Baker et al (2009) when analysed appropriately⁸⁵.

Interestingly we found that although mean %BM decreased ~3.5% (~2.4kg) during the 56km race; mean %TBW actually increased ~0.12% (~1.6kg). This finding is in agreement with those of Knechtle *et al* (2008) who found an increase in %TBW over the course of a 1200km multi-stage ultra-endurance race despite a loss of ~3kg in BM⁶⁰. This finding can be explained if the decrease in BM results from a loss of skeletal and fat mass with an increase in the water content of the remaining tissues at the end of exercise.

The origin of this added water might be the water originally bound to glycogen which is released as the glycogen is metabolized during exercise or possibly from fluid stored in the intestine^{73;87}. 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 adequately reflect exact changes in hydration status^{45;73}.

Furthermore, these results raise concern of the validity of fluid intake guidelines which recommend that athletes must not incur a BM loss >2% of starting BM during exercise^{17;36;103}. While changes in BM may be useful to predict fluid homeostasis in certain clinical scenarios at rest¹²⁹. During exercise the homeostatic controls that protect POsm may be associated with a decrease in BM without any negative physiological consequences^{27;51}. The failure of humans to replace 100% of their BM losses when they ingest fluid *ad libitum* during exercise has led to recommendation that athletes must drink beyond the sensations of thirst in order to prevent "voluntary dehydration"^{38;38}. However, the data presented here and in this thesis confirm our

other findings that *ad libitum* drinking will maintain %TBW with an insignificant decrease in absolute TBW even when there is a significant loss of BM. These data support our contention that BM is not the important physiologically-regulated variable during exercise^{78;80}. Strong evidence supporting the physiological regulation of BM had 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 other than to acute changes in fluid balance^{1;40}.

We are not the first to report dissociation between BM loss and TBW, Colt *et al* reported a 2.4% BM loss associated with a 2.4% increase in TBW in ten well-trained males running 16km. These results agree directly with the results of our 21.1km runners who showed a ~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 BM and TBW in both groups of our runners (Figure 5.2 & 5.3). Additionally Nolte *et al* (2010)⁸⁴ and Knechtle *et al* (2010)⁶¹ observed similar changes in BM and TBW over a 16km route-march and 100km ultra-endurance run in their cohort of athletes respectively.

It is perhaps counter-intuitive that there can be a reduction in BM with a maintained or even increased absolute or percentage TBW in subjects who drink less than they sweat during exercise and hence, in theory at least, must develop a reduction in TBW reserves. But this excludes 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 a significant absolute BM loss^{62;90;97}.

The only study which has critically assessed changes in BM, muscle glycogen content and TBW was performed by Olsson and Saltin in 1970. They demonstrated

that 3–4 grams of water may be complexed with each per gram of glycogen is stored in muscle (and liver)⁸⁷. Thus subjects 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” 2-3 days prior to exercise^{87;99}. Since the magnitude of this weight gain exceeds the body’s storage capacity for glycogen, it most likely includes some mass gain resulting also from water storage.

Although the POsm did rise significantly and certain values were above the “normal” range, none of the runners required medical assistance at the end of the race^{45;49}. This supports our hypothesis that athletes are able to regulate their plasma $[Na^+]$ and POsm despite the loss of some TBW and a greater than 2% loss of their BM during prolonged exercise^{33;46}.

Strengths and weaknesses of the study: This study provided a larger data set of TBW measurements and over a different distance compared to Chapter 3. Finally it is important to acknowledge that the post-race TBW measurement might be prone to a slight overestimation of the TBW pool compared to the pre-race measurement. Increased turnover of body water could result in possible isotope loss during the equilibration period ⁶. The isotope loss during the post-race equilibration period might be slightly higher compared to the pre-race equilibration period due to increased metabolic rates after exercise. This could result in isotope loss through sweat, respiratory water loss (RWL) as well as dilution of the isotope due to increased metabolic water production. Corrections for these variables remain approximates due to 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 pre-race measurements.

5.5) Conclusion

In conclusion this study has demonstrated that the significant decrease of ~2.5kg in BM occurs in endurance athletes who drink to the dictates of thirst during prolonged exercise but that changes in TBW were associated with a less significant decrease of ~1.4kg in TBW in runners during a 56km foot race. Adequate body fluid homeostasis was further confirmed with the maintenance of the blood biochemical markers POsm and plasma [Na⁺].

Therefore, the present study confirms our previous findings that the change in TBW during prolonged endurance exercise was substantially less than the significant ~3.5% BM loss measured in these runners. This finding further supports the conclusion that the body primarily defends POsm and plasma [Na⁺] – and **not** BM – during prolonged endurance exercise (56km).

CHAPTER 6

SUMMARY AND CONCLUSIONS

University Of Cape Town

The prescription of fluid intake during exercise has been a contentious issue for the past decade. What is known is that it is helpful to consume fluids for both performance and health protection during exercise especially during exercise lasting longer than 2 hours. The addition to this suggestion is that fluid containing carbohydrates and electrolytes have been encouraged to improve performance, slow the onset of fatigue and prevent dehydration¹⁰³.

But it is not the prescription of fluid itself that has been the main crux of the issue rather it is the question of how much should be ingested? Interestingly prior to the 1970s the principal recommendation was to “drink as little as possible”. Subsequently there was a rise of published recommendations that emphasized the detrimental effects of inadequate hydration and the need to prevent “dehydration”^{20;30;104}. These recommendations by authorities in the sports medicine field such as the ACSM began promulgating advice that athletes should “drink beyond thirst” and “as much as tolerable”²⁰.

This advice has been promoted despite the fact that field research has persistently proven that these recommendations, if strictly followed, can result in life-threatening issues such as EAH and death due to EAHE as documented from as early as 1985^{82;101}. Additionally it has been found that the best performing athletes lose significantly more BM during competition than those who perform less well^{55;108;109}. As a result some have proposed athletes should drink according to the physiologically-driven mechanism of thirst^{49;78;81}. Such advice has only recently been widely accepted by authorities such as the IMMUDA and USA Track and Field Association⁴⁹.

A decade later in 2007 the ACSM updated their guidelines for fluid replacement during exercise and have since proposed the recommendation that fluid should be

ingested to prevent >2% BM during exercise. These guidelines also promoted customized fluid replacement for individuals¹⁰³.

Subsequently this thesis intended to scientifically investigate this position stand and determine whether athlete's TBW loss during exercise is equivalent to the loss of BM, whether an *ad libitum* approach to fluid ingestion does indeed maintain an athlete's hydration state (most appropriately determined through POsm and plasma [Na⁺]) regardless of BM loss and lastly to correlate whether athletes losing the most BM suffer in performance (race time).

Accordingly the main findings of this thesis were the following:

- i) Despite variable BM loss in the 21.1km, 56km and 80km foot races, Fluid homeostasis was maintained by *ad libitum* drinking. This means that the body does not protect BM during exercise and that the advice to drink to protect BM is physiologically inappropriate and may have greatly contributed to the recent rise in cases of EAH and EAHE.
- ii) Changes in BM do not equate to changes in TBW (Figure 6.1). Figure 6.1 illustrates that a potential ~2kg decrease in BM will only result in ~1kg decrease in TBW as a result of exercise. This must be because there is general mass lost from irreversible loss of substrate oxidation. Whereas TBW will decrease less due to the contribution of fluid from the water complexed to glycogen and water formed from substrate oxidation. Where *ad libitum* fluid intake will encourage maintenance of body fluid homeostasis (regulation of POsm and plasma [Na⁺]) rather than that of BM loss.

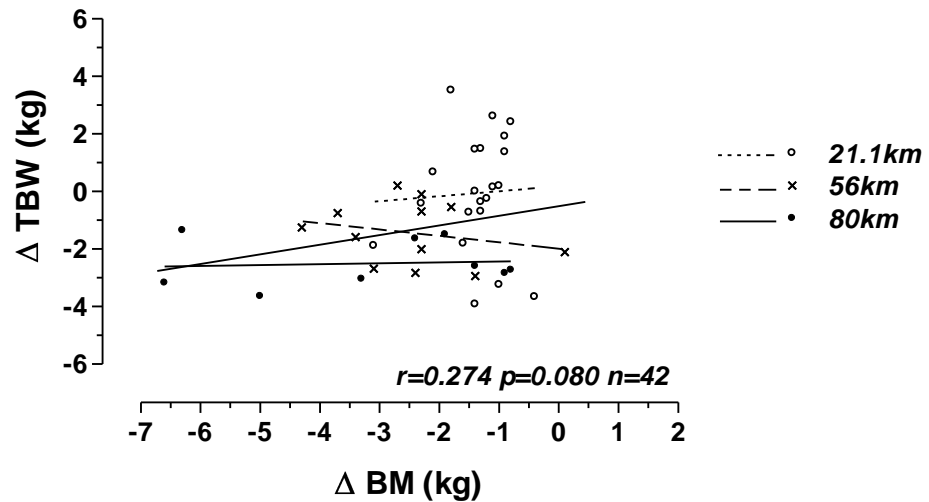


Figure 6.1 Change in (Δ) body mass (BM) in relation to Δ total body water (TBW) incorporating data from the 21.1km, 56km and 80km foot races. (-) represents the correlation between Δ BM and Δ TBW.

- iii) Athletes performing the best often experience the greatest BM loss during the 21.1km (with a similar trend in 56km race). We have suggested that this (in Chapter 4) is a result of both behavioural (fastest athletes don't have time to consume as much fluid as slower athletes) and physiological (maintenance of body fluid homeostasis, despite BM loss) reasons.
- iv) Lastly no medical complications were experienced in our cohort of athletes who drank *ad libitum*. This was further supported by the maintenance of body fluid homeostasis despite the significant loss in BM seen in the 21.1km, 56km and 80km races.

Future investigations should delve into the possible tightly controlled laboratory trials where most channels of fluid gain and loss can be tracked alongside the gross measurements of BM and TBW. This would better establish the internal contributions to fluid homeostasis and possible reason for the loss in BM during prolonged exercise without an equivalent loss of TBW

It should be acknowledged that fluid replacement guidelines have evolved and consequently only for the better since the detrimental 1996 ACSM Guidelines for Exercise and Fluid Replacement. Hopefully this body of work will provide the scientific community with practical, realistic and physiologically sound evidence to further evolve fluid replacement prescription, more specifically to disseminate practical and accurate advice to all athletes participating in athletic competition. These findings support the adoption of *ad libitum* fluid intake during exercise lasting from a 21.1km running race to more punishing ultra-endurance races lasting up to 12 hours. This drinking method ensures that athletes will indeed maintain their body fluid homeostasis and achieve their race goals without encountering avoidable medical complications.

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